

COLONIC EXPRESSION OF GENES ENCODING INFLAMMATORY MEDIATORS AND GELATINASES DURING *CAMPYLOBACTER JEJUNI* INFECTION OF CONVENTIONAL INFANT MICE

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Within 1 week following peroral *Campylobacter jejuni* infection, infant mice develop acute enteritis resolving thereafter. We here assessed colonic expression profiles of mediators belonging to the IL-23/IL-22/IL-18 axis and of matrix-degrading gelatinases MMP-2 and MMP-9 at day 6 post *C. jejuni* strain 81-176 infection. Whereas the pathogen readily colonized the intestines of infant IL-18^{-/-} mice only, colonic mucin-2 mRNA, a pivotal mucus constituent, was downregulated in IL-22^{-/-} mice and accompanied by increased expression of pro-inflammatory cytokines including IFN- γ , TNF, IL-17A, and IL-1 β . Furthermore, in both naive and infected IL-22^{-/-} mice, colonic expression of IL-23p19 and IL-18 was lower as compared to wildtype mice, whereas, conversely, colonic IL-22 mRNA levels were lower in IL-18^{-/-} and colonic IL-18 expression lower in IL-23p19^{-/-} as compared to wildtype mice. Moreover, colonic expression of MMP-2 and MMP-9 and their endogenous inhibitor TIMP-1 were lower in IL-22^{-/-} as compared to wildtype mice at day 6 postinfection. In conclusion, mediators belonging of the IL-23/IL-22/IL-18 axis as well as the gelatinases MMP-2 and MMP-9 are involved in mediating campylobacteriosis of infant mice in a differentially regulated fashion.

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

Introduction

Incidence and prevalence of human *Campylobacter jejuni* infections are progressively increasing worldwide [1–3]. Whereas *C. jejuni* can be regarded commensal bacteria within the intestinal tract of wild and domestic animals, humans usually become infected by consumption of contaminated products derived from livestock animals via the oral route [4, 5]. Infected patients suffer from gastroenteritis of varying degree ranging from mild malaise to severe ulcerative colitis. Whereas disease usually resolves spontaneously, post-infectious sequelae including peripheral neuropathies such as Guillain–Barré and Miller–Fisher syndromes as well as reactive arthritis might arise with a latency of weeks to months postinfection [3, 6, 7]. Our understanding of the molecular mechanisms underlying *Campylobacter*–host interactions has been hampered for a long time since appropriate *in vivo* models were not available [7]. Recently, our group showed that the infant mouse infection model mimics key features of human campylo-

bacteriosis. Upon peroral *C. jejuni* infection immediately after weaning, 3-week-old conventional infant mice develop self-limiting acute enteritis within 1 week [8–10]. *C. jejuni* induced increased colonic epithelial apoptosis and pro-inflammatory innate as well as adaptive immune responses [8–10]. Notably, infant mice exhibited much higher intestinal loads of commensal *E. coli* as compared to adult mice, thereby facilitating stable *C. jejuni* infection [8–10]. Overall, the infant mouse model has been proven suitable to investigate *Campylobacter*–host interactions in more detail [7].

In a very recent study [11], we investigated the role of IL-23, IL-22, and IL-18 during *C. jejuni* infection of conventional infant mice that were deficient in either cytokine gene. In fact, respective cytokines were differentially involved in mediating pro- and anti-inflammatory immune responses during the early (i.e., day 6 postinfection (p.i.)) and late (i.e., day 13 p.i.) phase of infection. For instance, as early as day 6 p.i., colonic numbers of neutrophils, T and B lymphocytes were lower irrespective of the genotype. By then, regulatory T cell (Treg) numbers were low-

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er in IL-23p19^{-/-} and IL-22^{-/-} as compared to wildtype (WT) mice that was accompanied by increased colonic IL-10 levels in IL-22^{-/-} mice at day 6 p.i. Pro-inflammatory cytokines including TNF, IFN- γ , IL-6, and MCP-1, however, were increased in large intestines of IL-23p19^{-/-} animals, whereas IL-18^{-/-} mice exhibited lower colonic cytokine concentrations as compared to WT controls, that were accompanied by lower colonic numbers of T and B cell as well as of neutrophils, macrophages, and monocytes. Later on (i.e., at day 13 p.i.), IL-22^{-/-} mice displayed lower colonic epithelial apoptotic cell numbers as compared to WT mice, whereas, conversely, colonic proliferating cells increased in infected IL-22^{-/-} and IL-18^{-/-} mice. As a follow-up study, we here dissected the contribution of defined molecules to the observed intestinal immune responses upon *C. jejuni* infection of conventional infant mice in more detail. We therefore surveyed the expression profiles of mucin-2, an integral part of the intestinal mucus layer and important for epithelial barrier function, and of distinct pro-inflammatory cytokines such as IFN- γ , TNF, and IL-1 β . We further focused on mediators that are pivotal for antimicrobial host immunity including the T helper (Th) -17 cell cytokines IL-22 and IL-17A and their key regulator IL-23p19, and finally analyzed the matrix-degrading matrix metalloproteinases (MMP) -2 and -9 (also termed gelatinases -A and -B, respectively) within the colon during *C. jejuni* infection of conventional infant mice that were gene-deficient for IL-23p19, IL-22, or IL-18.

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 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) [12]. Immediately after weaning, 3-week-old conventional infant mice were perorally infected with 10⁹ 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 [13].

Sampling procedures

Mice were sacrificed at day 6 p.i. by isoflurane treatment (Abbott, Greifswald, Germany). Colonic *ex vivo* biopsies were asserved under sterile conditions and collected in parallel for microbiological and immunological analyses.

