Larval *Echinococcus multilocularis* infection reduces dextran sulphate sodium-induced colitis in mice by attenuating T helper type 1/type 17-mediated immune reactions

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**Summary**

The tumour-like growth of larval *Echinococcus multilocularis* tissue (causing alveolar echinococcosis, AE) is directly linked to the nature/orientation of the periparasitic host immune-mediated processes. Parasite-mediated immune suppression is a hallmark triggering infection outcome in both chronic human and murine AE. So far, little is known about secondary systemic immune effects of this pathogen on other concomitant diseases, e.g. endogenous gut inflammation. We examined the influence of *E. multilocularis* infection on murine dextran sodium sulphate (DSS) - induced colitis. At 3 months after *E. multilocularis* infection (chronic stage), the mice were challenged with 3% DSS in the drinking water for 5 days plus subsequently with tap water (alone) for another 4 days. After necropsy, fixed tissues/organs were sectioned and stained with haematoxylin & eosin for assessing inflammatory reactions. Cytokine levels were measured by flow cytometry and quantitative RT-PCR. Colitis severity was assessed (by board-certified veterinary pathologists) regarding (i) colon length, (ii) weight loss and (iii) a semi-quantitative score of morphological changes. The histopathological analysis of the colon showed a significant reduction of DSS-induced gut inflammation by concomitant *E. multilocularis* infection, which correlated with down-regulation of T helper type 1 (Th1)/Th17 T-cell responses in the colon tissue. *Echinococcus multilocularis* infection markedly reduced the severity of DSS-induced gut inflammation upon down-regulation of Th1/Th17 cytokine expression and attenuation of CD11b+ cell activation. In conclusion, *E. multilocularis* infection remarkably reduces DSS-induced colitis in mice by attenuating Th1/Th17-mediated immune reactions.

**Keywords**: inflammation; parasitic-helminth; T helper type 1/type 17 cells.

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**Introduction**

Alveolar echinococcosis (AE) is a very severe zoonotic helminthic disease in humans, which is fatal if not appropriately treated.1 AE is characterized by chronic and progressively developing hepatic damage caused by the continuously proliferating parasite tissue (metacestode) of *Echinococcus multilocularis*,2 clinically mimicking a slowly growing and metastasizing liver cancer.3 The type of immune response elicited during *E. multilocularis* infection predetermines the outcome of infection (resistance versus susceptibility)4 and, if disease occurs, the kinetics of progression of disease.5 In humans, T helper type 2 (Th2) -oriented immunity is associated with increased
susceptibility to disease, leading to chronic AE, whereas Th1 cell activation has been linked to reduced or abrogated metacestode proliferation, or even protection, which occurs when the parasite becomes aborted, resulting in 'died-out' lesions.2,3 Experimental murine AE is immunologically characterized by an initial Th1-oriented response during the early stage of infection (till approximately 1 month after infection), which gradually acquires a greater Th2 emphasis alongside Th1 activation, so becoming a mixed Th1/Th2 response during the chronic phase of AE (2–4 months after infection). This mixed Th1/Th2 profile in immunocompetent mice is associated with the expression of pro-inflammatory cytokines in the periparasitic granuloma and partial/relative protective immunity (restriction of parasite growth) through fibrosis and necrosis,6 whereas in immunodeficient hosts uncontrolled susceptibility results in an extremely rapid progressing parasite proliferation.7–9 It has been previously reported that CD4+ CD25+ T regulatory (Treg) cells appeared quantitatively up-regulated in human AE. It was also shown that this up-regulation is associated with the blunting of the immune response to specific antigens, and/or to the suppression of the secretion of pro-inflammatory cytokines, especially through high interleukin-10 (IL-10) and transforming growth factor-β1 (TGF-β1) production in cystic echinococcosis.10 In the experimental mouse model, increased CD4+ CD25+ Treg cells were also observed within the peritoneal cell population. Such results concurred with other findings to demonstrate that E. multilocularis antigens promote T-cell differentiation into Treg cells11 and so finally induce immune tolerance and anergy towards the parasitic infection.

