

Increased susceptibility to *Strongyloides venezuelensis* infection is related to the parasite load and absence of major histocompatibility complex (MHC) class II molecules



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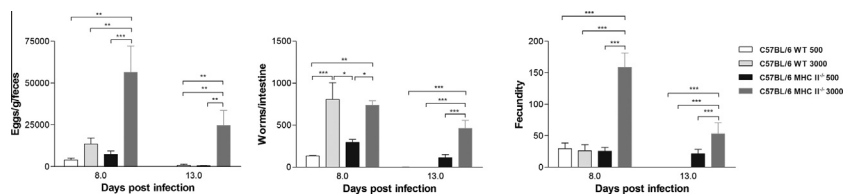
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HIGHLIGHTS

- 3000 L3 C57BL/6 MHC II^{-/-} infected mice are more susceptible to *S. venezuelensis* infection.
- C57BL/6 wild type (WT) mice are able to eliminate high and low levels of infection.
- MHC II is essential to *S. venezuelensis* control in high inoculum infection.

GRAPHICAL ABSTRACT



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ABSTRACT

In human and murine models strongyloidiasis induce a Th2 type response. In the current study we investigated the role of different loads of *Strongyloides venezuelensis* in the immune response raised against the parasite and the participation of the major histocompatibility complex (MHC) class II molecule in the disease outcome in face of the different parasite burden. The C57BL/6 wild type (WT) and MHC II^{-/-} mice were individually inoculated by subcutaneous injection with 500 or 3000 *S. venezuelensis* L3. The MHC II^{-/-} mice infected with 3000L3 were more susceptible to *S. venezuelensis* infection when compared with WT groups, in which the parasite was completely eliminated. The production of Th2 cytokines and specific IgG1 or IgE antibodies against parasite were significantly lowered in MHC II^{-/-} infected mice with different larvae inoculums. The infection of MHC II^{-/-} mice with *S. venezuelensis* induced slight inflammatory alterations in the small intestine, and these lesions were lower when compared with WT mice, irrespective of the parasite load utilized to infect animals. Finally, we concluded that MHC class II molecules are essential in the immune response against *S. venezuelensis* mainly when infection occurs with high parasite inoculum.

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1. Introduction

Strongyloides stercoralis is an intestinal nematode that causes human disease in most patients and potentially fatal disseminated

strongyloidiasis in immunocompromised individuals (Mejia and Nutman, 2012). The life cycle of this helminth is complex and the infection occurs by penetration of filariform larvae (L3) through the skin. Then, the parasite may replicate in the human host and produce autoinfection, and can persist in an individual for decades, resulting in a chronic disease (Siddiqui and Berk, 2001). The majority of the patients with strongyloidiasis are

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asymptomatic although patients with certain disorders such as hematological malignancies, HIV/AIDS infection, T cell leukemia virus type-1 (HTLV-1) infection and long-term corticosteroids use can suffer hyperinfection and even death (Montes et al., 2009; Win et al., 2011).

The immunodulatory mechanisms that regulate the interaction between this parasite and the host are not totally understood. In human and murine models the infection is characterized by systemic eosinophilia (Korenaga et al., 1991; Nawa et al., 1994; Onah and Nawa, 2000), production of cytokines such as IL-3, IL-4, IL-5 and IL-13 with subsequent secretion of specific IgM, IgG, IgG1, IgA and IgE, essential to the elimination of the parasite (Abe and Nawa, 1988; Onah and Nawa, 2000; Schilter et al., 2010; Gonçalves et al., 2012a). *S. venezuelensis* naturally infects rats and has been used in experimental models (Machado et al., 2005; Negrão-Correia et al., 2006) aimed to study the immunoprotection and immunoregulation aspects of the disease since it evokes similar innate and acquired immune response in mice as does *S. stercoralis* in humans (Rodrigues et al., 2009). Previous studies from our group demonstrated that intestinal establishment of *S. venezuelensis* is affected by the absence of major histocompatibility complex (MHC) class II molecules during parasite infection. The MHC class II deficient mice (MHC II^{-/-}) presented higher susceptibility to *S. venezuelensis* infection, with impaired responses to the worm, suggesting that the failure of MHC class II expression affects the antigen presentation to CD4⁺ T cells that is necessary for the initiation of an effective Th2 response against the parasite (Abe and Nawa, 1988; Gonçalves et al., 2008; Rodrigues et al., 2009).

