TREATMENT WITH INTERLEUKIN-18 BINDING PROTEIN AMELIORATES *TOXOPLASMA GONDII*-INDUCED SMALL INTESTINAL PATHOLOGY THAT IS INDUCED BY BONE MARROW CELL-DERIVED INTERLEUKIN-18

D. Struck¹, I. Frank¹, S. Enders², U. Steinhoff^{3**}, C. Schmidt⁴, A. Stallmach⁴, O. Liesenfeld^{1***} and M. M. Heimesaat^{1*}

¹ Institut für Mikrobiologie und Hygiene, Charité – Campus Benjamin Franklin, Berlin, Germany

² Institut für Laboratoriumsmedizin und Pathobiochemie, Charité – Campus Benjamin Franklin, Berlin, Germany

³ Abteilung Immunologie, Max-Planck Institut für Infektionsbiologie Berlin, Berlin, Germany

⁴ Abteilung für Gastroenterologie, Hepatologie und Infektiologie, Klinik für Innere Medizin II, Universitätsklinikum Jena der Friedrich Schiller Universität Jena, Jena, Germany

Received: June 19, 2012; Accepted: June 29, 2012

Peroral infection with *Toxoplasma gondii* results in a Th1-type immunopathology characterized by small intestinal necrosis and is dependent on IL-18. In the present study, we investigated whether treatment with IL-18 binding protein (IL-18bp) prevents ileal pathology. We observed increased expression of IL-18bp in intestinal biopsies of mice following infection. Whereas small intestines of control mice showed severe necrosis with complete destruction of the small intestinal architecture, mice treated with IL-18bp daily displayed only mild inflammatory changes including flattening of villi and edema in the space between the epithelium and lamina propria. Small intestinal parasite loads and concentrations of pro-inflammatory cytokines did not differ in control and IL-18bp-treated mice. Binding of IL-18 to immobilized IL-18bp revealed a remarkably slow dissociation rate, indicating high affinity. Using chimeric mice we observed that bone marrow-derived rather than stromal cells were the primary source of IL-18 that resulted in small intestinal pathology following peroral infection with *T. gondii*. In conclusion, the results presented here suggest that IL-18bp may be an effective and safe treatment for small intestinal inflammation. Antigen-presenting rather than epithelial cells appear to be the main source of IL-18 in *T. gondii*-induced small intestinal inflammation.

Keywords: *Toxoplasma gondii*, ileitis, IL-18, IL-18 binding protein, immunopathology

Introduction

Peroral infection with *Toxoplasma gondii* results in the development of massive necrosis of the villi and mucosal cells in the ilea [1]. We have identified Th1-cytokines including IFN- γ , TNF- α and CD4 T cells as essential mediators of small intestinal pathology following oral infection with *T. gondii* [1–3]. In addition, IL-18 and IL-12 as well as nitric oxide are mediating pathology [4] whereas TGF- β and IL-10 are anti-inflammatory chemokines [5, 6]. More recently, we identified IL-23 mediated IL-22-production as key mediators of intestinal pathology whereas IL-17A did not mediate pathology [7].

The anatomical location of pathological changes (the ileum) and the immunopathogenesis of this pathology resemble those described in patients with Crohn's disease or models of experimental intestinal inflammation [8–10].

IL-18, originally described as IFN- γ -inducing-factor, is structurally related to IL-1 and produced by both macrophages and non-immune cells, including epithelial cells [11–13]. IL-18 has been shown to act synergistically with IL-12 on the synthesis of IFN- γ [14]. IL-18 shares a role with IL-12 in the defense against certain intracellular bacteria, including mycobacteria and *Salmonella* [15, 16], and extracellular pathogens [17] whereas control of other intracellular pathogens including *T. gondii* does not appear

*These authors contributed equally to this work.

**Current addresses:

U. Steinhoff: Institut für Medizinische Mikrobiologie und Krankenhaushygiene der Philipps Universität Marburg, Hans-Meerwein Straße 2, 35032 Marburg, Germany

O. Liesenfeld: Medical and Scientific Affairs, Roche Molecular Diagnostics, Pleasanton, CA, USA

***Corresponding author: Oliver Liesenfeld, M.D.; Institut für Mikrobiologie und Hygiene, Charité – Campus Benjamin Franklin, Hindenburgdamm 27, D-12203 Berlin, Germany; Phone: +49-30-844-53628; Fax: +49-30-844-53620; E-mail: oliver.liesenfeld@charite.de

to depend on IL-18 [4]. The overproduction of IL-18 was reported in patients with inflammatory bowel diseases (IBD) and other autoimmune diseases [18–21], as well as in experimental models of IBD in the large [4, 22–25] and small intestine [4]; the cellular source of IL-18 in intestinal inflammation, however, has been discussed controversially [19, 20, 22].