The 'hygiene hypothesis' proposes that the stimulation of the immune system by microbes or microbial products protects from the development of inflammatory diseases. Reflecting the anti-inflammatory actions of parasite-derived immunomodulators, an inverse correlation exists between helminthic infections and the occurrence of autoimmune diseases, best documented in the industrially developed world.12 Furthermore, it has become evident that many helminthic infections are associated with reduced allergic reactivities, and most recent studies demonstrated an increased Treg cell activity in infected murine or human hosts.13 Crohn’s disease and ulcerative colitis are the main and distinct clinical and pathophysiological entities of inflammatory bowel diseases (IBD).14 Current evidence suggests that aberrant immune reactions against intestinal luminal-derived microbial antigens can lead to their onset in genetically susceptible individuals.15 During the last 15 years, the therapy of patients with IBD was revolutionized with the development of specific biologicals, notably of tumour necrosis factor (TNF) inhibitors of various types.16,17 However, approximately 20% of patients do not respond to anti-TNFs, and over 30% eventually lose response.16,18,19 In addition, these biological compounds and the often-associated chemical immunosuppressants have been shown to be responsible for adverse effects and especially for increasing the risk of infections and malignancies.20,21

Much experimental data support the hypothesis that helminths are able to modulate colitis. The possible beneficial effect of helminth infections on the development and course of colitis has been investigated in different animal models.22 It was shown that an infection with Schistosoma mansoni cercariae exerted preventive effects on the course of trinitrobenzene sulphonic acid (TNBS) -induced colitis in rats and dextran sodium sulphate (DSS) -induced colitis in mice.14,23,24 Furthermore, S. mansoni eggs prevented mice from developing TNBS-induced colitis, but did not prevent mice from developing DSS colitis.15,24 Schistosoma japonicum eggs also exerted preventive effects on TNBS-induced colitis in mice.6,17 A previous infection of mice with Trichinella spiralis larvae and Trichinella papuae larvae reduced the severity of DNBS-induced and DSS-induced colitis, respectively.18,19 Infection with Hymenolepis diminuta larvae in DNBS mice had a profound anti-colitis effect (both prophylactically and as a treatment), which was not seen in semi-permissive rats.20,21 In contrast, Heligmosomoides polygyrus bakeri larvae enhanced Citrobacter rodentium-induced infectious colitis in mice.25,26 and Hymenolepis diminuta infection caused an exacerbation of oxazolone-induced colitis in mice.27,28 Soon after the first promising findings of helminth infections on experimental colitis were published, clinical trials were started to explore whether helminths could alter the course of disease in patients, with the use of the pig whipworm Trichuris suis29–31 and larvae of the human hookworm Necator32. After these promising results, the US Food and Drug Administration requested the development of T. suis ova under good manufacturing practice and appropriate safety testing in order to continue clinical tests.33 Helminthic immunomodulators therefore may be highly attractive molecules with great potential to be used as therapeutic compounds for the treatment of colitis, as exemplified by the recent development of a transgenic probiotic secreting cystatin, an immunomodulator from nematodes, for site-directed treatment of gut inflammation.34 However, in clinical trials, the use of intestinal helminths/helminth eggs was not as convincing in patients with IBD as in the experimental models,35 this could be due to the schedule of administration and/or the voluntary use of low doses of worms to limit possible adverse effects. Other types of helminths and/or their products could be an alternative. Regarding the influence of cestode infections on immunologically mediated diseases, it has been shown that, in experimental models, Echinococcus granulosus infection reduced airway inflammation36.
and also alleviated the severity of experimental DSS-induced colitis.\textsuperscript{37} However, the influence of \emph{E. multilocularis}, which generates a more complex and sustained immunoregulatory profile than \emph{E. granulosus}, has never been studied, and the role of systemic T-cell regulation, its related cytokine production, and antigen-presenting process have not been clarified.

The major aims of the present study were: (i) to address whether infection by the cestode \emph{E. multilocularis} could modulate the phenotype of the well-known DSS-induced colitis; and (ii) to further explore possible mechanisms that would explain the immunotherapeutic properties of \emph{E. multilocularis} infection acting on DSS-induced colitis. To achieve these goals, we investigated the differential levels of pathological changes in the colon and its related pro-inflammatory/inflammatory cytokines, Th1/Th2 and Treg/Th17-related cytokine expression levels in both spleen and colon, together with CD11b\textsuperscript{+} CD11c\textsuperscript{+} cell activation in the spleen, in \emph{E. multilocularis}-infected mice subsequently challenged with a DSS-induced colitis.

\section*{Materials and methods}

\subsection*{Ethical statements}

The animal study was performed in strict accordance with the requirements of the Swiss Guidelines for the Care and Use of Laboratory Animals. The protocol was approved by the governmental Commission for Animal Experimentation of the Canton of Bern (approval nos BE103/11 and BE112/14).