Once most individuals infected with small number of parasites are asymptomatic or oligosymptomatic with gastrointestinal symptoms, it is still not clear if excessive parasite loads could also influence disease outcome during infection, a condition that could occur with individuals infected with a large number of parasites. In immunocompromised patients with excess parasites the disease may become potentially fatal due to hyperinfection syndrome and dissemination, besides being related to the highest mortality rate (Grove, 1996). However, there has been little investigation into the causes of this disease presentation form and the worst outcome (Machado et al., 2007).

Then, to investigate whether intensity of infection induced by different inoculums of *Strongyloides* could influence or be influenced by the host immune competence, we evaluated the role of two different loads of *S. venezuelensis* in the immune response raised against the parasite and the participation of the MHC class II molecule in the disease outcome in face of the different parasite inoculums.

2. Materials and methods

2.1. Animals

C57BL/6 wild type (WT) mice weighing 20–25 g were obtained from the animal facilities of the Centro Multidisciplinar para Investigação Biológica (CEMIB), Universidade Estadual de Campinas, SP, Brazil. C57BL/6 MHC II^{-/-} mice, lacking C₂TA transactivation molecule (B6.129 S2-CD4^{tm1mark}) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and maintained in the Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil. All animals used for the experiments were male and aged 8–10 weeks old. Experimental procedures were approved by the Animal Ethics Committee of the Universidade Federal de Uberlândia, Uberlândia, State of Minas Gerais, Brazil, under protocol number 004–2008.

2.2. Parasites and infection

S. venezuelensis strain was isolated from the wild rodent *Bolomys lasiurus* in April 1986 and since then it has been maintained

in the Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas, Brazil, by serial passages in Wistar rats (*Rattus norvegicus*). *S. venezuelensis* third-stage infective larvae (L3) were obtained from charcoal cultures of infected rat feces. The cultures were incubated at 28 °C for 72 h, and the infective larvae were collected and concentrated using a Baermann apparatus. The recovered larvae were then counted and the number was subsequently adjusted to 500 or 3000 L3. It was used six mice per group in three different moments (0, 8 and 13 days) with two infective doses (500 and 3000 L3), totaling 36 mice. As each experiment was repeated twice we used a final number of 72 C57BL/6 WT mice. The same number of animals was used for C57BL/6 MHC II^{-/-}. The C57BL/6 WT and MHC II^{-/-} mice were individually inoculated by subcutaneous (s.c.) injection with 500 or 3000 *S. venezuelensis* L3. Uninfected mice were used as controls (designated as day 0). All of the experiments in this study were repeated twice.

2.3. Egg and adult worm counts

Eggs per gram of feces were counted according to Machado et al. (2005) on days 8 and 13 post-infection (p.i.). The parasitological analysis was performed three times, and the average of the three results was recorded. At the time points described above, six mice per group were placed individually on clean, moist absorbent paper and allowed to defecate in order to allow the recovery feces. On days 8 and 13 p.i. mice were injected with anesthetics Ketamine (Syntec Brasil Ltda, SP, Brazil)/Xylazine (Schering-plough Coopers, SP, Brazil) by intraperitoneal route and blood samples were collected by retroorbital puncture. The serum samples obtained was stored at –20 °C until use. After euthanasia, the upper half of the small intestine from each infected mouse was removed, washed, cut open longitudinally, placed on Petri dishes containing saline (NaCl) at 0.85%, and incubated for 2 h at 37 °C. The worms were counted under light stereomicroscopy and worm fecundity was estimated by dividing the number of eggs eliminated in feces by the number of worms recovered from the intestine of each mouse at each time point. Infectivity rate was determined by assessing fecal eggs counted and numbers of adult worms recovered from the small intestine from six mice per group on days 8 and 13 p.i.

2.4. Alkaline parasite extracts preparation

S. venezuelensis alkaline extracts were prepared according to the protocol previously described (Machado et al., 2003). In brief, 1 ml of 0.15 M NaOH was added to ~300,000 *S. venezuelensis* L3 larvae, which were maintained under gentle shaking for 6 h at 4 °C. Subsequently 0.3 M HCl was added until reach pH 7.0. This preparation was then centrifuged at 12,400 x g for 30 min at 4 °C. Protein content of the supernatant was determined by the Lowry method (Lowry et al., 1951). The antigenic extract was used for enzyme-linked immunosorbent assay (ELISA).