Quantitative analysis of bacterial colonization

Viable *C. jejuni* was detected in feces over time p.i. or at time of necropsy (day 6 p.i.) in luminal samples taken from the colon, dissolved in sterile PBS and serial dilutions cultured on Karmali- and Columbia-Agar supplemented with 5% sheep blood (Oxoid) for 2 days at 37 °C under micro-aerobic conditions using CampyGen gas packs (Oxoid). 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 pathogens was 100 CFU per gram samples.

Real-time PCR

Total RNA was isolated from snap-frozen colonic *ex vivo* biopsies, reverse transcribed, and analyzed as described previously [12]. Briefly, mRNAs coding for murine mucin-2, IL-23p19, IL-22, IL-18, IL-17A, IL-1 β , TNF, IFN- γ , MMP-2, MMP-9, TIMP-1, and TIMP-3 were detected by real-time polymerase chain reaction (PCR) with specific primers and quantified by analysis with the Light Cycler Data Analysis Software (Roche). The mRNA of the house-keeping gene for hypoxanthine-phosphoribosyltransferase (HPRT) was used as reference; the mRNA expression levels of the individual genes were normalized to the lowest measured value and expressed as fold expression (arbitrary units).

IL-22 detection in supernatants of colonic ex vivo biopsies

Colonic *ex vivo* biopsies were cut longitudinally and washed in PBS. Strips of approximately 1 cm² intestinal tissue were placed in 24-flat-bottom well culture plates (Nunc, Wiesbaden, Germany) containing 500 μ l serum-free RPMI 1640 medium (Gibco, Life Technologies, Paisley, UK) supplemented with penicillin (100 U/ml) and streptomycin (100 μ g/ml; PAA Laboratories). After 18 h at 37 °C, culture supernatants were tested for IL-22 by ELISA (R&D Systems, Wiesbaden, Germany).

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

Time course of C. jejuni infection in conventional infant mice lacking IL-23p19, IL-22, or IL-18

Immediately after weaning, 3-week-old conventional infant mice that were gene-deficient for IL-23p19, IL-22,

or IL-18 and corresponding age-matched wildtype animals were perorally challenged with 10^9 CFU *C. jejuni* strain 81-176 on day 0 and day 1 by gavage as described in Heimesaat et al. [11]. As early as 48 h after the latest infection, up to three out of four mice had expelled the pathogen from the intestinal tract. More than 50% of IL-18^{-/-} mice, however, could be stably colonized by *C. jejuni* from day 3 until necropsy at day 6 p.i. (Fig. 1B) and exhibited significantly higher fecal pathogenic loads as compared to mice of the remaining groups ($p < 0.05$ – 0.001 ; Fig. 1).

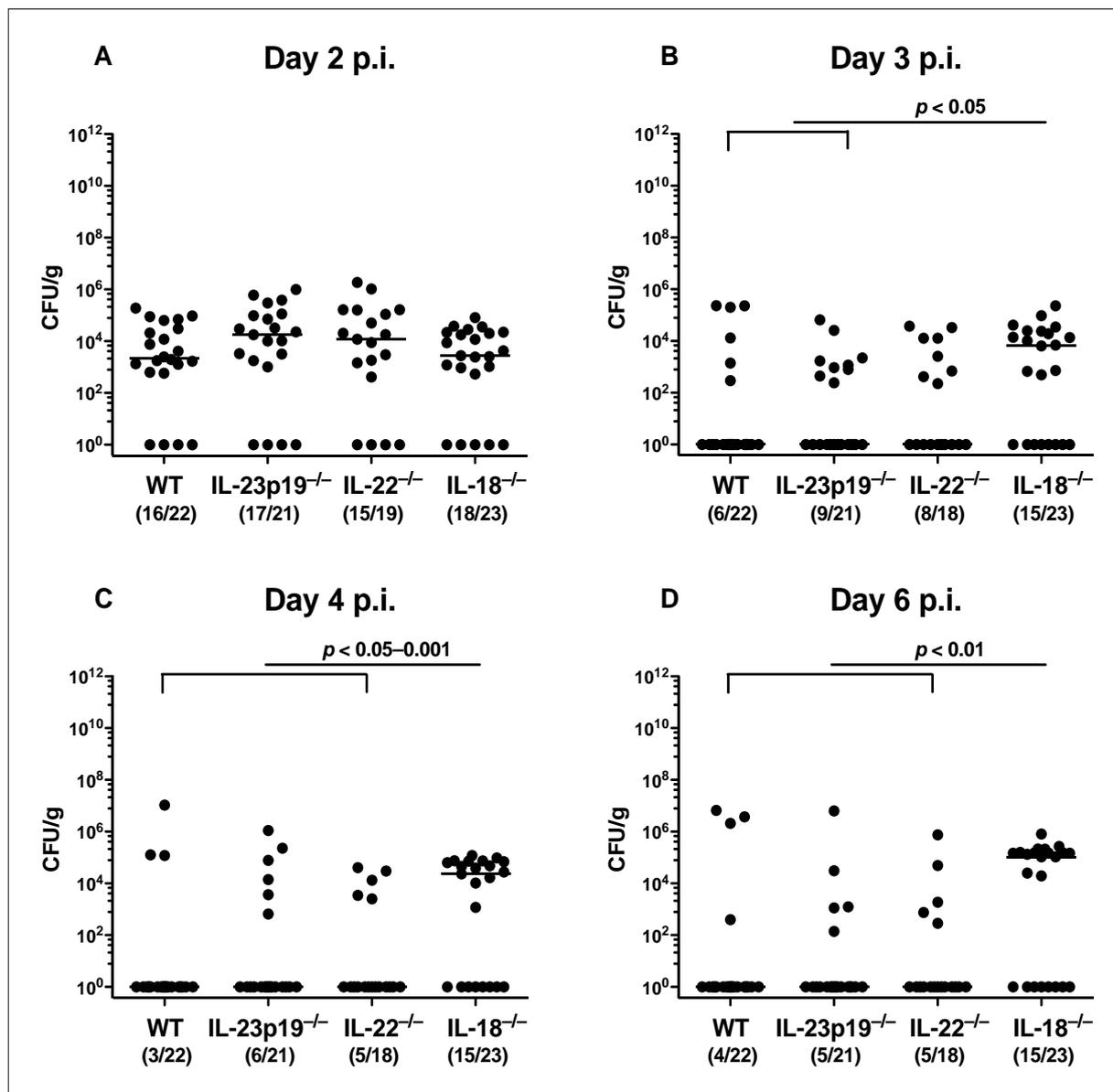
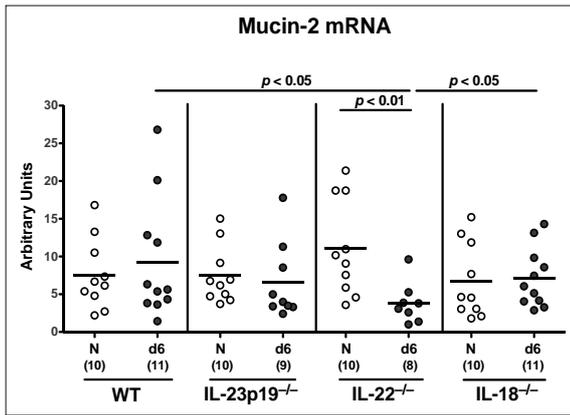