\subsection*{Experimental design, parasite sampling and histological examination}

\textit{Mice.} Female C57BL/6 mice, all aged between 8 and 10 weeks, were bred and housed under specific pathogen-free conditions according to recommendations of the Federation of European Laboratory Animal Science Association, and additionally monitored by daily inspection, including the assessment of the appearance of health status, putative weight loss or gain during the whole course of the experiment. Facility supervision for infectious diseases specifically included also the demonstration of absence of \emph{Helicobacter}, \emph{Citrobacter}, norovirus, rotavirus and \emph{Oxyuris}. All experiments with animals were performed within a laminar flow safety enclosure. The mice were randomly divided into four groups with six mice in each group comprising: (i) negative control group (NC); (ii) \emph{E. multilocularis} infection group (Em); (iii) DSS-induced colitis group (DSS); (iv) \emph{E. multilocularis} infection plus DSS-induced colitis group (Em+DSS).

\textit{Parasite and experimental infection.} \emph{Echinococcus multilocularis} (H95) was isolated and maintained by serial passages (vegetative transfer) in C57BL/6 mice as previously described.\textsuperscript{38} In order to prepare the infection material for mice, metacestode tissue was obtained from previously infected mice by aseptic removal from the peritoneal cavity. After grinding the tissue through a sterile 50-μm sieve, approximately 100 freshly prepared vesicular cysts were suspended in 100 μl RPMI-1640 (Gibco, Basel, Switzerland) and injected intraperitoneally.

\textit{DSS-induced colitis.} At 3 months after \emph{E. multilocularis} infection, or in non-infected mice used as controls, experimental acute colitis was induced by administration of 3% 36 000–50 000 MW DSS (MP Biomedical, Solon, OH) in the drinking water for 5 days, followed by 4 days of regular tap water, animals were finally killed at day 9.

\textit{Collection of host tissue.} Mice were killed by Isofluran anaesthesia followed by CO\textsubscript{2} euthanasia. Blood was collected by cardiac puncture, and serum samples were stored at –80°C.

\textit{Colonic cells.} Approximately 1.5-cm lengths of the anterior colons were opened longitudinally and cut into small pieces. The epithelium was removed by incubation in Hanks’ balanced salt solution/HEPES containing 5\% horse serum, 5 mM EDTA and 2 mM dithiothreitol at 37°C for 30 min under magnetic stirring. Colonic cells were obtained by subsequent digestion with 200 U/ml collagenase (Type IV; Sigma-Aldrich, St Louis, MO, USA) and 50 U/ml DNase (Type I, grade II; Roche, Basel, Switzerland) for 45 min, then filtered through a 40-μm cell strainer, counted using Trypan Blue staining and further characterized by quantitative RT-PCR.

\textit{Histopathology and histopathological grading.} Sternum, thymus, part of the spleen, mesenteric lymph nodes, and small and large intestine from each mouse were fixed in 10\% neutral-buffered formalin for 24 hr and embedded in paraffin. Blocks were sectioned and slides were stained with haematoxylin & eosin. On haematoxylin & eosin-stained sections, morphological changes of all tissues were recorded and a semi-quantitative grading was used to score bone marrow, spleen and colon as listed in Table 1. The disease activity index (DAI) for the DSS-induced colitis scoring system is summarized in Table 2. The macroscopic and microscopic evaluations were performed in a blinded fashion by European-board-certified veterinary pathologists.

\textit{Cell preparations.} Spleen cells were collected by grinding individual organs separately with 5 ml RPMI-1640. Cells were subsequently washed twice and resuspended in RPMI-1640 (Gibco). Macrophages were removed from each group of mice by plastic adhesion after incubation of spleen cell suspension in 15 ml RPMI-1640 + 20%
fetal calf serum in a Petri dish for 2 hr at 37 °C in an atmosphere containing 5% CO2. Subsequent to incubation, non-adherent cells were separated from macrophage-enriched adherent cells, and this new cell suspension was used for FACS analyses.

Flow cytometry

Aliquots of 10^5 cells/100 µl of staining buffer per well were incubated each with 1 µg of purified anti-CD16/CD32 for 20 min in the dark to block non-specific binding of antibodies to the FcγIII and FcγII receptors. Subsequently, these cells were separately stained with the following surface markers for 15 min with 1 µg of primary antibodies: allophycocyanin-labelled anti-CD4, anti-CD80, anti-CD86; phycoerythrin-labelled anti-CD11b, anti-CD11c. All antibodies were from eBioscience (San Diego, CA). For intracellular staining, peritoneal exudate cells (PECs) or spleen cells were first incubated with Inside Fix (Miltenyi Biotec, Bergisch Gladbach, Germany) for 20 min at room temperature and subsequently stained with phycoerythrin-labelled anti-interferon-γ (IFN-γ), anti-IL-4, anti-IL-17A, anti-IL-10 and anti-Foxp3 (eBioscience) in Inside Perm (Miltenyi Biotec) for 15 min in the dark. Corresponding fluorochrome-labelled isotype control antibodies were used for staining controls. Cells, resuspended in 300 µl of buffer (0.15 M NaCl, 1 mM NaH2PO4 H2O, 10 mM Na2HPO4 2H2O and 3 mM NaN3) were analysed in a flow cytometer (Becton Dickinson, Heidelberg, Germany) using the corresponding CELL QUEST software.