IL-18 binding protein (IL-18bp) was reported to counter-regulate the biological activity of IL-18. In mucosal tissue, IL-18bp is mainly produced by epithelial cells and macrophages [3], and its induction depends on IFN- γ [26]. Patients with Crohn's disease display increased expression and secretion of IL-18 and IL-18bp mRNA [18, 19, 27]. Treatment with anti-IL-18 mAbs as well as administration of IL18bp-Fc inhibited weight loss and intestinal inflammation in DSS- and TNBS-induced colitis [23, 25].

In the present study, we therefore investigated whether treatment with IL-18bp prevents ileal pathology following oral infection with *T. gondii*. Furthermore, we determined the cellular source of IL-18 using bone-marrow chimeric mice. Results of the present study indicate that administration of IL-18bp prevents the development of small intestinal pathology without impairing antimicrobial responses and depending on bone-marrow derived IL-18. Thus, antigen-presenting rather than stromal cells are the main source of IL-18 in small intestinal inflammation.

Results

Development of intestinal immunopathology following oral infection with T. gondii is associated with increased IL-18bp expression

Elevated levels of IL-18bp have been detected in inflamed intestinal tissues in models of experimental colitis. To determine whether IL-18bp expression is upregulated after oral infection with *T. gondii*, we determined mRNA expression of IL-18bp in ilea on days 0, 3, 5, and 7 following

infection compared to uninfected mice using qPCR. IL-18bp mRNA levels increased after infection and peaked on day 5, the day when intestinal pathology starts to develop (Fig. 1). Similarly, using Affymetrix genome arrays, infected wildtype mice revealed a more than 6-fold up-regulation of IL-18bp mRNA in their small intestines 7 days following infection compared to small intestines of uninfected control mice (data not shown).

IL-18bp prevents T. gondii-induced ileal immunopathology

To determine whether administration of IL-18bp protects mice against IL-18-dependent small intestinal inflammation, mice were infected with *T. gondii* and treated with recombinant IL-18bp. Whereas small intestines of control mice treated with rat IgG showed complete destruction of the small intestinal architecture characterized by necrosis of villi and the mucosa, mice treated with 90 μ g of IL-18bp intraperitoneally displayed only mild changes, including flattening of villi and edema in the space between epithelium and lamina propria but no necrosis (Fig. 2a, b). IL-18^{-/-} mice used as an additional control were also protected against ileal pathology as previously described [4]. In contrast, mice infected and treated with a lower daily dose of 50 μ g IL-18bp developed small intestinal pathology as did control animals (data not shown).

IL-18bp does not impact resistance against T. gondii

To examine whether treatment with IL-18bp impairs host antimicrobial defenses, we determined numbers of inflammatory foci in livers and the parasite burden in the gut of infected mice. Mice treated with IL-18bp did not exhibit increased numbers of parasite-associated inflammatory foci in their livers as compared to control animals treated with rat IgG (Fig. 3). Seven days after infection, the numbers of parasitophorous vacuoles did not differ

Fig. 1. Kinetics of IL-18bp-expression in the ileum following oral infection with 100 cysts of the ME49 strain of *T. gondii*. IL-18bp mRNA concentrations were determined in ileal biopsies at indicated time points by qPCR. Results shown are from one representative experiment with two (days 0 and 3) to three (days 5 and 7) mice per group

Fig. 2. Histological changes in the ilea of control mice and mice treated with 90 μ g of IL-18bp daily following infection with 100 cysts of the ME49 strain of *T. gondii*. Small intestines were obtained 7 days after infection and stained with H&E. (a) Representative H&E-stained section of an infected control mouse (A), a mouse treated with IL-18bp (B), and an IL-18^{-/-} mouse (C). (b) Histological score of ileal immunopathology; a score of less than 3 indicates the lack of necrosis in the entire ileum. Results shown are pooled data from three experiments (four to five mice per group per experiment)

in the small intestines of mice treated with IL-18bp and controls (data not shown). Parasites were not detectable in livers of infected control mice or infected mice treated with IL-18bp.