Fig. 1. Intestinal *C. jejuni* strain 81-176 loads over time in perorally infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Pathogenic loads were determined in fecal samples (CFU, colony forming units per gram) at A) day 2, B) day 3, C) day 4, and D) day 6 postinfection as indicated by culture. Medians (black bars) and level of significance (p value) determined by Mann–Whitney U test are indicated. 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



Colonic mucin-2 and pro-inflammatory cytokine expression in C. jejuni-infected infant mice lacking IL-23p19, IL-22, or IL-18

Mucins comprise important constituents of the mucus layer and are pivotally involved in maintaining intestinal epithelial barrier function [14]. Among these, mucin-2 is predominantly expressed in the small as well as large intestine [15]. We therefore investigated whether *C. jejuni* infection affects mucin-2 expression in the colon of infant mice and might subsequently cause compromised intestinal barrier function. In fact, in IL-22^{-/-} mice, colonic mucin-2 expression was downregulated at day 6 p.i. ($p < 0.01$; Fig. 2), and mRNA levels were lower as compared to infected WT and IL-18^{-/-} animals ($p < 0.05$; Fig. 2). Conversely, colonic expression of the pro-inflammatory cytokines IFN- γ , TNF, IL-17A, and IL-1 β was upregulated in *C. jejuni*-infected IL-22^{-/-} mice and the latter cytokine additionally in IL-23p19^{-/-} mice ($p < 0.05$ – 0.01 ; Figs. 3 and 4). Moreover,

Fig. 2. Mucin-2 mRNA expression levels 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. Mucin-2 mRNA expression levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR and expressed as arbitrary units (fold expression). 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 two independent experiments

at day 6 p.i., colonic IFN- γ and IL-1 β mRNA levels were higher in IL-23p19^{-/-} and IL-22^{-/-} mice ($p < 0.05$ – 0.001 ; Figs. 3A and 4B), whereas IFN- γ and IL-17A mRNA expressions were lower in large intestines of IL-18^{-/-} as compared to WT mice ($p < 0.01$; Figs. 3A and 4A). Interestingly, already in the naive state, IFN- γ and IL-1 β mRNA expressions were higher in IL-23p19^{-/-} ($p < 0.05$ and $p < 0.01$, respectively; Fig. 3A and 4B), whereas IFN- γ levels were lower in IL-18^{-/-} mice as compared to WT control animals ($p < 0.01$; Fig. 3A).

Colonic cytokines of the IL-23/IL-22/IL-18 axis in C. jejuni-infected infant mice lacking IL-23p19, IL-22, or IL-18

We next surveyed mRNA expression of cytokines belonging to the IL-23/IL-22/IL-18 axis in *C. jejuni*-infected conventional infant mice. As expected, the respective interleu-