2.5. ELISA for measurement of specific IgG1 and IgE antibodies in serum samples

High-binding microtiter plates (Corning-Costar, Laboratory Sciences Company, NY, USA) were coated with 10 µg/ml of *S. venezuelensis* alkaline extract in 0.06 M carbonate buffer (pH 9.6) for 18 h at 4 °C. After three washes in phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T), nonspecific binding sites were blocked with PBS-T plus 1% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO, USA) (PBS-T-BSA) for 1 h at 37 °C. Diluted serum samples (1/10 in PBS-T-BSA, 50 µl/well) were used for IgG1 and IgE quantification. After incubation for 1 h at 37 °C, plates were washed and peroxidase goat anti-mouse IgG1 (1/

4000; Santa Cruz Biotechnology, California, USA) conjugates were added to the plates and incubated for 2 h at 37 °C. For IgE detection, biotinylated goat anti-mouse IgE (1/250; Caltag Lab, San Francisco, CA, USA) was added and incubated for 2 h at 37 °C, followed by streptavidin-peroxidase conjugate (Sigma) (1/1000), for 30 min at 37 °C. The reaction was developed with 0.03% H₂O₂ in 0.1 M citrate–phosphate buffer (pH 4.5) containing 3,3',5,5'-tetramethylbenzidine (Sigma). For developing IgG1 reaction, the enzyme substrate consisting of 0.05% H₂O₂ in 0.1 M citrate–phosphate buffer (pH 5.0) containing 0.4 mg/ml of o-phenylenediamine (Sigma–Aldrich Co., Deisenhofen, Germany) was added to the plates. Absorbance was read at 450 (IgE) or 492 nm (IgG1) in a plate reader (Titertek Multiskan, Flow Laboratories, McLean, VA, USA). The results were expressed as ELISA index (EI), as follows: EI = absorbance of test sample/cut-off, where cut-off was the mean absorbance of three negative sera plus three standard deviations according to Ferro et al. (2002). Serum samples from non-infected mice were used as control. Values of EI > 1.0 were considered positive.

2.6. Experimental procedure and histological analysis

The small intestine samples were collected from other experimental groups, fixed in 10% buffered formalin and processed for paraffin embedding. The whole small intestine was cut into four pieces (duodenum, proximal jejunum, distal jejunum and ileum) and rolled on itself to make a “Swiss roll”. Tissue sections (4 µm) were stained with hematoxylin and eosin. The entire length of the small intestine, (the four pieces as described above) was examined histologically and the inflammatory infiltrate was graded according to leukocyte infiltration in the lamina propria (LP), epithelium and submucosa as previously described (Rodrigues et al., 2009). The inflammatory score was represented by arbitrary units: 0–2, mild; 2–4, moderate; 4–6, severe; above 6, very severe, as previously described (Rodrigues et al., 2009).

2.7. Cytokine quantification

Cytokines were quantified in homogenates prepared from duodena. Gut samples were obtained by homogenizing 100 mg of the lower upper part of the duodena from WT or MHC II^{−/−} mice collected on days 8 and 13 p.i. in 500 µl of PBS added by 0.05% Tween 20 containing protease inhibitors (1.6 mM phenylmethylsulfonyl fluoride, 100 µg/ml leupeptin hemisulfate, 10 µg/ml aprotinin, 1 mM benzamidinium hydrochloride hydrate, 10 mM EDTA). Each sample was homogenized and then centrifuged for 10 min at 3000 × g, and the supernatant was used for the assays. Concentration of IL-4, IL-5, IL-12, IFN-γ, IL-17, IL-10 and TGF-β were determined by ELISA using commercially available kits (DuoSet, R&D Systems, Minneapolis, USA), according to manufacturer's instructions.

2.8. Statistical analysis

Data were reported as mean ± Standard Error of Mean (SEM) and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test. Student's *t* test was used in the analysis of parasite and egg number. Values of *p* < 0.05 were considered statistically significant.

3. Results

3.1. Influence of 500 or 3000 L3 parasite loads in the number of eggs in the feces and female worm fecundity in mice infected by *S. venezuelensis*

To verify if different parasite loads could influence the helminthiasis outcome, the number of eggs in feces, adult worms recovered

from the small intestine and fecundity rate were evaluated at days 8 and 13 p.i. in mice infected with 500 or 3000 L3 (Fig. 1). The results demonstrated an augmented number of eggs at day 8 p.i. recovered from the feces of WT mice infected with 3000 L3 when compared to WT infected with 500 L3. At 13 days p.i. a significant reduction in the number of eggs was observed in these animals despite the parasite load during infection (Fig. 1A). In addition, mice deficient for MHC II infected with 3000 L3 larvae had a huge augmentation in the number of eggs in the feces at day 8 p.i. when compared with MHC II^{−/−} infected with 500 L3 or WT mice infected with 500 or 3000 L3 (Fig. 1A). Furthermore, eggs were recovered from feces of knockout mice infected with 3000 L3 on day 13 p.i. The number of *S. venezuelensis* eggs eliminated in both infections (500 or 3000 L3) reflected the kinetics of the presence of adult worms in the intestine (Fig. 1A and B) and a significant increase was found in the number of adult worms recovered from the small intestine of WT infected mice with 3000 L3 when compared with WT infected with 500 L3 at 8 days p.i. (Fig. 1B). *S. venezuelensis* infection was almost completely eliminated at 13 days p.i. in WT infected groups. Even though there was no difference in the number of adult worms in the gut on day 8 between WT and MHC II^{−/−} mice, we observed a delay in the worm elimination in the absence of MHC II molecules, in special in those animals infected with 3000 L3 (Fig. 1B). These results could be related to the increased fecundity rate detected in the high-inoculums infected MHC II^{−/−} mice on days 8 and 13 p.i. (Fig. 1C).