IL-18bp does not alter cytokine responses following infection with T. gondii

To determine the mechanism by which IL-18bp prevents the development of ileal necrosis, we examined systemic and local levels of Th1-cytokines in sera and organs of mice. In the sera of mice treated with IL-18bp concentrations of IFN- γ , TNF- α , and IL-12 were 13.6 ± 2.7 , 4.9 ± 4.5 , and 47.3 ± 39.5 ng/ml, respectively, and these did not differ from those in control mice treated with rat IgG. Local cytokine concentrations in the ileum and MLN did not differ significantly between mice treated with IL-18bp and control animals (Fig. 4a, b). Interestingly, systemic and local

IL-18 levels were not altered by treatment with IL-18bp. IL-18bp did not appear to block the capacity of the ELISA to detect IL-18 since detection of IL-18 by ELISA was not blocked by pre-incubation of IL-18 with IL-18bp before or during incubation in the ELISA (data not shown).

Binding of IL-18 to IL-18bp

To determine the affinity of IL-18 to IL-18bp surface plasmon resonance measurements were performed. Initially, a Biacore sensor chip with immobilized protein A/G was used to bind IL-18bp by its Fc fusion part. Subsequently, mouse IL-18 was passed over the surface for binding human IL-18bp. As shown in Figure 5, mouse IL-18 bound human IL-18bp. The binding of increasing amounts of IL-18 to immobilized human IL-18bp revealed a remarkable slow dissociation rate (k_{off}) of 9.72×10^{-5} /s, indicating a very high affinity.

Fig. 3. Numbers of inflammatory foci in livers of control mice and mice treated with IL-18bp following oral infection with 100 cysts of the ME49 strain of *T. gondii*. Livers were obtained on day 7 after infection. Numbers of inflammatory foci were determined in sections stained with H&E ($\times 100$ magnification). Results shown are pooled data from three experiments (four to five mice per group per experiment)

Bone marrow derived cells are the major source of IL-18 in *T. gondii*-induced ileitis

Since the cellular source of IL-18 in models of inflammatory bowel disease is still under discussion [19, 20, 22], we determined the cellular source of IL-18 that induces small intestinal immunopathology following oral infection with *T. gondii*. Seven days following infection, irradiated wildtype and IL-18^{-/-} mice reconstituted with cells derived from wildtype mice developed severe signs of necrosis as did infected wildtype control mice (Fig. 6a–c). In contrast, transfer of cells isolated from IL-18^{-/-} mice into wildtype or IL-18^{-/-} mice only resulted in minor inflammation but did not result in the development of ileal immunopathology (Fig. 6d–e). Histological changes in these mice were rather mild and similar to those observed in IL-18^{-/-} control mice (Fig. 6f). The development of small intestinal pathology in chimeric mice with intact bone marrow-derived IL-18 production and in control mice was associated with significantly elevated IL-18 concentrations in MLN and spleens 7 days after infection as compared to chimeric

mice with a deficiency in bone marrow derived IL-18 and IL-18^{-/-} mice (Fig. 7). IL-18 levels in the ilea of mice did not differ significantly between chimeric mice regardless of the cellular source of reconstitution.

Discussion

IL-18 has been identified as a key molecule in the immunopathogenesis of murine experimental inflammation in mice and in human IBD [19, 21, 23–25, 30]. Neutralization of IL-18 or administration of IL-18bp may therefore be an effective treatment strategy. In the present study, we investigated whether IL-18bp effectively inhibits small intestinal inflammation that develops in an IL-18-dependent manner following oral infection of mice with *T. gondii* [4]. Daily administration of 90 µg IL-18bp prevented the development of small intestinal necrosis. At the same time, the neutralization of IL-18 by IL-18bp did not impair immune responses against the parasite given that both concentrations of local and systemic pro-inflammatory cytokines

Fig. 4. Concentrations of IFN-γ, IL-12, and IL-18 in MLN (a) and ileum (b) of mice infected with 100 cysts of the ME49 strain of *T. gondii*. Mice were treated with IL-18bp, or left untreated; organs were obtained on day 7 after infection. Cytokine concentrations were determined by ELISA. Data are representative of three independent experiments (five mice per group each)