Cytokine gene-expression analyses by quantitative RT-PCR

Total RNA was extracted from spleen or colon tissue previously put into TRIzol (Invitrogen) according to the manufacturer’s instructions. The cDNA was synthesized using the Omniscript Reverse Transcription kit (Qiagen, Hilden, Germany). SYBR-Green Mix-based quantitative RT-PCR was carried out on a Rotor-Gene 6000 quantitative PCR detection system (Corbett, Sydney, Australia) with the FastStart Essential DNA Green Master (Roche) following the manufacturer’s instructions. PCR cycling was performed in triplicates in final volumes of 20 µl containing 2 µl cDNA and 10 µM of each primer (Cycle scheme: initial denaturation at 95°C for 15 min, 45 cycles of 95°C for 15 seconds, 55°C for 30 seconds and 72°C for 30 seconds). Fluorescence was measured in every cycle, and a melting curve was analysed after the PCR by increasing the temperature from 55°C to 95°C in 0.5°C increments. The primers used were described earlier, and cytokine mRNA levels were quantified relative to the mRNA level of the housekeeping gene β-actin. Respective mean values from triplicate determinations from six individual mice in each group were taken for the calculation of relative cytokine mRNA levels in relation to β-actin mRNA levels.

Statistical analyses

All data were analysed by SPSS 17.0 (IBM Corporation, Armonk, New York, USA). The results are presented as means ± SD. Normality of data was assessed by D’Agostino & Pearson and Shapiro–Wilk test. For normally distributed groups of data, one-way analysis of variance followed by Bonferroni’s post-test or unpaired two-tail Student’s t-test were used to compare the differences between groups. Significance was defined as \( P < 0.05 \) for all tests, except those subsequently corrected by Bonferroni.
Results

*Echinococcus multilocularis* infection reduces severity of DSS-induced colitis in mice

To evaluate the benefits of *E. multilocularis* infection in abrogating or down-regulating colitis, we subjected mice to DSS-induced colitis 3 months after *E. multilocularis* infection. With administration of 3% DSS, the Em + DSS groups (*E. multilocularis* infection combined with challenge with 3% DSS) exhibited less severe symptoms including body weight losses, DAI scores and colon length changes compared with the DSS group (challenge with 3% DSS) (Fig. 1). The body weight in the DSS group (acute colitis model) decreased significantly when compared with the negative control (NC) groups, whereas body weight loss was much lower in the Em+DSS group (Fig. 1a). The DAI scores were significantly lower in the Em+DSS group when compared with those of the DSS group (Fig. 1b). Similarly, the average colon length of the DSS group was shortened by approximately 24% when compared with that of the NC groups, whereas it was only reduced by about 4% in mice of the Em+DSS group (Fig. 1c). These results suggest that *E. multilocularis* infection reduces the severity of colitis.

![Figure 1](image-url)

**Figure 1.** Mice chronically infected with *Echinococcus multilocularis* are protected from dextran sodium sulphate (DSS) -induced colitis. (a) Weight loss relative to the initial body weight. Mean values of *n* = 15 to *n* = 18 mice analysed per group are shown with error bars indicating the SD. At 3 months after *E. multilocularis* infection, experimental colitis was induced by administration of 3% DSS in the drinking water for 5 days followed by 4 days of regular tap water. Day 1, 2, 3, 4 indicates the days after tap water. (b) Disease activity index (DAI) changes among groups after 9 days 3% DSS treatment at the end time-point. Data represents the mean ± SD (*n* = 15 to *n* = 18). (c) Colon lengths were determined in individual mice. Data show mean values for each group of mice. (d–g) Individual parameters of histopathological scoring. (d) Lymph follicles in the colon. (e) Increase in granulopoiesis in the bone marrow. (f) Lymphoid structures in the spleen. (g) Extramedullary haematopoiesis in the spleen. Histopathological scores were determined for individual mice by a pathologist according to parameters defined in the Materials and methods section. Columns show mean values for *n* = 5 or *n* = 6 mice analysed per group and error bars indicate the SD. One representative experiment out of three independent experiments is shown. HPF, high-power field; EMH, extramedullary haematopoiesis. *P* < 0.05.
Histopathological effects of *E. multilocularis* infection on DSS-induced colitis in mice