3.2. Different parasite inoculum does not influence MHC II dependent humoral response to *S. venezuelensis*

We compared the parasite-specific IgG1 and IgE antibodies among the infected groups and controls. We found that, as expected, mice infected with *S. venezuelensis* developed IgG1 and IgE responses to the parasite, especially when infected with the higher inoculum (3000 L3) (Fig. 2A and B). Mice deficient for MHC II molecules behave similarly to the WT low-inoculum infected group and could not respond to the infection by producing elevated levels of these antibodies, independently on the parasite load during infection (Fig. 2A and B).

3.3. Cytokine production in the intestine of mice infected with different parasite inoculums of *S. venezuelensis*

To verify whether the presence of adult worms in the intestine and thus the intensity of infection induced by different parasite loads were also associated to the local immune response in the gut, we next quantified the cytokines produced in the duodena during infection. WT mice infected with 500 and 3000 L3 presented the greatest increase in IL-4 and IL-5 at day 8 p.i. when compared to the other groups (Fig. 3A and B), although the levels of IL-5 were significantly higher in WT mice infected with 500 L3 when compared to those infected with 3000 L3. We found that production of these cytokines in the duodena was dependent on MHC II and in this case was not altered by different parasite inoculums (Fig. 3A and B). Moreover, at day 8 p.i. IL-12 levels were significantly elevated in WT mice infected with 3000 L3 when compared with WT mice infected with 500 L3, whereas the production of this cytokine was abrogated in the absence of MHC II molecules, independently on the parasite inoculum in the initial infection (Fig. 3C). In addition, the IFN-γ concentration was also significantly increased in WT mice infected with 3000L3 when compared with mice infected with 500 or 3000 L3 in the absence of MHC II at day 8 p.i. (Fig. 3D). The infection with the parasite induced IL-17 production and it was observed a trend towards an increase in the levels of this cytokine at 8 days p.i. in MHC II^{−/−} mice infected with 500 or 3000 L3 as well as in MHC II^{−/−} mice on day 13 p.i. with

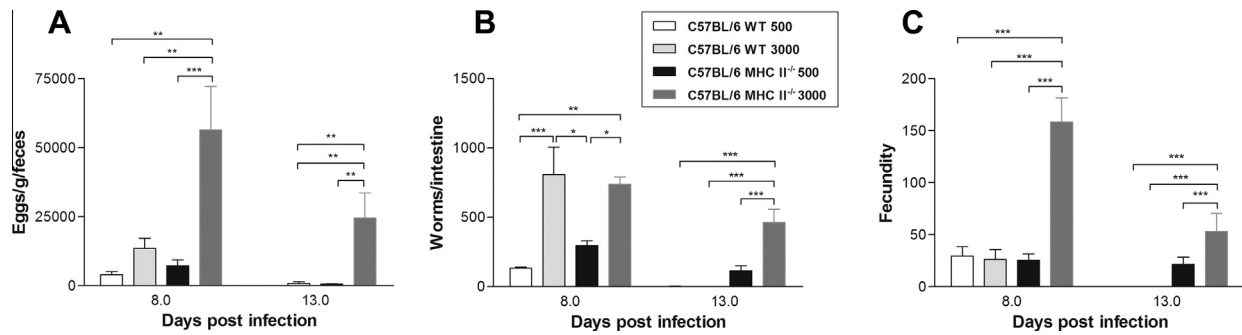


Fig. 1. Parasite burden in C57BL/6 wide type (WT) and C57BL/6 MHC class II (MHC II^{-/-}) mice after subcutaneous (s.c.) infection with 500 or 3000 *Strongyloides venezuelensis* L3 larvae. Number of eggs/g of feces eliminated in feces (the average of the three results was recorded) (A), adult worms recovered from the small intestine (B), and the fecundity rate (C) were evaluated at 8 and 13 days post-infection. Data are expressed as mean $\pm$ SEM ($n = 6$). Results are representative of the average of two independent experiments. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