Fig. 5. Kinetics of binding affinity of mouse IL-18 to human IL-18bp. Different concentrations of recombinant IL-18 were passed over immobilized IL-18bp on a Biacore sensor chip as recommended by the manufacturer (see Materials and methods for details)

and parasite numbers were unchanged as compared to control mice. The lack of impairment of the antiparasitic immune response following administration of IL-18bp is in contrast to the neutralization of other key mediators of small intestinal inflammation including TNF- α since we have previously shown that neutralization of TNF- α effectively suppressed small intestinal necrosis but resulted in increased parasite numbers resulting in death of mice [2]. Administration of IL-18bp (600 μ g IL-18bp.Fc/day) has previously been reported to attenuate inflammation during DSS-induced colitis [25]. Thus, IL-18bp represents a candidate for the safe and efficacious treatment of small and large intestinal inflammation and should be further investigated in patients with IBD. In this regard, the influence of IL-18bp on infection with other pathogens, i.e. those with

extracellular pathogens (*Yersinia enterocolitica*, *Streptococcus pneumoniae*, or *Pseudomonas aeruginosa*) should be investigated to further underline the safe use of IL-18bp.

The efficacy of IL-18bp in preventing the development of small intestinal necrosis is likely dependent on the strong binding capacity for IL-18. The affinity of IL-18 to immobilized IL-18bp was characterized by a low dissociation constant of 2.16×10^{-11} M with a markedly slow dissociation rate of 9.72×10^{-5} /s. A similar slow dissociation rate has been described for the affinity of IL-18bp to IL-18 by Kim et al. [31].

The administration of IL-18bp did not affect the concentrations of pro-inflammatory cytokines. This comes as a surprise since the amelioration of pathologic effects was marked and significant. However, cytokine concentra-

Fig. 6. Histological changes in ilea of bone-marrow chimeric mice (see Materials and methods for details). Mice were sacrificed 7 days after infection, and ileal sections were stained by H&E ($\times 100$ magnification). (a) Wildtype mice reconstituted with wildtype cells, (b) IL-18 $^{-/-}$ mice reconstituted with wildtype cells, (c) control wildtype mice, (d) wildtype mice reconstituted with IL-18 $^{-/-}$ cells, (e) IL-18 $^{-/-}$ mice reconstituted with IL-18 $^{-/-}$ cells, and (f) IL-18 $^{-/-}$ control mice. Pictures are representative for three independent experiments (three to six mice/group)

Fig. 7. IL-18 levels in spleen, MLN, and ileum of wildtype and IL-18^{-/-} bone marrow chimeric mice 7 days following infection with 100 cysts of *T. gondii* (see Materials and methods for details). Representative data of three independent experiments (three to six mice/group) are shown (*, $p < 0.05$ vs. mice reconstituted with IL-18^{-/-} cells; **, $p < 0.05$ vs. IL-18^{-/-} control mice).

tions were measured on a tissue biopsy but not on a cellular level. It is tempting to speculate that the secretion of pro-inflammatory cytokines by individual cellular components in the small intestines was affected by the administration of IL-18bp.

Most studies show a role for IL-18 in intestinal inflammation. However, the cellular source of IL-18 has been discussed controversially; either macrophages [20] or epithelial cells [22, 32] were reported to be the source of IL-18 in models of experimental colitis. Using confocal laser scanning microscopy, IL-18 expression was localized in epithelial cells during DSS colitis [22]. Similarly, during T cell dependent colitis in SCID mice epithelial cells were identified as the major source of IL-18 by immunohistology [32]. In contrast, macrophage depletion using anti-Mac-1-saporin antibodies pointed towards macrophages as the major source of IL-18 [20]. We here present evidence that bone-marrow derived cells are the major source of IL-18 during small intestinal inflammation following oral infection with *T. gondii*. Using bone-marrow chimeric mice, we observed the development of small intestinal necrosis exclusively in mice harboring bone marrow-derived IL-18-competent cells whereas mice with IL-18-competent stromal cells but bone-marrow-derived IL-18-deficient cells developed minor inflammation but not small intestinal necrosis. These findings were confirmed by the presence of IL-18 exclusively in the MLN and serum of those mice that had bone-marrow-derived IL-18-competent cells. Thus, bone-marrow-derived cells, most likely macrophages and/or dendritic cells but not epithelial cells, are sufficient to drive small intestinal inflammation by secretion of IL-18.

