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Immune-protective effect of echinococcosis on colitis experimental model is dependent of down regulation of TNF-α and NO production

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ABSTRACT

Hydatid disease (echinococcosis) is a chronic, endemic helminthic disease caused by the larval stage of the tapeworm, *Echinococcus granulosus*. This disease is endemic in many parts of the world, such as the Mediterranean area, and in particular in Algeria. Helminth parasites have developed complex strategies to modulate the immune responses of their hosts through versatile immune-regulatory mechanisms. These mechanisms may regulate immune responses associated with inflammatory diseases such as inflammatory bowel diseases (IBD). the goal of this study was to investigate the effect of *Echinococcus granulosus* infection on the development of dextran sulfate sodium (DSS)-induced colitis. Our results demonstrated that *E. granulosus* infection significantly improved the clinical symptoms and histological scores observed during DSS-induced colitis, and also maintained mucus production by goblet cells. Interestingly, this infection reduced Nitric oxide (NO) and tumor necrosis factor α (TNF-α) production and attenuated inducible nitric oxide synthase (iNOS) and nuclear factor-κB (NF-κB) expression in colonic tissues. Collectively, our data support the hygiene hypothesis and indicate that prior infection with *E. granulosus* can effectively protect mice from DSS-induced colitis by enhancing immune-regulatory mechanisms.

1. Introduction

Hydatid disease (echinococcosis) is a chronic, endemic helminthic disease caused by the larval stage of the tapeworm, *Echinococcus granulosus*. This disease is endemic in many parts of the world, such as the Mediterranean area, and in particular in Algeria (Dakkak, 2010; Mc Manus et al., 2003; Zhang et al., 2014). One significant feature of cystic echinococcosis is that the larval cysts of *E. granulosus* are able to evade the host immune response by modulating it (Wang et al., 2014).

Helminth parasites have developed complex strategies to modulate the immune responses of their hosts through versatile immune-regulatory mechanisms, in order to avoid immune effector cells and molecules. Chronic helminth infections lead to a strong T-helper 2 (Th2) response that opposes the T-helper 1 (Th1) response and promotes secretion of regulatory cytokines by regulatory T cells. These cytokines may heighten Th2 responses, contributing to asthma and food allergies (Wang et al., 2014), and regulate excessive Th1 responses associated with inflammatory diseases such as inflammatory bowel diseases (Elliott and Weinstock, 2012; Ledesma-Soto et al., 2015).

Inflammatory bowel diseases (IBD) is the collective name for a family of chronic, relapsing inflammatory conditions, clinically subdivided into Crohn's disease (CD) and ulcerative colitis (UC) (Podolsky, 2002; Smith et al., 2007; Strober et al., 2009).

Many studies conducted in animal models, human genetics, in basic science and clinical trials have provided important insights into the pathogenesis of inflammatory bowel diseases. Crohn's disease and ulcerative colitis are heterogeneous diseases characterized by a dysregulated immune response to the normal gut flora, which is aggravated by environmental factors in genetically predisposed individuals (Cortota et al., 2009; Sartor, 2006).

The incidence and prevalence of IBD have increased substantially over the past 50 years in industrialized countries, but remains low in developing areas (Sutton et al., 2008). Currently, some developed countries report an incidence of one out of every 250 people (Kappelman et al., 2007; Ledesma-Soto et al., 2015). In Europe, up to 0.3% of the population suffers from IBD (Burisch et al., 2013; Ledesma-Soto et al., 2015).

Epidemiological studies conducted on inflammatory bowel diseases have indicated a north-south gradient of IBD prevalence in both North America and Europe, with a lack of these diseases in developing countries. One explanation for this trend is known as

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the "hygiene hypothesis" (HH) (Kiloski et al., 2008). This hypothesis correlates the epidemiological trend with the improvement in general hygienic conditions, such as free access to clean water, running hot water, and smaller family sizes. It is hypothesized that in countries with improved hygiene, the dramatic reduction in childhood exposure to bacteria and parasites, such as helminths, results in an immunological over-reaction following microbial contact (Castiglione et al., 2012; Sutton et al., 2008). This suggests an association between the absence of helminth infection and the increase in cases of IBD (Elliott and Weinstock, 2012).

Several experimental studies reported that infection with a number of parasitic worms could reduce the severity of colitis in various animal models (Elliott et al., 2004). These experimental animal studies have now been extended to include worm infections in humans. UC or CD patients infected with Trichuris suis, were shown disease activity indices reduction (Smith et al., 2007; Summers et al., 2005). Additionally, infection with the human hookworm, Necator americanus, in patients with CD, has also shown potential therapeutic effects (Croese et al., 2006; Smith et al., 2007).

An immunological study conducted in our laboratory has shown that the hydatid laminated layer of *E. granulosus* has a beneficial effect in dextran sulfate sodium (DSS)-induced experimental colitis (Soufli et al., 2015). In our study, we used a secondary *E. granulosus* infection mouse model to evaluate the effect of this parasitic infection on the development of the inflammatory response during dextran sulfate sodium (DSS)-induced colitis, and to situate a part of its immunological mechanism. On the basis of our previous work, we enlarged our objectives and we investigated the effects of *E. granulosus* infection on TNF-α and NO pathways in our colitis model.

