SURVEY OF EXTRA-INTESTINAL IMMUNE RESPONSES IN ASYMPTOMATIC LONG-TERM *CAMPYLOBACTER JEJUNI*- INFECTED MICE

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Received: July 9, 2013; Accepted: July 16, 2013

Campylobacter jejuni is among the most frequently reported bacterial pathogens causing diarrhea in humans worldwide. We recently reported a murine infection model mimicking key features of human campylobacteriosis. Six days following oral *C. jejuni* infection immediately after weaning, infant mice developed acute enterocolitis resolving within 2 weeks. Thereafter, *C. jejuni* could still be isolated from the intestines of asymptomatic mice at low levels accompanied by distinct immune responses, both at intestinal and extra-intestinal locations. We here show that, at day 103 post infection (p.i.), long-term *C. jejuni*-infected mice exhibited higher numbers of T lymphocytes in liver, lung, kidneys, and cardiac muscle as compared to uninfected controls. In addition, B lymphocytes were slightly higher, but macrophage numbers were significantly lower in liver and lung of *C. jejuni*-infected versus naive mice. As compared to uninfected control animals, proliferating cells were significantly lower in liver, lung, kidneys, cardiac muscle, and spleen at day 103 p.i., whereas more apoptotic cells were abundant in the spleen with predominance in the red pulp. This study underlines that post-infectious, immunological sequelae at extra-intestinal locations are of importance even in asymptomatic long-term *C. jejuni* carriers and need to be further studied in order to unravel the underlying molecular mechanisms.

Keywords: *Campylobacter jejuni*, infant mice, long-term *C. jejuni* infection, intestinal microbiota, colonization resistance, host–pathogen interaction, innate and adaptive immunity, extra-intestinal immune cell influx, proliferative cells, apoptotic cells, macrophages, T lymphocytes, B lymphocytes

Introduction

We have previously shown that following peroral infection with *Campylobacter jejuni* strain B2 immediately after weaning, infant mice display an acute ulcerative enterocolitis resolving within 2 weeks post infection [1]. The clinical course of disease and *C. jejuni* induced pro-inflammatory immune responses in the murine intestinal tract were mimicking key features of human campylobacteriosis. Interestingly, infected mice were carrying live *C. jejuni* B2 at relatively low levels in their intestines for more than 100 days post infection (p.i.). Furthermore, even though no overt intestinal *C. jejuni*-related symptoms such as watery or even bloody diarrhea could be observed, long-term infected mice exhibited distinct intestinal pro-inflammatory immune cell responses. As compared to uninfected controls, numbers of apoptotic cells, T and B lymphocytes,

regulatory T cells, as well as neutrophils were higher in the colonic mucosa of *C. jejuni*-infected mice at day 103 p.i. Remarkably, asymptomatic long-term *C. jejuni* carriers displayed also a marked influx of immune cells into extra-intestinal compartments. We here present a comprehensive survey of the distinct immune cell populations as well as apoptotic and proliferative cells in affected extra-intestinal organs such as liver, lung, kidneys, cardiac muscle, and spleen.

Materials and methods

Ethics statement

All animal experiments were conducted according to the European Guidelines for animal welfare (2010/63/EU)

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Fig. 1A. T lymphocytes in livers of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from livers at day (d) 103 post infection (p.i.) (black circles) were stained against CD3 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of T lymphocytes from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of CD3+-stained liver sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 1B. B lymphocytes in livers of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from livers at day (d) 103 post infection (p.i.) (black circles) were stained against B220 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of B lymphocytes from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of B220+-stained liver sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 1C. Macrophages in livers of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from livers at day (d) 103 post infection (p.i.) (black circles) were stained against F4/80 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of macrophages from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of F4/80+-stained liver sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 1D. Proliferating cells in livers of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from livers at day (d) 103 post infection (p.i.) (black circles) were stained against Ki67 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of proliferating cells from at least six high-power fields (HPF, 400× magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (*p* values) as determined by the Student's *t*-test are indicated. For three independent experiments, representative photomicrographs of Ki67+-stained liver sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100× magnification, scale bar 100 µm; lower panel: 400× magnification, scale bar 20 µm)

with approval of the commission for animal experiments headed by the “Landesamt für Gesundheit und Soziales” (LaGeSo, Berlin, Germany). Animal welfare was monitored twice daily by assessment of clinical conditions.