In conclusion, results of the present study identify bone marrow-derived cells as the main source of IL-18 that induces small intestinal pathology in mice following oral infection with *T. gondii*. IL-18bp effectively prevented the development of intestinal necrosis and did not impair

antimicrobial immune responses. Therefore, the IL-18/IL-18bp axis should be further investigated as a potential target for the treatment of human IBD.

Materials and methods

Mice

Female C57BL/6 and IL-18^{-/-} mice on the C57BL/6 background (a kind gift by A. Takeda) were bred and maintained in the animal facility of the Charité Campus Benjamin Franklin under specific pathogen-free conditions. All mice were 8–12 weeks old when used. There were at least three mice in each experimental group for histological and immunological studies. All experiments were repeated at least twice as indicated and conducted according to the German animal protection laws.

Infection

Cysts of the *T. gondii* ME49 strain were obtained from brains of NMRI mice that had been infected intraperitoneally with ten cysts for 2–3 months. For peroral infection, mice were infected with 100 cysts by gavage in a volume of 0.2 ml in PBS.

Treatment with IL-18bp

Beginning 1 day after infection, mice were treated daily with either 50, 90, or 200 μ g of IL-18bp or 100 μ g rat IgG administered by intraperitoneal injection. 18bpa-IgG2b fusion proteins were generated as follows. The cDNA coding for IL-18bpa was ligated to cDNA coding for the Fc part of human IgG2b. cDNA was amplified by PCR

using pORF-hIL18bpa (kindly provided by Dr. H. Mühl, Pharmazentrum Frankfurt, Johann Wolfgang Goethe Universität) as template and the primer oligonucleotides introducing a *SpeI* and *BamHI* site. The IL-18bpa PCR products were digested with *BamHI* and *SpeI*, and inserted into the *XbaI/BamHI*-digested CD16-IgG2b-CDM8 vector DNA [28] containing the IgG2b cDNA fused to IL-2 cDNA, thereby replacing the IL-2 cDNA by the IL-18bpa cDNA. For protein expression, 293 cells (2×10^6 cells) were transfected with 2 µg of plasmid DNA, and the culture supernatant was harvested at day 7. Fusion protein from the supernatant was bound to protein A-Sepharose CL4B (0.5 g/L) (Pharmacia Biotech, Piscataway, NJ) for 16 h at 4 °C, pH 7.4, washed with 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, and eluted with 1 M acetic acid. Purified proteins were free of detectable amounts of endotoxins (Kinetic-Chromogenic Lysate, BioWhittaker Inc., Walkersville, MD).

Histopathology

Mice were sacrificed by cervical dislocation following anesthesia with isofluran (Abbott, Wiesbaden, Germany) at day 9 after oral infection with *T. gondii*. Livers, spleens, mesenteric lymph nodes (MLN), and small intestines (about 10 cm rolled on itself to make a "Swiss role") were removed and immediately fixed in a solution containing 5% formalin. Two to four 5-µm-thick sections (50 or 100 µm distance between sections) were stained with H&E and examined by light microscopy. A standardized histological inflammation score ranging from 0 to 6 (0, normal; 1, edematous blubbing; 2, cell-free exudate into the lumen, but intact epithelium; 3, cellular shedding into the lumen; 4, beginning epithelial disintegration; 5, mucosal destruction <50% of small intestine length; 6, complete destruction >50% of small intestine length, severe necroses) was used for blinded duplicate evaluation (I.F. and O.L.) [29]. Sections of livers were evaluated for the numbers of inflammatory foci at $\times 100$ magnification in five optical fields chosen at random. Ileal sections were evaluated for numbers of parasitophorous vacuoles in two randomly chosen areas of 1 cm length, each under $\times 400$ magnification.