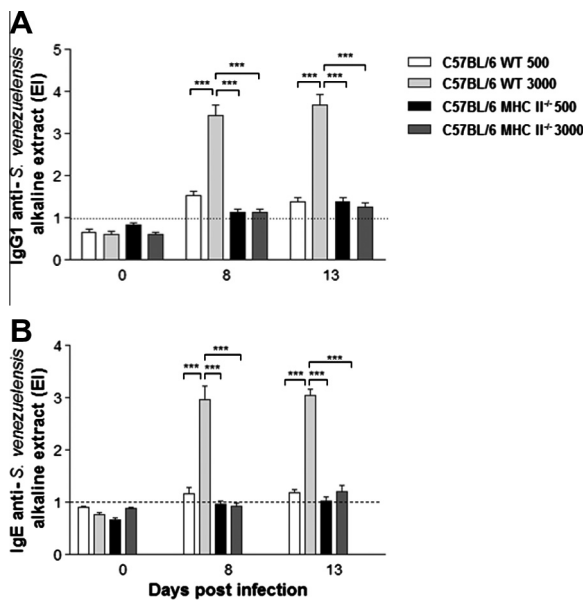


Fig. 2. Levels of specific IgG1 (A) and IgE (B) in serum samples of C57BL/6 wide type (WT) and C57BL/6 MHC class II (MHC II^{-/-}) mice, were measured using alkaline extract of *S. venezuelensis* after subcutaneous (s.c.) infection with 500 or 3000 *Strongyloides venezuelensis* L3 larvae. The results are expressed as ELISA index (EI) (values of EI > 1.0 are considered positive, horizontal dashed line) and showed as mean $\pm$ SEM ($n = 6$). Results are representative of two independent experiments. ** $p < 0.001$; *** $p < 0.0001$.

500 L3 (Fig. 3E) when compared to C57BL/6 WT infected mice. In addition, it was verified an increase in IL-10 levels on day 8 p.i. irrespective of the parasite load utilized to infect animals (Fig. 3F). Also, the results showed that on day 13 p.i. there was a significant decrease in IL-10 production in the gut of WT and MHC II^{-/-} mice infected with 3000 L3 compared with the other groups infected with 500 L3, suggesting that IL-10 production is dependent on the parasite inoculum but independent on the presence of MHC II molecules (Fig. 3F). Moreover, we observed a significant increase in TGF- β in the gut of WT and MHC II^{-/-} mice on day 8 p.i. and in the gut of MHC II^{-/-} mice infected with 3000 L3 compared with the other groups on day 13 p.i. suggesting that the production of this cytokine is constrained in the presence of lower parasite inoculums or MHC molecules at this time point (Fig. 3G).

3.4. The inflammatory changes in the small intestine are related to *S. venezuelensis* infection in spite of the parasite inoculum used to infect mice

The infection of WT mice with 500 or 3000 larvae induced a similar inflammatory infiltration in the lamina propria (LP) and

submucosa of the small intestine at day 8 and 13 p.i. which was constituted by polymorphonuclear eosinophils and mononuclear cells (Fig. 4A–D). The inflammatory lesions were localized mainly in the duodena. The lesions were associated with elevated number of worms in the mucosa besides the inflammatory reaction and the presence of oedema in LP at the end of the villi. The infection of MHC II^{-/-} mice with *S. venezuelensis* induced slight inflammatory alterations in the small intestine, and these lesions were lower in MHC II^{-/-} compared to WT animals at days 8 and 13 p.i. irrespective of the parasite inoculum utilized to infect animals, 500 or 3000 larvae (Fig. 4E).

4. Discussion

The aim of this study was to verify whether intensity of infection induced by different inoculums could influence or be influenced by the host immune competence in the control of *S. venezuelensis* infection. It was hypothesized that in infection settings with different larvae doses there would be enhanced survival and development of this parasite in mice lacking MHC class II molecules. It is known that both in human hosts and experimental models the infection with *Strongyloides* sp is characterized by tissue eosinophilia (Korenaga et al., 1991; El-Malky et al., 2003) and intestinal mastocytosis (Abe and Nawa, 1988; Nawa et al., 1994; Kobayashi et al., 1998), with production of Th2-type antibodies (Abe and Nawa, 1988; Machado et al., 2005; Rodrigues et al., 2007; Gonçalves et al., 2012b) and cytokines (Maruyama et al., 2000). Here we demonstrated that MHC II^{-/-} mice infected with 3000 L3 exhibited a higher number of eggs recovered from the feces and delayed elimination of adults worms besides increased worm fecundity compared with the other groups, suggesting that *S. venezuelensis* migration, expulsion or intestinal establishment are affected not only by the host immune competence but also by the amount of parasites to which mice are exposed during infection.