Mice

All animals were bred and maintained in the facilities of the “Forschungsinstitut für Experimentelle Medizin” (FEM, Charité – Universitätsmedizin, Berlin, Germany), under specific pathogen-free (SPF) conditions. In the experiments, female C57BL/6j wildtype mice age matched 3 weeks (right after weaning) and 3–4 months of age (conventional adult controls) were used.

C. jejuni infection of mice

Mice were infected with viable approximately 10^9 colony forming units of *C. jejuni* strain B2 (kindly provided by Prof. Dr. Uwe Groß, University of Göttingen, Germany) in a total volume of 0.3 ml phosphate buffered saline (PBS) on three consecutive days by gavage as described previously [1–3].

Sampling procedures and immunohistochemistry

Mice were sacrificed by isofluran treatment (Abbott, Germany). Tissue samples from liver, lung, kidneys, cardiac muscle, and spleen were removed under sterile conditions. *In situ* immunohistochemical analyses of paraffin sections (5 µm) derived from liver, lung, kidneys, cardiac muscle, and spleen were performed as described previously [1, 2, 4, 5]. Primary antibodies against CD3 (#N1580, Dako, Denmark, dilution 1:10), B220 (eBioscience, San Diego, CA, USA, 1:200), F4/80 (#14-4801, clone BM8, eBioscience,

1:50), Ki67 (TEC3, Dako, 1:100), and cleaved caspase-3 (Asp175, Cell Signaling, USA, 1:200) were used. The average number of positively stained cells within at least six representative high-power fields (HPF, 0.287 mm²; 400× magnification) was determined microscopically by two independent double-blinded investigators and subjected to statistical analysis as indicated.

Statistical analysis

Mean values and levels of significance were determined by Mann–Whitney *U* test. Two-sided probability (*p*) values ≤ 0.05 were considered significant. Experiments were repeated twice.

Results

In the study presented here, we detected immune responses in extra-intestinal compartments of asymptomatic long-term *C. jejuni*-infected mice. Infant mice harboring a conventional intestinal microbiota were infected with *C. jejuni* strain B2 immediately after weaning. At day 103 p.i., mice were sacrificed and paraffin sections derived from liver, lung, kidneys, cardiac muscle, and spleen were stained with antibodies against CD3 (T lymphocytes), B220 (B lymphocytes), F4/80 (macrophages), Casp3 (apoptotic cells), and Ki67 (proliferating cells). Quantification of these cells revealed that, at day 103 p.i., *C. jejuni* B2 carrying mice exhibited almost two-fold higher T lymphocyte numbers in the liver parenchyma and periportal fields as compared to uninfected infant and adult control mice ($p < 0.005$; Fig. 1A), which held also true for B lymphocyte numbers versus adult animals ($p < 0.05$; Fig. 1B). Interestingly, naive 3-week-young infant mice exhibited highest numbers of macrophages and proliferating cells in the liver. With increasing age, both numbers declined ($p <$

0.05 and $p < 0.0005$, respectively; Fig. 1C and D), which was even more pronounced upon *C. jejuni* B2 infection ($p < 0.0005$ and $p < 0.0001$, respectively; Fig. 1C and D). Of note, hepatocytes were Ki67-positive in naive infant mice, whereas at day 103 p.i., mostly lymphocytes in inflammatory foci were positively stained (Fig. 1D, photomicrograph left and right panel, respectively). *In situ* immunohistochemical analyses of lungs derived from long-term infected mice revealed similar results (Fig. 2). Whereas at day 103 p.i., higher T and B lymphocyte counts could be observed in the lung parenchyma as compared to naive adult control mice ($p < 0.005$ and $p < 0.05$, respectively; Fig. 2A and B), numbers of macrophages and proliferating cells were lower (Fig. 2C and D). Notably, in lungs of uninfected infant mice, higher total Ki67-positive counts were due to higher numbers of positively stained cells in the lung parenchyma as well as in alveolar epithelial cells (Fig. 2D, photomicrograph left panel, respectively).