Cytokine ELISA

Homogenates of MLN and spleen were prepared as previously described [29]. Briefly, organs were homogenized in 1 ml PBS using frosted microscope-slides (Corning, Wiesbaden, Germany). Homogenates were centrifuged for 10 min at 3,000 rpm. Supernatants were collected and frozen at -70 °C. Concentrations of IL-12, TNF-α, and IFN-γ were determined by ELISA using reagents and protocols from BD Biosciences (Heidelberg, Germany). Briefly, purified anti-cytokine antibodies against IL-12, TNF-α, and IFN-γ were used as primary antibodies and incubated overnight. Biotinylated rat anti-mouse secondary antibodies

were added. Finally, streptavidin-conjugated peroxidase (Amersham, Little Chalfont, UK) was added as the developing reagent with 3,3',5,5'-tetramethylbenzidine tablets (TMB) as substrate (Sigma, Deisenhofen, Germany). IL-18 concentrations were determined using anti-mouse-IL-18 ELISA kits (R&D Systems, Wiesbaden, Germany/MBL, Tokyo, Japan). Absorbance was determined at 450 nm with an ELISA reader (Tecan, Crailsheim, Germany). Cytokine concentrations were determined by reference to standard curves. Serum levels of cytokines were measured in the same manner.

Analysis of IL-18bp expression

Total RNA was isolated from ileum with the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The concentration of isolated RNA was determined using the ratio of absorbance (A260/A280) (Eppendorf BioPhotometer 6131). RNA was subjected to the SuperScript™III Platinum® One-Step Quantitative RT-PCR System (Invitrogen) on a LightCycler (Roche). Exon spanning primers were chosen for both the IL-18bp gene (sense: 5'-AGAAGTGCCACTGAATGGAAGT-3', antisense: 5'-TGTTCCTGTGCTCGCGAC-3', FL-probe: 5'-AGGATGCTGAAGTAGGGGAAGC-FL-3', LC Red640-probe: 5'-LC-CTGCAGGCAGTACAGGACAAGGTC-PH-3') and the housekeeping gene HPRT (sense: 5'-gTTggATACAggCCAgACTTTgT-3', antisense: 5'-CACAggACTAgAACACCTgC-3', FL-probe: 5'-AAAgCCTAAgATgAgCgCAAgTTgA-FL-3', LC Red 705-probe: 5'-LC-TCTgCAAATACgAggAgTCCTgTTg-PH-3') to avoid amplification of genomic DNA. All primers were designed by TIB MOLBIOL (Berlin, Germany) for use in the LightCycler. Each PCR reaction was performed in a total volume of 10 µl in glass capillaries containing 1 µl RNA sample, 3.5 mM MgSO₄, 5% BSA, 0.5 µM primer, 0.15 µM probes, 4% SuperScript™III RT/Platinum® Taq-Mix, and 2× reaction mix (Invitrogen, Karlsruhe, Germany). One cycle at 60 °C for 30 min, one cycle at 95 °C for 2 min, 45 cycles at 95 °C for 5 s/58 °C for 7 s/72 °C for 5 s were run for HPRT with a single fluorescence detection point at the end of the cycle. Distilled water was used as negative control in each PCR. Fluorescence was analyzed by LightCycler Data Analysis software 3.5 (Roche). Crossing points (Cp) and target gene expressions for internal standards were established using the second derivative method. Target transcripts were analyzed relatively to an internal standard for the same sample. Samples were normalized through a calibrator introduced in each run. Results were expressed as the target/internal standard concentration ratio of the sample divided by the target/internal standard concentration ratio of the calibrator. Five different dilutions of the calibrator RNA were quantified in quadruplicate to establish PCR efficiency. Expression of Cp versus log of concentration was used to draw a calibration curve by linear regression. PCR efficiency was equal to 10⁻¹/slope. These efficiencies were

taken into account in relative quantification, performed using the RelQuant software (Roche).

Generation of bone marrow chimeras

Recipient wildtype and IL-18^{-/-} mice were given lethal total body irradiation (800 rad, 7.5 min). Within 24 h, they were reconstituted intravenously with 0.75×10^7 bone marrow cells and 0.75×10^7 spleen cells in a mixed suspension in 0.2 ml PBS. Irradiated IL-18^{-/-} mice received wildtype cells; a control group of irradiated IL-18^{-/-} mice received IL-18^{-/-} cells as control. Irradiated wildtype mice received IL-18^{-/-} cells while a control group received wildtype cells. Bone marrow cell suspensions were prepared from donor tibial and femoral bones by flushing with RPMI 1640 (GIBCO) supplemented with penicillin (100 U/ml) and streptomycin (100 U/ml) (Biochrom AG, Berlin) using a 26-gauge needle syringe. Irradiated and reconstituted mice were given neomycin (2 g/ml) (Charité pharmacy, Berlin, Germany) in drinking water for 3 weeks. Thereafter, they received sterile drinking water, to avoid any impact of antibiotic treatment on experimental infection with *T. gondii*. Unless otherwise stated, chimerism was analyzed 8–9 weeks after bone marrow cell transfer using flow cytometry and ELISA. For each infection experiment, groups of non-irradiated wildtype and IL-18^{-/-} animals were included as positive and negative controls, respectively.