The role of MHC class II in the induction of the immune response against parasites was previously demonstrated in studies with other experimental nematode infections (Abe and Nawa, 1988; Mawhorter et al., 1993; Geiger et al., 2003; Holland et al., 2005). Our results pointed to the production of parasite specific antibodies as important effector mechanisms to induce an effective control of worms in the WT mice infected with 3000 L3. On the other hand, in WT animals infected with 500 L3, which were able to efficiently eliminate the parasite, the production of Th2 specific antibodies was low and similar to that found in MHC II^{-/-} mice; however, the infection in WT mice was controlled in contrast to the absence of complete parasite elimination in knockout mice, suggesting that other immune response mechanisms may be

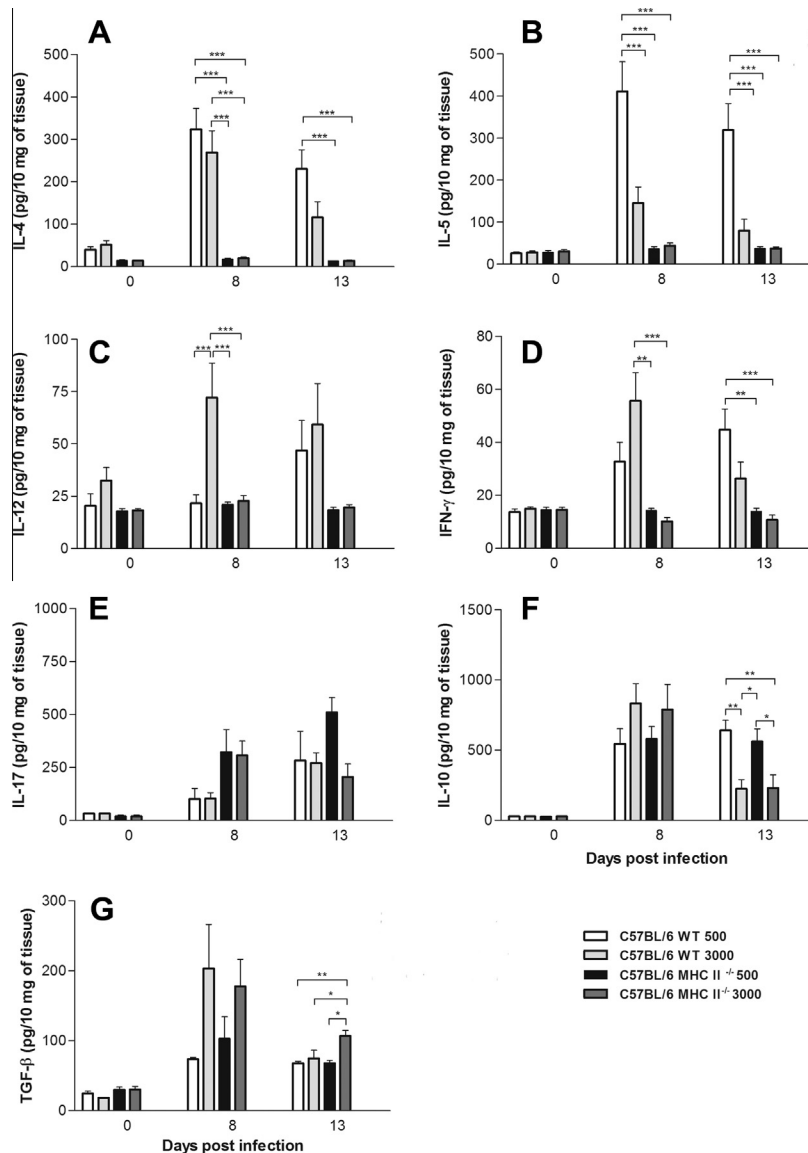


Fig. 3. Levels of IL-4 (A), IL-5 (B), IL-12 (C), IFN- γ (D), IL-17 (E), IL-10 (F) and TGF- β (G) determined by ELISA in small intestine homogenates of C57BL/6 wide type (WT) and C57BL/6 MHC class II (MHC II^{-/-}) mice, after subcutaneous (s.c.) infection with 500 or 3000 *Strongyloides venezuelensis* L3 larvae. Analyses were performed at 8 and 13 days post infection. Data are expressed as mean $\pm$ SEM (n = 6). Results are representative of two independent experiments. *p < 0.05; **p < 0.01; ***p < 0.0001.

responsible for *Strongyloides* control in WT animals infected with low parasite load. Nevertheless, further studies are still necessary to better clarify these statements. As expected, the production of Th2 specific antibodies was similar in either 500 or 3000 L3 infected animals in the absence of MHC II molecules, showing that in these animals the lack of polarization to type-2 response could be contributing to the impediment of the adult worm elimination. Moreover the Th2 data are in accordance with results of previous studies showing the participation of IgG1 and IgE in the immune response to *S. venezuelensis* infection (Machado et al., 2003; Fernandes et al., 2008; Rodrigues et al., 2009).