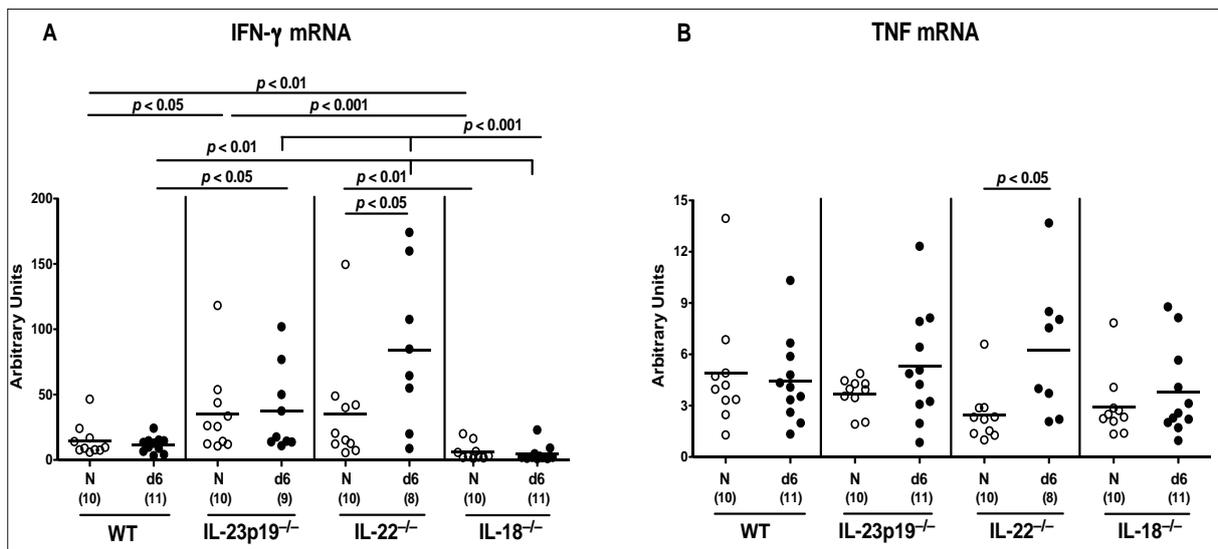


Fig. 3. Expression levels of TNF and IFN- γ mRNA in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22 or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-} and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) TNF and B) IFN- γ mRNA expression levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR and expressed as arbitrary units (fold expression). 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 two independent experiments

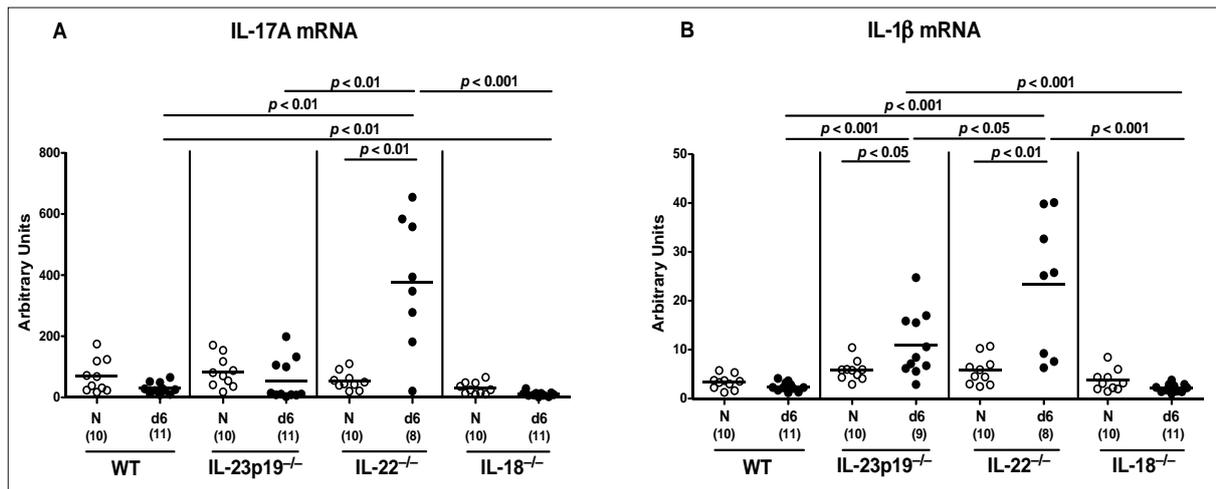


Fig. 4. Expression levels of IL-17A and IL-1 β mRNA in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) IL-17A and B) IL-1 β mRNA expression levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR and expressed as arbitrary units (fold expression). 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 two independent experiments