2. Materials and methods

2.1. Experimental animals

Female BALB/c mice, aged 4–8 weeks (approximately 16 g in weight), were purchased from the Pasteur Institute (Algiers, Algeria). Mice were kept under normal conditions with a 12 h dark/light cycle with ad libitum access to food and water, and allowed to acclimate for 1 week prior to the start of the experiments. This study was approved by the National Thematic Agency Research in Health (N°43-ANDRS-2011).

2.2. E. granulosus *protoscoleces*

E. granulosus protoscoleces (PSC) were collected as described by Amri et al. (2007). Briefly, PSCs were obtained by aseptic puncture of fertile human pulmonary hydatid cysts (Department of Surgery, Mustapha Pacha Hospital, Algiers, Algeria). PSCs were washed with sterile phosphate buffered saline (PBS), pH 7.5. PSC viability was assessed prior to inoculation and determined by eosin exclusion. All samples had viability of >98% at the time of the experiment. BALB/c mice were inoculated intraperitoneally (IP) with 2000 viable PSCs suspended in sterile PBS (Urrea-Paris et al., 2002).

2.3. Experimental design

BALB/c mice were randomly divided into four groups (n = 6–9 mice per group) as follows: (1) the negative control group (PBS) which received an intraperitoneal injection with PBS, (2) the *E. granulosus* infected group (Eg) which received an intraperitoneal injection with a suspension of 2000 viable PSCs in 200 μl of sterile PBS, (3) the DSS group (DSS) which were given 3.5% (w/v) DSS (MW 36 000–44 000) (TdB Consultancy, Sweden) in their drinking water ad libitum for seven days as described by Okayasu et al. (Okayasu et al., 1990) in order to induce acute colitis, and (4) the Eg + DSS group which was given 3.5% (w/v) DSS (MW 36 000–44 000) in their drinking water ad libitum for seven days after three months of parasite infection. All mice were euthanized three months and seven days post-PSC inoculation. The experimental design is represented in (Fig. 1).

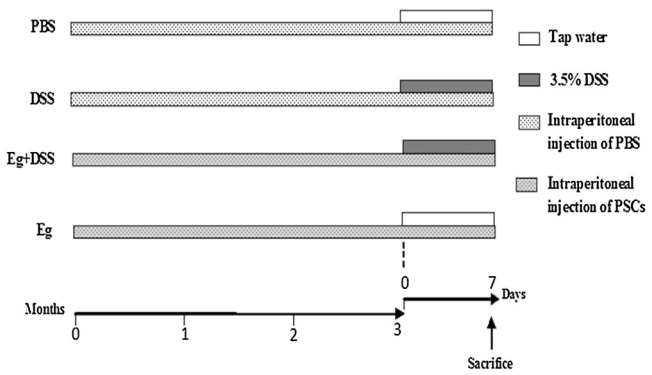

Fig. 1. Experimental design.

2.4. Disease activity index (DAI) assessment

A Disease Activity Index (DAI) was used to evaluate the grade and extent of intestinal inflammation (Azuma et al., 2010a). To assess the severity of colitis: body weight, stool consistence, and blood in the stool were determined daily during the experiment, using a previously published grading system (Azuma et al., 2010b). Weight loss was scored as follows: score 0, none; score 1, 1–5%; score 2, 5–10%; score 3, 10–20%; score 4, >20%. Diarrhea was scored as follows: score 0, normal; score 2, loose stools; score 4, watery diarrhea. Blood in stool was scored as follows: score 0, normal; score 2, slight bleeding; score 4, gross bleeding. The total score of DAI ranged from 0 to 12.

2.5. Plasma collection

At the end of the experiment, mice were anesthetized with chloroform, and blood was collected via cardiac puncture. Blood was centrifuged to isolate plasma which was kept at −20 °C until use.

2.6. Preparation of peritoneal macrophages and cell culture

Macrophages were obtained by peritoneal lavage of the mice using 5 ml of sterile PBS (pH = 7.4). Cellular suspensions were washed three times with sterile PBS by centrifugation at 2800 rpm for 5 min, and then the pellets were resuspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 20 mM L-glutamine. Cells were cultured at a density of 5×105 cells/well. After two hours, the wells were washed using sterile PBS to remove non-adherent cells. Peritoneal macrophages (pMΦ) were incubated in complete DMEM at 37 °C and 5% CO2 for 24 h. The cells used for all experiments were cultured in duplicate. After 24 h, the supernatants were removed and stored at −20 °C until further use.

2.7. Nitrite level measurement

Nitrite production, an indicator of NO synthesis, was measured in all culture supernatants and plasma using a modified Griess reaction as described by Touil-Boukoffa et al. (1998). In brief, 100 ml of culture supernatant was mixed with 100 ml of Griess reagent (5% sulfanilamide, 0.5% napthylethylenediamine dihydrochloride, and 20% HCL), and 800 ml of distilled water. The resulting solution