Significant morphological changes were recorded in the bone marrow and lymphoid organs of the Em group and of the Em+DSS group when compared with the NC and DSS groups (Fig. 1d–g). In detail, the ratio of granulocytic to erythrocytic precursor cells in the bone marrow was scored 0 (no pathological change) in the NC group, 2 (mild increase) in the DSS group and grade 3 (moderate increase) in groups with Em or Em+DSS, respectively (mean values) (Fig. 1e). Reduced numbers of periarteriolar lymphoid sheaths were noted in the Em and Em+DSS groups in comparison to the NC and DSS groups (Fig. 1f). Additionally, a significant increase in extramedullary haematopoiesis was noted in the spleen of all animals from the Em and Em+DSS groups compared with the NC and DSS groups (Fig. 1g), corresponding to a marked increase in organ weight (data not shown). The large intestine was significantly altered in all animals from the DSS group, displaying multifocal epithelial necrosis and mucosal ulceration, loss of goblet cells, loss and/or proliferation of crypts, crypt abscesses and marked infiltration of the lamina propria by macrophages, neutrophils and lymphocytes, and finally fibrosis (grade 2, Fig. 2). Besides these findings, five animals also revealed epithelial dysplasia/proliferation (grade 3, Fig. 2). In comparison, the large intestine from animals of groups Em and Em+DSS presented fewer lymph follicles in the mucosa and/or submucosa (grade 1, Fig. 2), and no pathological changes were noted in the NC group (grade 0) (Figs 1d and 2).

**Echinococcus multilocularis** infection reduces DSS-induced inflammatory/pro-inflammatory cytokine expression in the colons of mice

Cytokines are principal mediators of the innate and adaptive arms of the immune responses in mucosal inflammation. To analyse the influence of the cytokine patterns in DSS-induced colitis mice and the effect of *E. multilocularis* infection on DSS-induced colitis pathogenesis, the cytokine profile in the colon of the mice was measured by quantitative RT-PCR. In general, a marked up-regulation in the pro-inflammatory/Th1 cytokine (IL-12, IFN-γ, IL-1β, IL-6, TNF-α) and Th17 cytokine (IL-17A) mRNA expression level was observed in the DSS group (Fig. 3). Interestingly, a significant decrease of those cytokine expression levels (IL-12, IFN-γ, IL-6, TNF-α, IL-17A,) was noted in the colon of the Em+DSS group, when compared with the DSS group. Interleukin-1β, responsible for inducing the inflammatory cascade, was not differentially expressed in the colon of the Em+DSS group, when compared with DSS group (Fig. 3). There was no difference in IL-4 mRNA level between the colons of the Em+DSS and the DSS groups (data not shown).

**Echinococcus multilocularis** infection changed immune direction in the spleen from mice with DSS-induced colitis

To further explore the effect of *E. multilocularis* infection on the systemic T-cell immune response in DSS-induced colitis in mice, T helper-related cytokines were comparatively assessed in all the groups (Em, DSS, Em+DSS, and
in their respective NC controls). Flow cytometry revealed that Th cells from the Em group were oriented towards the Treg/Th2 pathway at the chronic stage of *E. multilocularis* infection (Em group), with high expression levels of IL-10, Foxp3 and IL-4 (Fig. 4), whereas DSS induced significantly increased expression levels of IFN-γ when compared with the NC group. CD4+ T cells from Em+DSS mice were oriented towards a Treg pathway, with high expression levels of IL-10 and Foxp3 in the spleen. Conversely, a lower IFN-γ expression level was found in the Em+DSS group when compared with the DSS group (Fig. 4).

**Echinococcus multilocularis** infection reduces antigen-presenting cell activation and co-stimulation in the spleen from mice with DSS-induced colitis

To further explore the effect of *E. multilocularis* infection on antigen-presenting cell activation and co-stimulation signals for T cells, we measured CD80, CD86 and CD40 in CD11b+ and CD11c+ cells. Flow cytometry showed that the frequency of maturation markers CD80 and CD86 was higher in CD11c+ cells (Fig. 5a–d), and the frequency of T-cell co-stimulation marker CD40 was lower in CD11c+ cells in the Em group than in the NC group (Fig. 6a,b). However, there was no difference either in CD80 and CD86 frequency (Fig. 5a–d), or in CD40 frequency among CD11b+ cells in these groups (Fig. 6a, b). However, DSS challenge led to a significantly increased expression level of CD80, CD86 and CD40 in CD11b+ cells when compared with the NC group (Figs 5 and 6). Conversely, in the Em+DSS group, CD80, CD86 and CD40 levels in CD11b+ cells were significantly lower when compared with the DSS group (Figs 5 and 6). However, there was no difference in the CD80, CD86 and CD40 frequencies among CD11c+ cells between groups DSS and Em+DSS (Figs 5 and 6).