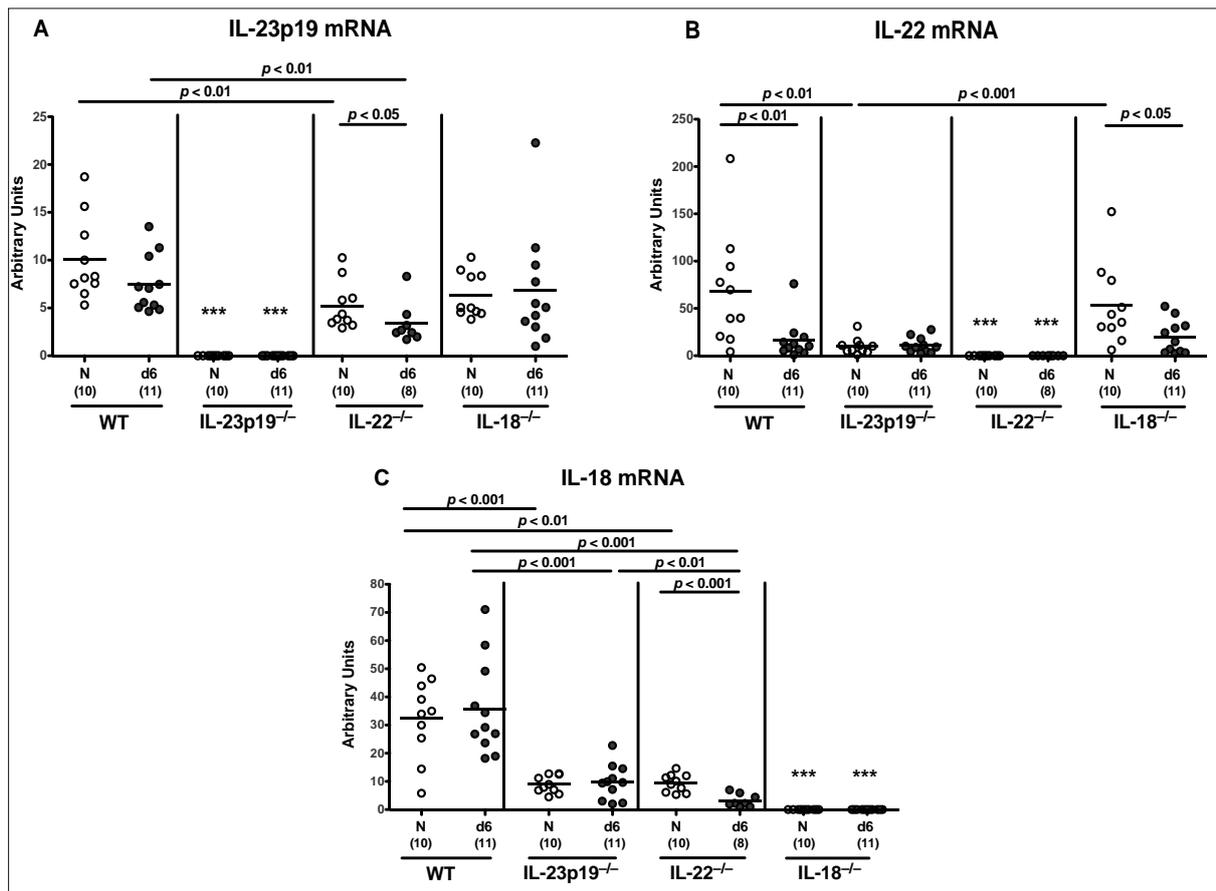


Fig. 5. Expression levels of IL-23p19, IL-22, and IL-18 mRNA in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) IL-23p19, B) IL-22, and C) IL-18 mRNA expression levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR and expressed as arbitrary units (fold expression). 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 two independent experiments

kins were undetectable in the corresponding gene-deficient animals at either time point (Fig. 5). In naive and infected IL-22^{-/-} mice, colonic IL-23p19 mRNA levels were lower as compared to WT controls ($p < 0.01$; Fig. 5A) and were downregulated upon *C. jejuni* infection ($p < 0.05$; Fig. 5A), whereas, the other way round, IL-22 mRNA was lower in naive IL-23p19^{-/-} versus WT mice ($p < 0.01$; Fig. 5B). This was further confirmed by measuring IL-22 protein secretion in colonic *ex vivo* biopsies, given that only basal and significantly lower IL-22 concentrations were determined in large intestines of IL-23p19^{-/-} as compared to WT mice at days 0 and 6 p.i. ($p < 0.001$ and $p < 0.05$, respectively; Fig. 6). In both IL-23p19^{-/-} and IL-22^{-/-} mice, however, large intestinal IL-18 mRNA was lower at either time point when compared to WT mice ($p < 0.01$ – 0.001 ; Fig. 5C). During *C. jejuni* infection, colonic IL-22 mRNA decreased in WT and IL-18^{-/-} mice ($p < 0.01$ and $p < 0.05$, respectively; Fig. 5B), and conversely, IL-18 mRNA in IL-22^{-/-} mice ($p < 0.001$; Fig. 5C). Furthermore, IL-23p19 mRNA levels decreased in large intestines of IL-22^{-/-} mice at day 6 p.i. ($p < 0.05$; Fig. 5A). Hence, the data point

towards a differentially regulated role of the IL-23/IL22/IL-18 axis in mediating *C. jejuni* infection of conventional infant mice.

Colonic gelatinase expression in C. jejuni-infected infant mice lacking IL-23p19, IL-22, or IL-18

Since gelatinases, particularly MMP-2, have been shown to be involved in mediating intestinal immunopathogenesis in *C. jejuni*-infected infant mice [16], we determined large intestinal gelatinase expression levels in infected infant mice lacking IL-23p19, IL-22, or IL-18. Upon *C. jejuni* infection, MMP-2 mRNA expression was lower in IL-23p19^{-/-} and IL-22^{-/-} mice as compared to WT and IL-18^{-/-} animals ($p < 0.05$ – 0.01 ; Fig. 7A). Six days p.i., MMP-9 mRNA expression was upregulated in WT and IL-18^{-/-} ($p < 0.01$ and $p < 0.05$), but downregulated in IL-22^{-/-} mice ($p < 0.05$), with a trend towards lower MMP-9 mRNA levels in IL-23p19^{-/-} animals (n.s.) (Fig. 7B). Moreover, *C. jejuni*-infected gene-deficient mice

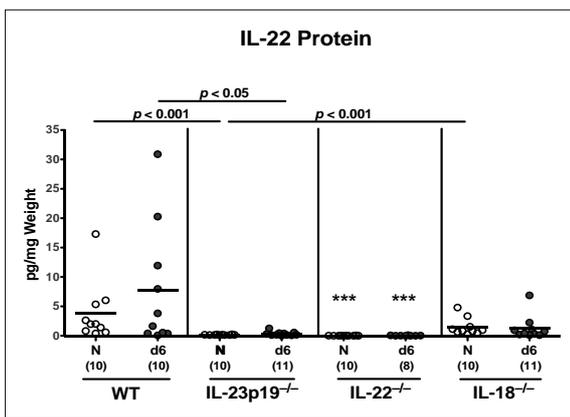


Fig. 6. IL-22 secretion in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. IL-22 protein levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by ELISA. 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 two independent experiments