We also investigated the cytokine production induced by the different parasite burden. It was observed a tendency to decreasing IL-4 and IL-5 production in the gut of higher parasite inoculated WT mice, indicating that other local mediators or systemically produced cytokines could be involved in the augmented humoral Th2 response, such as specific IgG1 and IgE, in these animals. In addition to presenting lower levels of Th2 cytokines, WT mice infected with 3000 L3 produced increased amounts of the Th1 related cyto-

kines IL-12 and IFN- γ on day 8 p.i. which could be related to the elevated number of worms recovered from their gut at day 8 p.i. In this study we also observed that WT mice inoculated with higher parasite inoculums presented a mixed cytokine profile, although it was found that these animals have a predominant Th2 immune response, suggesting that increased IFN- γ could influence infection control. In the present study we observed that TGF- β concentration in the gut of the MHC II^{-/-} infected mice with 3000 L3 is elevated on 13 day p.i. and the reasons for such an increase are still not known. It was also demonstrated that at later periods (13 days p.i.), WT or MHC II^{-/-} 3000 L3 infected mice presented reduced levels of IL-10. When analyzing the cytokine production in mice infected with 500 L3, it was observed that WT mice had greater production of Th2 cytokines, IL-4 and IL-5, at days 8 and 13 day p.i. when compared with the other animals. It was also observed elevated levels of IL-10 in WT or MHC II^{-/-} mice infected with 500 L3 on day 13 p.i. suggesting that this cytokine production could be favored in lesser infected mice. Previous study demonstrated the role of IL-10 in the regulation of the production and activity