**Discussion**

Inflammatory bowel diseases are complex diseases caused by a deregulated immune response to intestinal luminal antigens,15 and have increased at an alarming rate in the past two decades.40–45 Symptoms of IBD are the result of
complex interactions among genetic and environmental factors, and the immune response. Nowadays, therapy with immunomodulating biological agents is considered the mainstay for moderate-to-severe Crohn’s disease and severe ulcerative colitis. The anti-TNFs infliximab and adalimumab have been extensively used and shown to induce clinical and endoscopic remission in both diseases. Nevertheless, some patients do not respond initially or, more frequently, lose response. Recently, other biological agents targeting other immunological pathways were approved for the treatment of IBD, such as vedolizumab, an $\alpha_4\beta_7$ anti-integrin that impairs gut homing of lymphocytes in Crohn’s disease and ulcerative colitis, and ustekinumab, a bivalent anti-IL-12–IL-23 monoclonal antibody for Crohn’s disease. Other promising therapeutic biologicals targeting specific cellular metabolic pathways are currently in phase III stage of research, such as orally administered Janus kinase inhibitors (tofacitinib) in ulcerative colitis, or a SMAD7 oral anti-sense oligonucleotide (Mongersen) that down-regulates inflammatory cytokine production by restoring TGF-$\beta_{1}$/Smad signalling in Crohn’s disease. However, altogether these drugs have potential deleterious and sometimes unpredictable paradoxical effects on the immune system and new therapeutic approaches are eagerly awaited. Faecal transplantation has shown promising results in ulcerative colitis and opens a new ‘pathophysiological era’ in IBD treatment management but its long-term efficacy and modalities of administration are debated. Therefore, it is still important to look for other therapeutic pathways.
against IBD, and systemic immune modulation induced by parasite-derived compounds is one of these innovative pathways. Epidemiological observations showed an association between (i) the decrease in infectious diseases, including parasite infections, use of antibiotics, vaccinations and a general improvement in food, water and housing sanitary conditions; and (ii) an increase in the incidence of allergic diseases, and also autoimmune and chronic inflammatory disorders. This finding forms the basis of the continually revisited ‘hygiene hypothesis’. Within this hypothesis, intestinal worms could play a role by modulating the composition/function of the gut microbiota, which is fundamental to the ‘education’ of the immune system after birth; all types of helminths, including their larval stage in organs other than the gut, might also play a role in the systemic immune balance that prevents abnormal inflammation. Several experimental studies using different animal models of colitis have shown the ability of parasitic worms to attenuate intestinal inflammation, for example *T. suis* exerts a therapeutic effect in patients with ulcerative colitis and Crohn’s disease. Although a large body of evidence indicates that regulatory mechanisms that reduce IBD severity may