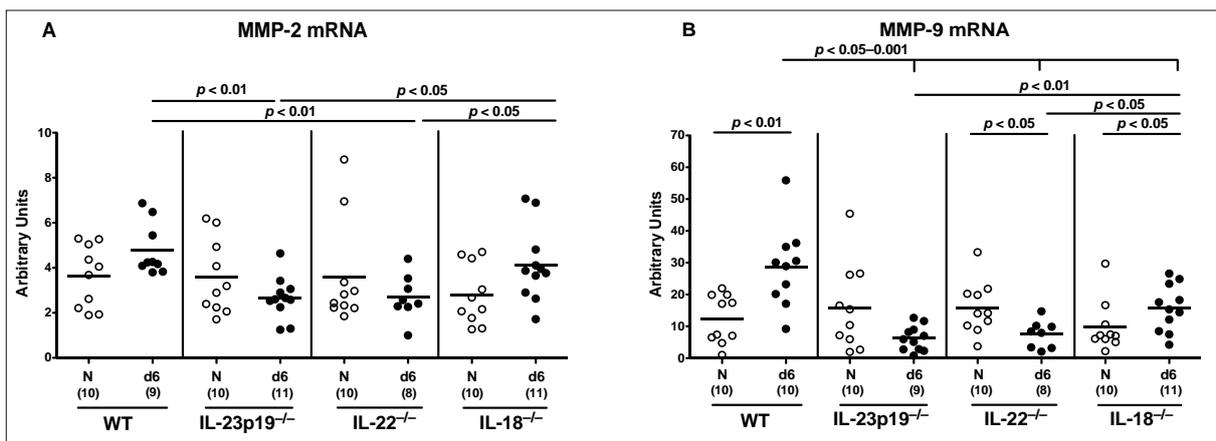


Fig. 7. Expression levels of MMP-2 and MMP-9 mRNA in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) MMP-2 and B) MMP-9 mRNA expression levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR and expressed as arbitrary units (fold expression). 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 two independent experiments

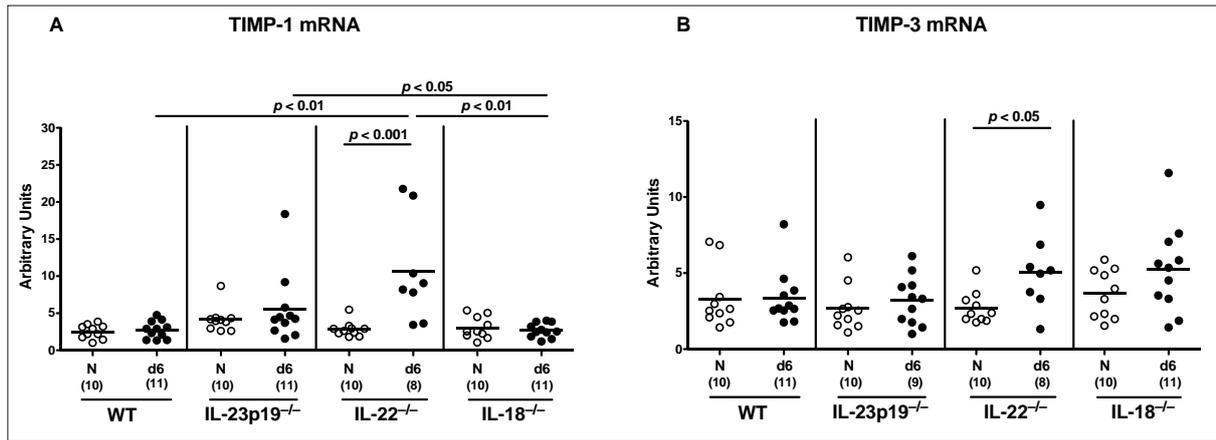


Fig. 8. Expression levels of TIMP-1 and TIMP-3 mRNA in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) TIMP-1 and B) TIMP-3 mRNA expression levels were determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR and expressed as arbitrary units (fold expression). 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 two independent experiments

exhibited lower colonic MMP-9 mRNA expression rates as compared to WT controls ($p < 0.05$ – 0.001 ; Fig. 7B). In IL-22^{-/-} mice, colonic TIMP-1 and TIMP-3, both of which act as endogenous inhibitors of MMPs [17, 18], were upregulated during *C. jejuni* infection ($p < 0.001$ and $p < 0.05$, respectively; Fig. 8). At day 6 p.i., IL-23p19^{-/-} and IL-22^{-/-} mice displayed higher TIMP-1 mRNA expression levels in the large intestines as compared to WT and IL-18^{-/-} mice ($p < 0.05$ – 0.01 ; Fig. 8A).

Given that biologically active MMPs (including MMP-2 and MMP-9) and their endogenous inhibitors such as TIMP-1 and TIMP-3 are expressed in a fine-tuned, balanced equilibrium under physiological conditions [17, 18], we assessed whether the respective

MMP/TIMP ratios had shifted during *C. jejuni* infection of infant mice. Ratios of mRNA expression levels of MMP-2 and MMP-9 either with TIMP-1 or TIMP-3 decreased in infected IL-22^{-/-} mice (Figs. 9 and 10). At day 6 p.i., colonic MMP-2/TIMP-1, MMP-2/TIMP-3, and MMP-9/TIMP-3 mRNA ratios were lower in either gene-deficient as compared to WT mice ($p < 0.01$ – 0.001 ; Figs. 9 and Fig. 10), whereas infected IL-23p19^{-/-} and IL-22^{-/-} mice displayed lower MMP-9/TIMP-1 mRNA ratios in their large intestines as compared to infected WT and IL-18^{-/-} mice ($p < 0.001$ – 0.001 ; Fig. 10A). Moreover, MMP-9/TIMP-1 and MMP-9/TIMP-3 mRNA ratios increased in WT mice, but decreased in IL-22^{-/-} and, for the latter, also in IL-23p19^{-/-} mice upon *C. jejuni* infec-