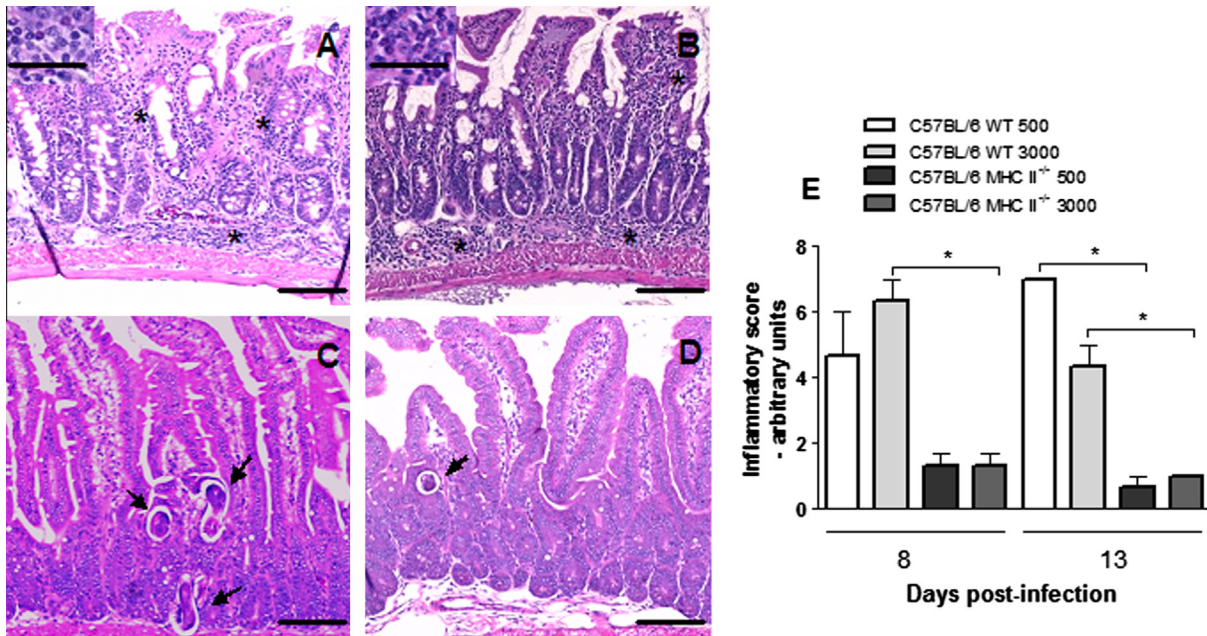


Fig. 4. Photomicrographs of hematoxylin and eosin staining and inflammatory score of the small intestine of C57BL/6 wild type (WT) and C57BL/6 MHC class II (MHC II^{-/-}) mice infected with 500 or 3000 *Strongyloides venezuelensis* L3 larvae. The asterisks show the infiltration of inflammatory cells in the LP and submucosa of the duodena of C57BL/6 WT mice on day 8 (A) and 13 (B) of infection with 500 L3 and the insert show the polymorphonuclear eosinophils. The arrows show worms in the duodena of C57BL/6 MHC II^{-/-} mice on days 8 (C) and 13 (D) of infection with 500 L3. Inflammatory score on days 8 and 13 p.i. was represented as arbitrary units (A.U.): 0–2, mild; 2–4, moderate; 4–6, severe; above 6, very severe (E). Data are expressed as mean $\pm$ SEM ($n = 6$). Results are representative of two independent experiments. Bar scale, 100 μ m and insert 50 μ m. The brackets and the symbol * $p < 0.05$ indicate the statistical significance between the groups.

of IL-4 during *S. venezuelensis* infection (Ferreira et al., 2007). On the other hand, in *S. ratti* infected mice the suppression of IFN- γ was correlated to IL-10 production (Eschbach et al., 2010). Interestingly was the fact that in infected animals, detectable levels of IL-17 were verified. In MHC II^{-/-} infected mice with 500 L3 there was a tendency of higher levels of IL-17 at 13 days p.i. when compared to the others groups; however, the role of IL-17 cytokine production in the helminth infections is not totally understood yet. It is known that IL-17 induces neutrophilia, participate in the response against certain bacterial and fungal infection and is involved in the pathogenesis of multiple autoimmune diseases (Bonne-Annee et al., 2011). Mice deficient in the receptor for IL-17A and IL-17F, upstream mediators of CXCR2 ligand production, infected with *S. stercoralis* larvae presented no difference in larval survival, neutrophil recruitment, or production of CXCR2 ligands compared with wild type mice (O'Connell et al., 2011). In contrast, although C57BL/6 WT mice presented lower IL-17 levels and were more efficient in the worm elimination in our study, it seemed that in the absence of MHC II molecules the cytokine IL-17 could be a critical mediator for *S. venezuelensis* control. Indeed, although not statistically significant, our findings pointed to lower IL-17 production in the gut of 3000 L3 MHC II^{-/-} infected mice on day 13 p.i. when compared with the 500 L3 inoculum, suggesting that the delay in worm elimination by the 3000 L3 MHC II^{-/-} group could be related to the reduced levels of IL-17. It is of note that there was an overall reduced level of cytokines in this group, mainly on day 13 p.i. demonstrating that these animals presented a global defect in cytokine production, with consequent failure in mounting effective immune responses. Furthermore, the high TGF- β production could also account for this defective effector response resulting in increased parasitism and a delayed elimination of adult worms. Nevertheless, further studies are still necessary to explain the role of IL-17 in the strongyloidiasis infection.

The histopathology and the analyzes of the inflammatory score showed localized lesions mainly in the duodena of both WT mice

infected with 500 or 3000 L3, in contrast to the slight inflammatory alterations in the small intestine of MHC II^{-/-} animals. Although 3000 L3 MHC II^{-/-} infected mice seemed to be more susceptible to *S. venezuelensis* infection, our results did not point to increased inflammatory infiltration in this experimental group. On the contrary, the data suggested that the lack of MHC class II molecules might have affected the immune response necessary to the adult worm elimination. Previous studies have demonstrated that the MHC II molecule activation drives specific Th2 cytokine production in helminth infection (Mawhorter et al., 1993; Fowell et al., 1997; Rodrigues et al., 2009), inducing the differentiation and activation of basophils, mast cells and eosinophils (Miter-mitre et al., 2004; Thienemann et al., 2004), as well as stimulating B-cells to produce specific IgG1 and IgE (Finkelman et al., 1997; Faquim-Mauro et al., 1999), required to expel gastrointestinal nematodes (Machado et al., 2005; Negrão-Correa et al., 2006). Altogether, our results indicated that *S. venezuelensis* infection induced a strong Th2 response that lead to the elimination of this parasite from the small intestine in immunocompetent hosts. Finally, we concluded that the MHC class II molecules are essential for a cascade of events during the immune response induced for the elimination of adult worm, especially when infection occurs with high parasite inoculum.

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Conflict of interest

The authors declare that there is no conflict of interest.

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