Figure 5. Antigen-presenting cell (APC) activation was attenuated in the spleen of mice with alveolar echinococcosis (AE) upon dextran sodium sulphate (DSS)-induced colitis. Cells were first gated on size and singularity followed by DAPI exclusion to identify live cells for further analysis. Live cells were gated on CD11b or CD11c expression to first identify CD11b+ and CD11c+ cells. Then phycoerythrin-labelled CD80, CD86 expression was identified as CD80, CD86 frequency within CD11c+ or CD11b+ cells. (a) CD80 frequency within CD11c+ and CD11b+ cells, CD80 mean fluorescence intensity (MFI) within CD11b+ cells in spleen cells from each group. (b) Representative images of CD80+ within CD11b+ cells in spleen cells from each group. (c) CD86 frequency within CD11c+ and CD11b+ cells, CD86 MFI within CD11b+ cells in spleen cells from each group. (d) Representative images of CD86+ within CD11b+ cells in spleen cells from each group. Comparison between groups was performed using a one-way analysis of variance with Bonferroni’s multiple comparison post-test for statistical analysis. *P < 0.004.
be triggered by intestinal helminths, and especially nematode infections, far less is known about larval cestodes, which cannot exert a direct effect on the gut. Previous experiments using a mouse model of cystic echinococcosis (E. granulosus infection) have suggested that concomitant infection with E. granulosus improved the clinical score, ameliorated the DAI, and prevented the shortening of the colon in experimental DSS-induced colitis; such improvement was associated with a reduced nitric oxide and TNF-α production in the plasma of experimental E. granulosus-infected mice with DSS-induced colitis and decreased inducible nitric oxide synthase and nuclear factor-κB expression in colonic tissue. It is interesting to note that a crude extract of the laminated layer from an E. granulosus cyst was as able as active E. granulosus, because it is chronically sustained and because the rodents are the natural intermediate host of the parasite, hence making the mouse experimental model highly relevant. We therefore hypothesized that the negative immune regulation observed when E. multilocularis infection is fully established would also down-regulate DSS-induced colon inflammation, and so prevent or repair DSS-induced damage; this model would also allow us to explore the T-cell-dependent immunoregulatory mechanisms, which have been well delineated in the chronic infection by E. multilocularis in mice. The results of our first experiments fully confirm our hypothesis; taken together, our observations suggest that a pre-established E. multilocularis infection protects mice from DSS-induced colitis. DSS-exposed E. multilocularis-infected mice exhibited significantly less severe colitis than those animals without E. multilocularis infection: colonic improvement included maintained colorectal lengths, and microscopically normal mucosal structures with a nearly normal number and size of goblet cells secreting mucus, including a down-regulation of periparasitic and systemic immunity against the metacestode both in humans and in experimentally infected mice. Mechanisms of the immune tolerance involved in Echinococcus spp. infection has received more attention in AE, caused by E. multilocularis, than in cystic echinococcosis, caused by E. granulosus, because it is chronically sustained and because the rodents are the natural intermediate host of the parasite, hence making the mouse experimental model highly relevant. We therefore hypothesized that the negative immune regulation observed when E. multilocularis infection is fully established would also down-regulate DSS-induced colon inflammation, and so prevent or repair DSS-induced damage; this model would also allow us to explore the T-cell-dependent immunoregulatory mechanisms, which have been well delineated in the chronic infection by E. multilocularis in mice. The results of our first experiments fully confirm our hypothesis; taken together, our observations suggest that a pre-established E. multilocularis infection protects mice from DSS-induced colitis. DSS-exposed E. multilocularis-infected mice exhibited significantly less severe colitis than those animals without E. multilocularis infection: colonic improvement included maintained colorectal lengths, and microscopically normal mucosal structures with a nearly normal number and size of goblet cells secreting mucus,
E. multilocularis infection reduces colitis

in marked contrast to non-infected animals with DSS-induced colitis, suggesting that E. multilocularis, like other helminths, can help in preserving/restoring these cells. Goblet cells are involved in regulating both the mucosal barrier and the relative composition of the luminal microbiota by mucin production. The production of mucus by these cells could limit bacterial access to epithelial cells and prevent chronic inflammation.