In lungs of *C. jejuni* carriers, however, overall numbers of proliferating cell numbers, particularly within the lung parenchyma, were lower versus naive controls (Fig. 2D), whereas in leukocytic infiltrates, focal accumulations of Ki67-positive cells could be observed (Fig. 2D, right panel of photomicrograph). Long-term *C. jejuni* infection was also accompanied by a distinct influx of T lymphocytes into kidneys and cardiac muscle ($p < 0.005$ and $p < 0.01$, respectively; Fig. 3A and 4A), whereas numbers of proliferating cells were lower compared to respective controls ($p < 0.01$ and $p < 0.0001$; Fig. 3B and 4B). Furthermore, naive infant mice exhibited higher Ki67-positive cells in their cardiac muscle as compared to naive adult and *C. jejuni*-infected mice ($p < 0.05$ and $p < 0.0001$; Fig. 4B). Numbers of B lymphocytes and macrophages did not differ in kidneys and cardiac muscle of the respective groups (data not shown). Remarkably, mean total numbers of apoptotic cells in spleen of *C. jejuni* carriers were up to

Fig. 2A. T lymphocytes in lungs of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from lungs at day (d) 103 post infection (p.i.) (black circles) were stained against CD3 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of T lymphocytes from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of CD3+-stained lung sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 2B. B lymphocytes in lungs of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from lungs at day (d) 103 post infection (p.i.) were stained against B220 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of B lymphocytes from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of B220+-stained lung sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 2C. Macrophages in lungs of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from lungs at day (d) 103 post infection (p.i.) (black circles) were stained against F4/80 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of macrophages from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of F4/80+-stained lung sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 2D. Proliferating cells in lungs of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from lungs at day (d) 103 post infection (p.i.) (black circles) were stained against Ki67 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of proliferating cells from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of Ki67+-stained lung sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

three times higher as compared to uninfected infant and adult controls ($p < 0.0001$ and $p < 0.0005$, respectively; Fig. 5A). At day 103 p.i., high splenic Casp3-positive cell counts were detected rather in the red pulp where macrophages are located than in distinct T and B cell zones of the white pulp (Fig. 5A, right panel of photomicrographs). Whereas naive infant mice harbored by far the highest numbers of proliferating splenic cells ($p < 0.0005$ and $p < 0.0001$, respectively; Fig. 5B), Ki67-positive cells counts declined with age and were lowest upon *C. jejuni* infection at day 103 p.i. (Fig. 5B; right panel of photomicrographs).

Discussion

We previously showed that the infant mouse model is excellently suited to unravel the clinical course and underlying pro-inflammatory intestinal immune responses of human

campylobacteriosis [1]. Interestingly, conventional 3-week-young mice orally infected with *C. jejuni* immediately after weaning not only developed acute ulcerative enterocolitis resolving within 2 weeks upon infection, but also harbored the pathogen in their intestines at low levels for several months thereafter. Even though clinically asymptomatic, mice carrying *C. jejuni* displayed distinct immunopathological features in the intestinal tract, but, remarkably, also at extra-intestinal locations [1]. This prompted us to perform a comprehensive survey of the immune cell composition in spleen, liver, lung, kidneys, and cardiac muscle of long-term *C. jejuni*-infected mice by applying *in situ* immunohistochemical analyses. Results revealed that more than 100 days following oral infection, *C. jejuni* carriers exhibited higher numbers of CD3-positive T lymphocytes in their liver, lung, kidneys, and cardiac muscle as compared to uninfected control animals. In addition, B220-positive B lymphocytes were slightly higher in liver and lung of *C. je-*

Fig. 3A. T lymphocytes in kidneys of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from kidneys at day (d) 103 post infection (p.i.) (black circles) were stained against CD3 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of T lymphocytes from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of CD3+-stained kidney sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 3B. Proliferating cells in kidneys of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from kidneys at day (d) 103 post infection (p.i.) (black circles) were stained against Ki67 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of proliferative cells from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments representative photomicrographs of Ki67+-stained kidney sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 4A. T lymphocytes in cardiac muscle of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from cardiac muscle at day (d) 103 post infection (p.i.) (black circles) were stained against CD3 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of T lymphocytes from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of CD3+-stained cardiac muscle sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 4B. Proliferating cells in cardiac muscle of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from cardiac muscle at day (d) 103 post infection (p.i.) (black circles) were stained against Ki67 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of proliferating cells from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of Ki67+-stained cardiac muscle sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 5A. Apoptotic cells in spleens of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from spleens at day (d) 103 post infection (p.i.) (black circles) were stained against Casp3 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of apoptotic cells from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of Casp3+-stained spleen sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