Binding affinity of IL-18 to IL-18bp

Surface plasmon resonance measurements were carried out on a Biacore X instrument (Biacore AB, Uppsala, Sweden) at 25 °C. Protein A/G (Pierce Chemicals, Rockford, IL, USA) was immobilized on a sensor chip CM5 using the amine coupling kit (Biacore AB) according to the manufacturer's instructions. Injections of 100 µg/ml protein A/G diluted in 10 mM sodium acetate, pH 4.5, were passed over the surface to immobilize 2100 RU of protein A/G. Unreacted NHS esters were blocked using ethanolamine provided in the coupling kit. The surface was then conditioned with three injections of 100 mM phosphoric acid to stabilize the modified surface. A reference surface was prepared by excluding the injection of protein A/G. A 30-µl injection of a 10 µg/µl solution of human IL-18bp as described above was passed over the surface at 30 µl/min, resulting in a baseline shift of approximately 2300 RU. After the new baseline had stabilized (1 min), binding of mouse IL-18 was recorded in running buffer (PBS, 0.001% Tween 20, 3 mM EDTA) at a flow rate of 30 µl/min. One hundred microliters of IL-18 solution were passed over the surface using the injection command. After the injection, dissociation data (measured in response units [RU]) were collected over 600 s to compare dissociation rates. Regeneration of the surface was done by a single injection of 4 M MgCl₂ at 10 µl/min for 360 s. After regeneration, the next measurement started

again with binding of human IL-18bp to protein A/G at same concentrations as described above, followed by the binding of mouse IL-18 to IL-18bp. Mouse IL-18 was used at five concentrations (25 pM, 50 pM, 75 pM, 100 pM, 125 pM); one of the concentrations (50 pM) was measured twice. The buffer lacking IL-18 was taken as a negative reference. Rate constants were calculated by using BIAevaluation software 4.1, assuming a 1:1 mode of binding.

Statistical analysis

Levels of significance for differences in numbers of parasitophorous vacuoles containing tachyzoites, numbers of inflammatory foci in livers, cytokine levels in organ homogenates and sera, and *Toxoplasma* DNA were determined using Student's *t*- and Mann-Whitney-U-test. A *p*-value of <0.05 was considered significant.

Acknowledgements

The authors thank the staff of the animal facility of the Charité-FEM for their expertise and support. We are grateful to Andrea Maletz, Solvy Wolke, and Uwe Lohmann for technical assistance.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

References

1. Liesenfeld O, Kosek J, Remington JS, Suzuki Y: Association of CD4+ T cell-dependent, interferon-gamma-mediated necrosis of the small intestine with genetic susceptibility of mice to peroral infection with *Toxoplasma gondii*. *J Exp Med* 184, 597–607 (1996)
2. Liesenfeld O, Kang H, Park D, Nguyen TA, Parkhe CV et al.: TNF-alpha, nitric oxide and IFN-gamma are all critical for development of necrosis in the small intestine and early mortality in genetically susceptible mice infected perorally with *Toxoplasma gondii*. *Parasite Immunol* 21, 365–376 (1999)
3. Khan IA, Schwartzman JD, Matsuura T, Kasper LH: A dichotomous role for nitric oxide during acute *Toxoplasma gondii* infection in mice. *Proc Natl Acad Sci U S A* 94, 13955–13960 (1997)
4. Vossenkamper A, Struck D, Alvarado-Esquivel C, Went T, Takeda K et al.: Both IL-12 and IL-18 contribute to small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*, but IL-12 is dominant over IL-18 in parasite control. *Eur J Immunol* 34, 3197–3207 (2004)
5. Suzuki Y, Sher A, Yap G, Park D, Neyer LE et al.: IL-10 is required for prevention of necrosis in the small intestine and mortality in both genetically resistant BALB/c and susceptible C57BL/6 mice following peroral infection with *Toxoplasma gondii*. *J Immunol* 164, 5375–5382 (2000)