Polymorphonuclear leukocytes such as neutrophils play a critical role in the maintenance of intestinal homeostasis. They display defence mechanisms to eliminate microbes that have translocated across the single layer of mucosal epithelial cells, which form a critical barrier between the gut lumen and the underlying tissue. During the inflammatory response, neutrophils also contribute to the recruitment of other immune cells and facilitate mucosal healing by releasing mediators necessary for the resolution of inflammation. Neutrophil infiltration is a key event in inflammation of the colon. Here we found that E. multilocularis infection during DSS-induced colitis generated a greater infiltration by monocytes than neutrophils, the latter being the main cell type detected in the absence of helminth infection. Inflammatory monocytes accumulate in response to infection or tissue injury, and in most cases they help to clear pathogens. However, in IBD, the recruitment of inflammatory monocytes into damaged tissue frequently worsens the inflammatory response. Neutrophil infiltration is a key event in inflammation of the colon.53 Here we found that E. multilocularis infection during DSS-induced colitis generated a greater infiltration by monocytes than neutrophils, the latter being the main cell type detected in the absence of helminth infection. Inflammatory monocytes accumulate in response to infection or tissue injury, and in most cases they help to clear pathogens. However, in IBD, the recruitment of inflammatory monocytes into damaged tissue frequently worsens the inflammation. Macrophages from E. multilocularis-infected mice have been shown to exhibit a reduced ability to present a conventional antigen, such as ovalbumin to specific responder lymph node T cells, when compared with normal macrophages from non-infected mice; this triggers an unresponsiveness of T cells, which in turn leads to the suppression of their clonal expansion during the chronic phase of E. multilocularis infection. High periparasitic nitric oxide production by peritoneal exudate cells, mainly macrophages, were also shown to contribute to E. multilocularis-induced immunosuppression. Functionally modified macrophages might therefore play a critical role in avoiding colonic inflammation and perhaps inhibiting recruitment of inflammatory cells into the lamina propria of the colon; the relevance of this hypothesis is also supported by the results obtained in the studies with E. granulosus infection. CD4⁺ T cells and their associated cytokines play an important role in the development of DSS-induced experimental colitis, however, their role was not studied in the E. granulosus model. In E. multilocularis infection, in resistant hosts, Th1 cytokines induce a protective immunity that involves IFN-α and IL-12 as initiating cytokines, and IFN-γ and TNF-α as effector cytokines. The key-role for TNF-α in the protection of mice against E. multilocularis infection was demonstrated by the extreme susceptibility of TNF-α-deficient (knockout) mice to the infection. The down-regulation of TNF-α observed both in the E. granulosus model and our E. multilocularis model, associated with reduced intensity of the DCC-induced colitis, fits well with such observations and with the pathophysiology of experimental colitis, as well as with the spectaculvar effect of anti-TNF-α biological agents in patients with Crohn’s disease. In patients with AE and in experimental susceptible mice, the sustained inhibition of Th1-type effector cells as well as pro-inflammatory and Th1-type cytokines at the chronic stage of E. multilocularis infection is associated with up-regulation of CD4⁺ CD25⁺ Treg cells and of Th2/Treg-cell-associated cytokines and regulatory factors. Our results in the non-DSS-challenged E. multilocularis-infected mice confirmed these observations; conversely, our observations in DSS-challenged mice confirmed the up-regulation of Th1-type cytokine mRNA in acute DSS-induced colitis, including IL-12, IFN-γ, IL-1β, IL-6, TNF-α and the Th17 class cytokine IL-17A. In addition to TNF-α inhibition, we could show that E. multilocularis infection markedly reduced the expression level of Th1/Th17-related cytokines, including IFN-γ, IL-6 and IL-17A in mice with associated experimental colitis. Our results showing that CD4⁺ T cells in the spleen from Em⁺DSS mice are oriented towards a Treg cell pathway, with low IFN-γ and high IL-10 and FoxP3 expression, strongly suggest that the clinical and histopathological effects on colitis are actually mediated through changes in the systemic immune profile of the mice, as was suggested in experiments with other helminthic infections. The reduced levels of the co-stimulation molecules CD80, CD86 and CD40 that we observed on CD11b⁺ dendritic cells in E. multilocularis infected mice with colitis compared with the non-infected mice similarly challenged with DSS, could be involved, at least partially, in skewing the immune profile. Our knowledge of TGF-β and TGF-β/Smad signalling in E. multilocularis infection and of the efficacy of new biological agents that interfere with Smad signalling in Crohn’s disease suggests that further studies on the mechanisms of E. multilocularis-mediated protection against DSS-induced colitis should focus on the TGF-β/Smad pathway.

Even more than nematode worms such as Trichuris spp., which have a potential for pathogenicity, Echinococcus spp. metacestodes are associated with potentially severe diseases and concomitant infection cannot represent an alternative to the currently used therapies in IBD. Cestode-derived compounds, and especially those derived from E. multilocularis, might be more appropriate than real infection with nematodes for subsequent therapeutic application in human patients with IBD. Parasite metabolites and/or secreted or excreted parasite molecules appear as crucial elements in the immune modulation at the host–parasite interface to promote parasite survival. For E. multilocularis, immune modulatory effects have been mainly attributed to E. multilocularis vesicular fluid, and also to the laminated layer, which represents a highly
immunogenic mucopeysaccharid interface between the germinal layer of the parasite and adjacent host tissue.69 Experiments using extracts of the non-infectious laminated layer of E. granulosus are encouraging.69 At this stage, identification and purification of the bioactive molecule(s) responsible for the effective anti-inflammatory and thera-
peutic effects of Echinococcus spp. in colitis as well as exploration of their safety aspects will therefore be the next steps towards promising therapeutic agents.

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Disclosures
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References
E. multilocularis infection reduces colitis


48 Hsu SJ, Tseng PH, Chen PJ.

49 Soufli I, Toumi R, Rafa H, Amri M, Labsi M, Khelifi L

50 Deng Q, Chen H, Liu Y, Xiao F, Guo L, Liu D


57 Mejri N, Gottstein B. Intraperitoneal Echinococcus multilocularis infection in C57BL/6 mice affects CD240 and B7 costimulator expression on peritoneal macrophages and impairs peritoneal T cell activation. Parasite Immunol 2006; 28:373–85.