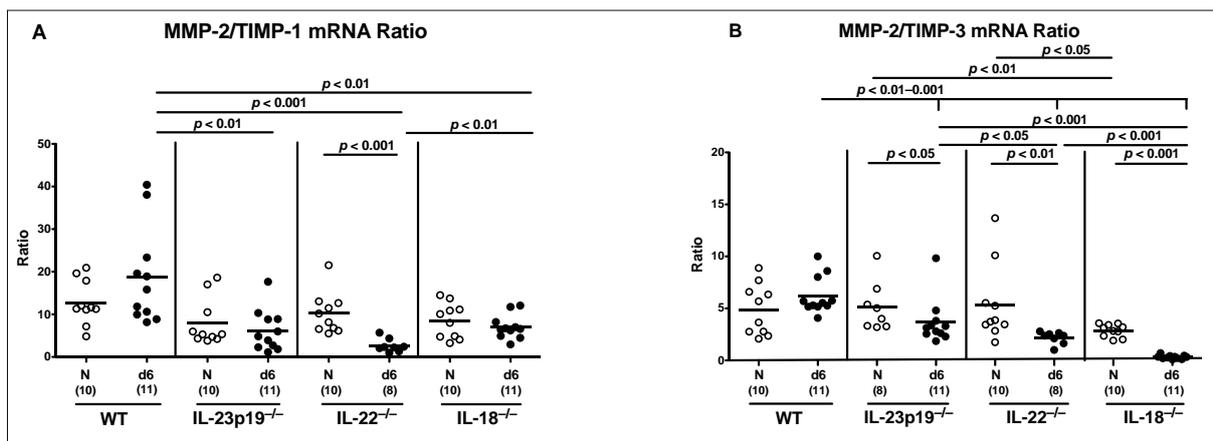


Fig. 9. Colonic MMP-2/TIMP-1 and MMP-2/TIMP-3 mRNA expression ratios in *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Ratios of A) MMP-2 and TIMP-1, and B) MMP-2 and TIMP-3 mRNA expression levels as determined in colonic *ex vivo* biopsies at day (d) 6 post-infection (black circles) by real-time PCR are indicated. 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 given. Data were pooled from two independent experiments

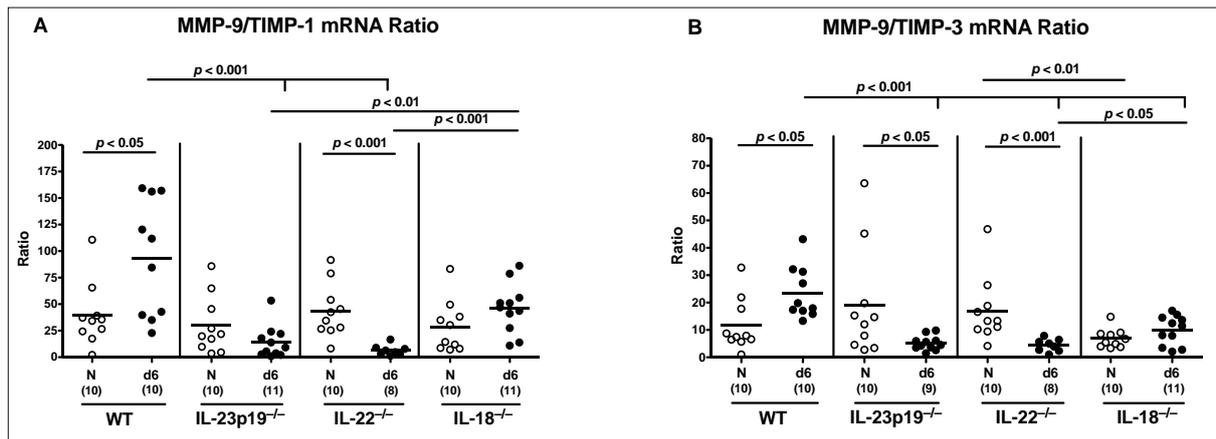


Fig. 10. Colonic MMP-9/TIMP-1 and MMP-9/TIMP-3 mRNA expression ratios in *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19^{-/-}, IL-22^{-/-}, and IL-18^{-/-} infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Ratios of A) MMP-9 and TIMP-1, and B) MMP-9 and TIMP-3 mRNA expression levels as determined in colonic *ex vivo* biopsies at day (d) 6 postinfection (black circles) by real-time PCR are indicated. 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 given. Data were pooled from two independent experiments

tion ($p < 0.05$ – 0.001 ; Fig. 10). Hence, lower MMP/TIMP ratios point towards lower colonic MMP and/or higher TIMP expression upon *C. jejuni* infection of infected gene-deficient (particularly IL-22^{-/-}) as compared to WT mice.