6. Buzoni-Gatel D, Debbabi H, Mennechet FJ, Martin V, Lepage AC et al.: Murine ileitis after intracellular parasite infection is controlled by TGF-beta-producing intraepithelial lymphocytes. *Gastroenterol* 120, 914–924 (2001)
7. Muñoz M, Heimesaat MM, Danker K, Struck D, Lohmann U et al.: 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, 3047–3059 (2009)
8. Liesenfeld O: Oral infection of C57BL/6 mice with *Toxoplasma gondii*: a new model of inflammatory bowel disease? *J Infect Dis* 185, S96–S101 (2002)
9. Podolsky DK: Inflammatory bowel disease. *N Engl J Med* 347, 417–429 (2002)
10. Xavier RJ, Podolsky DK: Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 448, 427–434 (2007)
11. Stoll S, Jonuleit H, Schmitt E, Muller G, Yamauchi H et al.: Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC-derived IL-18 enhances IL-12-dependent Th1 development. *Eur J Immunol* 28, 3231–3239 (1998)
12. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A et al.: Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 378, 88–91 (1995)
13. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H.: Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 19, 423–474 (2001)
14. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E et al.: IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. *Immunity* 7, 571–581 (1997)
15. Sugawara I, Yamada H, Kaneko H, Mizuno S, Takeda K et al.: Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. *Infect Immun* 67, 2585–2589 (1999)
16. Mastroeni P, Clare S, Khan S, Harrison JA, Hormaeche, CE et al.: Interleukin 18 contributes to host resistance and gamma interferon production in mice infected with virulent *Salmonella typhimurium*. *Infect Immun* 67, 478–483 (1999)
17. Huang X, McClellan SA, Barrett RP, Hazlett, LD: IL-18 contributes to host resistance against infection with *Pseudomonas aeruginosa* through induction of IFN-gamma production. *J Immunol* 168, 5756–5763 (2002)
18. Kanai T, Watanabe M, Okazawa A, Nakamaru K, Okamoto M et al.: Interleukin 18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. *Gastroenterol* 119, 1514–1523 (2000)
19. Pizarro TT, Michie MH, Bentz M, Woraratanadharin J, Smith, MF Jr et al.: IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. *J Immunol* 162, 6829–6835 (1999)
20. Kanai T, Watanabe M, Okazawa A, Sato T, Yamazaki M et al.: Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. *Gastroenterol* 121, 875–888 (2001)
21. Liew FY: The role of innate cytokines in inflammatory response. *Immunol Lett* 85, 131–134 (2003)
22. Siegmund B, Fantuzzi G, Rieder F, Gamboni-Robertson F, Lehr HA et al.: Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. *Am J Physiol Regul Integr Comp Physiol* 281, R1264–1273 (2001)
23. Ten Hove T, Corbaz A, Amitai H, Aloni S, Belzer I et al.: Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. *Gastroenterol* 121, 1372–1379 (2001)
24. Lochner M, Forster I: Anti-interleukin-18 therapy in murine models of inflammatory bowel disease. *Pathobiol* 70, 164–169 (2002)
25. Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TI et al.: Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. *Gut* 50, 812–820 (2002)
26. Paulukat J, Bosmann M, Nold M, Garkisch S, Kampfer, H et al.: Expression and release of IL-18 binding protein in response to IFN-gamma. *J Immunol* 167, 7038–7043 (2001)
27. Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A et al.: Bioactive IL-18 expression is up-regulated in Crohn's disease. *J Immunol* 163, 143–147 (1999)
28. Seed B, Aruffo A: Molecular cloning of the CD2 antigen, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. *Proc Natl Acad Sci U S A* 84, 3365–3369 (1987)
29. Heimesaat MM, Bereswill S, Fischer A, Fuchs D, Struck D et al.: Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*. *J Immunol* 177, 8785–8795 (2006)
30. Kanai T, Uraushihara K, Totsuka T, Okazawa A, Hibi T et al.: Macrophage-derived IL-18 targeting for the treatment of Crohn's disease. *Curr Drug Targets Inflamm Allergy* 2, 131–136 (2003)
31. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D et al.: Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc Natl Acad Sci U S A* 97, 1190–1195 (2000)
32. Wirtz S, Becker C, Blumberg R, Galle PR, Neurath MF: Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. *J Immunol* 168, 411–420 (2002)