Fig. 5B. Proliferating cells in spleens of chronically *C. jejuni*-infected infant mice. Infant mice 3 weeks of age were infected with *C. jejuni* B2 right after weaning. Paraffin sections of *ex vivo* biopsies taken from spleens at day (d) 103 post infection (p.i.) (black circles) were stained against Ki67 as described in *Materials and methods*. Uninfected infant (open circles) and adult mice (crossed circles) served as negative controls. The average numbers of proliferating cells from at least six high-power fields (HPF, 400 $\times$ magnification) per animal were determined microscopically in immunohistochemically stained sections (upper graph). Numbers of animals are given in parentheses. Means (black bars) and levels of significance (p values) as determined by the Student's t -test are indicated. For three independent experiments, representative photomicrographs of Ki67+-stained spleen sections at day 103 p.i. (right) versus naive infant (left) and adult (middle) controls are shown (upper panel: 100 $\times$ magnification, scale bar 100 μ m; lower panel: 400 $\times$ magnification, scale bar 20 μ m)

jejuni-infected versus naive mice, whereas numbers of F4/80-positive macrophages were significantly lower. Interestingly, compared to naive controls, Ki67-positive proliferating cells were significantly lower in liver, lung, kidneys, cardiac muscle, and spleen at day 103 p.i., whereas in the spleen, additionally more Casp3-positive apoptotic cell numbers could be detected. Interestingly, apoptotic cells were most abundant in the red pulp of the spleen, the area where macrophages are located but not in the white pulp comprising T and B lymphocyte zones.

The observed decline in macrophage numbers in the extra-intestinal locations of *C. jejuni*-infected mice is supported by our previous finding that, during the early course of *C. jejuni* infection, myeloid cells were less abundant in spleens of conventional IL-10 deficient mice with chronic colitis as early as day 3 p.i. when comparing with uninfected control animals [3]. Furthermore, the *Campylobacter* invasion antigen (Cia) protein has been shown to play an important role in invasion of mammalian cells and macrophage apoptosis [6, 7]. As a consequence, enteric disease is incited and pathogen spread from the intestinal tract to deeper tissue sites facilitated [6, 7]. At the first glance, this might be reasonable for the early course of an acute *C. jejuni* infection. It is tempting to speculate that *C. jejuni*-laden macrophages migrating from the intestinal tract via the blood stream or lymphatic vessels further broaden the path for the observed extra-intestinal sequelae in chronic *C. jejuni* carriers. Apoptotic macrophages would provide a niche for *C. jejuni* and furthermore expose the pathogen to adaptive immune cells, which in turn eliminate the pathogen. This could explain the observed sterile inflammatory responses in the respective organs. In our previous studies, live *C. jejuni* could be cultured from mesenteric lymph nodes draining the intestines but virtually never from extra-intestinal organs such as spleen, liver, kidneys, and cardiac blood following infection of conventional, germfree, or with human microbiota re-associated mice [1–3, 8].

Even though human campylobacteriosis is self-limiting in the vast majority of cases, sequelae such as Guillain-Barré and Reiter's syndrome, affecting peripheral nerves and joints, respectively, may occur [9–11]. These post-infectious complications are due to antibodies generated against sialylated *C. jejuni* lipooligosaccharide which cross-reacts with structurally similar glycopeptides on peripheral nerves or joints [11–13]. Only very sporadic cases of extra-intestinal *C. jejuni*-associated disease manifestations affecting the liver (e.g., hepatitis), lung (e.g., pneumonia, empyema), heart (e.g., endocarditis), or spleen (e.g., splenic rupture) have been reported to date, particularly in immunocompromized patients with *C. jejuni* bacteremia [14–16].

In conclusion, post-infectious, immunological sequelae at extra-intestinal locations such as liver, lung, kidneys, cardiac muscle, and spleen should be considered in asymptomatic long-term *C. jejuni*-infected/carrying individuals and need to be further studied in order to unravel the underlying molecular mechanisms.

Acknowledgements

This work was supported by grants from the German Research Foundation (DFG) to U.B.G. (GO363/12-1, CampyGerm; SFB633, TP A7), S.B. and A.F. (SFB633, TP A7), A.A.K. (SFB633, TP Z1), M.M.H. (SFB633, TP B6), and L.M.H. and B.O. (SFB633, Immuco) and from the German Federal Ministry of Education and Research (BMBF) to S.B. ("Lab in a hanky" projects TP 1.1 and TP 8.2). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

We thank Michaela Wattocht, Ursula Rüschenhoff, Gernot Reifemberger, Alexandra Bittroff-Leben, and the staff of the animal research facility for excellent technical assistance and animal breeding. We are grateful to Simone Spieckermann for immunohistochemical staining of tissue sections.

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