Discussion

In the present study, we performed a comprehensive survey of large intestinal gene expression levels of molecules that are involved in mediating host resistance against pathogens such as *C. jejuni* and infection-induced inflammatory sequelae including tissue destruction. We were able to show that, despite sporadic *C. jejuni* colonization, distinct pathogen-induced changes in expression of the colonic mucus constituent mucin-2 and of pro-inflammatory and regulatory cytokines as well as of matrix degrading gelatinases could be observed in large intestines of *C. jejuni* strain 81-176 infected conventional infant mice lacking IL-23p19, IL-22, or IL-18 and of corresponding WT control animals. Following peroral infection, pathogens are separated from the mucosal epithelial tissue by the mucus layer, thereby protecting the epithelial lining from damage and preserving epithelial barrier function [19]. Mucins including mucin-2 are secreted complex glycoproteins, give mucus its viscous consistency, and act as first line defense against pathogens [19]. Mucins have further been shown to act as major chemoattractants for bacterial pathogens such as *C. jejuni* [20]. Upon binding, *C. jejuni*–mucin-2 interaction results in reduced pathogenic growth, and besides other transcriptomic changes, in enhanced transcription of mucin-degrading enzymes in *C. jejuni* [21]. In our study, colonic mucin-2 mRNA expression was in fact downregulated, but in IL-22^{-/-} infant mice only. We

could recently show that *C. jejuni* infection of conventionally colonized adult IL-10^{-/-} mice was accompanied by a decrease in intestinal mucin-2 mRNA expression [22], which was also true for *Arcobacter butzleri*-infected gnotobiotic (i.e., secondary abiotic) IL-10^{-/-} animals [23, 24]. Downregulated mucin-2 expression in the large intestines of IL-22^{-/-} mice at day 6 p.i. was accompanied by increased pro-inflammatory cytokines including IFN- γ , TNF, IL-17A, and IL-1 β , which might have additionally contributed to a compromised epithelial barrier function upon infection. Notably, neither macroscopic nor microscopic sequelae of *C. jejuni* infection, however, were more pronounced in IL-22^{-/-} as compared to mice of the remaining genotypes at day 6 p.i. [11]. In fact, later during the course of infection (i.e., day 13 p.i.), IL-22^{-/-} mice displayed even lower colonic apoptotic cell numbers, whereas proliferating cells (as a potential measure to counteract infection-induced cell damage) were increased in the large intestines as compared to WT mice [11]. Moreover, colonic mRNA expression levels of the matrix-degrading gelatinases MMP-2 and MMP-9 and their endogenous inhibitor TIMP-1 were lower in IL-22^{-/-} as compared to WT mice. Interestingly, the respective ratios of the gelatinases (MMP-2 or MMP-9; numerator) with TIMP-1 and TIMP-3 (denominator) were lower in gene-deficient as compared to WT mice at day 6 p.i. indicative for less distinct tissue turnover post infection.

Both innate and adaptive immune responses following *C. jejuni* infection are characterized by increased expression of genes coding for T helper cell (Th) -17 cytokines including IFN- γ , IL-22, and IL-17A as shown in a human *ex vivo* gut infection model [25]. This triad of cytokines exerts effective antimicrobial defense mechanisms against *C. jejuni* such as enhanced β -defensin production, for instance [26]. As early as 4 days following *C. jejuni* infec-

tion, increased IL-22, IL-17A, and IL-1 β concentrations could be determined in large intestines of IL-10^{-/-} mice [27]. Whereas IL-17A induction was associated with early neutrophil recruitment to infection sites [28], increased intestinal IL-17A and IFN- γ levels resulted in tissue damage upon *C. jejuni* infection [27]. The increased expression levels of colonic IFN- γ , IL-17A, and IL-1 β in infected IL-22^{-/-} mice, however, were neither associated with more severe histopathological sequelae nor with a more distinct colonic abundance of neutrophils. In fact, converse to IL-18^{-/-} and WT animals, numbers of neutrophilic granulocytes did not differ in large intestines of naive and infected IL-22^{-/-} and also IL-23p19^{-/-} mice [11]. Furthermore, in both naive and infected IL-22^{-/-} mice, colonic expression of IL-23p19 and IL-18 was downregulated, whereas – vice versa – IL-22 mRNA levels were lower in large intestines of IL-18^{-/-}, but not IL-23p19^{-/-}, mice. In colons of IL-23p19^{-/-} mice, however, IL-18 mRNA levels were lower as compared to WT controls. IL-23 has been highlighted as a master regulator of mucosal immune responses upon intestinal infection and inflammation [29]. We have recently shown that, following *Arcobacter butzleri* infection, IL-23 was upregulated depending on the respective strain, the intestinal compartment, and the time course of infection [24]. Furthermore, in acute *Toxoplasma gondii*-induced ileitis, immunopathology was characterized by an IL-23-dependent upregulation of both IL-22 and MMP-2, leading to small intestinal necrosis [12]. Hence, IL-22 exerts its dichotomous mode of action in the gut in a tissue-dependent manner, given that, in the small intestines, IL-22 acts as a pro-, but in the colon, as an anti-inflammatory mediator [12, 30–32]. Moreover, a mutual regulation between IL-22 and IL-18 could be demonstrated in bacterial and parasitic induced inflammation recently, given that IL-22 induced IL-18 expression in epithelial cells following *Citrobacter rodentium* and *T. gondii* infection, whereas, conversely, IL-18 amplified IL-22 secretion during Th1 type immunopathology [30]. It is therefore tempting to speculate that such an orchestrated regulation might also be true for *C. jejuni* infection.

In conclusion, our data indicate that cytokines belonging to the IL-23/IL-22/IL-18 axis, particularly IL-22, and the gelatinases MMP-2 and MMP-9 as well as their endogenous inhibitors are involved in mediating *C. jejuni*-induced inflammatory responses in infant mice in a differentially regulated/orchestrated fashion. Future studies need to dissect the exact regulatory interactions to improve our understanding of the molecular mechanisms underlying campylobacteriosis.

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Competing interests

The authors declare that no competing interests exist.

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