

# Histopathologic aspects of experimental cysticercosis and *in situ* cytokines profile in C57BL/6 mice

Aspectos histopatológicos da cisticercose experimental e perfil de citocinas *in situ* em camundongos C57BL/6

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## ABSTRACT

Neurocysticercosis (NCC) is one of the parasitic infections that most affects the central nervous system. The knowledge regarding its immunopathogenesis and pathophysiology needs broadening. *Taenia crassiceps* cysticerci are used as the NCC experimental model. The aim of this work was to describe the general pathological processes and the *in situ* cytokine profile in C57BL/6 mice inoculated intracranially with viable *T. crassiceps* cysticerci. The histopathology analysis showed cysticerci in the extraparenchymal and intraventricular region, mononuclear inflammatory infiltration surrounding the parasite, microgliosis and meningitis. The analysis of the *in situ* immune profiles showed a predominance of the Th2 response. The IL-4 and IL-10 dosages were significantly increased in the infected group. The decrease in the INF- $\gamma$  dosage reflects the immunomodulation from the cysticerci. In conclusion, a *T. crassiceps* NCC infection in C57BL/6 mice triggers an inflammatory response, a predominance of Th2 type *in situ* profile, with mononuclear inflammatory cell infiltration, meningitis and microgliosis.

**Keywords:** neurocysticercosis; neglected diseases; Taenia.

## RESUMO

Neurocisticercose (NCC) é uma das doenças parasitárias que mais afeta o sistema nervoso central. É necessário aprofundar o conhecimento em relação à sua imunopatogênese e patofisiologia. Os cisticercos de *Taenia crassiceps* são utilizados como modelo experimental para estudos da NCC. O objetivo deste trabalho foi descrever os processos patológicos gerais e o perfil de citocinas *in situ* em camundongos C57BL/6 inoculados via intracerebral com cisticercos viáveis de *T. crassiceps*. A análise histopatológica demonstrou cisticercos nas regiões extra-parenquimatosa e intraventricular, infiltrado inflamatório de células mononucleares ao redor do parasita, microgliose e meningite. A análise *in situ* do perfil de citocinas mostrou uma predominância da resposta Th2. As dosagens de IL-4 e IL-10 foram significativamente maiores no grupo infectado. Conclui-se que a NCC por *T. crassiceps* em camundongos C57BL/6 induz uma resposta inflamatória com predominância *in situ* de citocinas do perfil Th2, com infiltrado inflamatório de células mononucleares, meningite e microgliose.

**Palavras-chave:** Neurocisticercose; doenças negligenciadas; Taenia.

Taeniasis is the infection by the adult worm of the *Taenia* spp., while cysticercosis is the infection with the larval form of the parasite. Human cysticercosis is caused by the presence of *T. solium* cysticerci<sup>1</sup>. The World Health Organization considers both diseases to have been neglected<sup>1,2</sup>. The taeniasis/cysticercosis complex is a prominent cause of chronic debilitating illness, morbidity and mortality<sup>3</sup>, with worldwide prevalence, especially in rural communities of developing countries<sup>2</sup>.

Neurocysticercosis (NCC) is the most frequent parasitic infection of the central nervous system (CNS) and the main preventable cause of acquired epilepsy<sup>4</sup>. Up to 50% of the NCC cases are asymptomatic. The symptoms vary from seizures, headaches, focal neurologic deficits, cognitive decline and intracranial hypertension. The clinical manifestations depend on factors related to the parasite, such as size, location, and development stage, or factors related to the host, such as the immune response<sup>4,5,6</sup>. The great variety of clinical

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forms are related to the modulation of the immune profile due to the presence of viable cysticerci in the brain (asymptomatic NCC), or to an intense inflammatory response (symptomatic NCC)<sup>7,8,9</sup>.

Usually, when an organism is exposed to a pathogen it generates an immunologic response aimed at the pathogen's destruction. The protective or pathogenic efficacy of this innate immune response greatly depends on the generation of unspecific inflammatory phenomena in the surroundings of the pathogen. The adaptive immune response relies on the selective clonal proliferation of systemic lymphoid cells followed by a differentiation of effective Th1, Th2, Treg and Th17 cells, among other profiles that induce cytokine or antibody production<sup>5,6,7,8,9</sup>.

The presence of the viable *T. solium* cysticercus in the brain tissue of humans triggers the increase of local inflammatory cells through the main pathways of leukocyte infiltrations, which are the parenchymal vessels, subarachnoid space and choroid space<sup>10</sup>. Within the CNS, the parasite may install itself in the brain parenchyma, subarachnoid space, ventricular system<sup>11</sup> and, less frequently, it can be found in the brainstem<sup>12</sup> and in the spinal cord<sup>13</sup>.

One of the experimental models used to study *T. solium* cysticercosis is *T. crassiceps* due to their epitope similarity<sup>14</sup>. The immune response in mice infected with *T. crassiceps* is not well described in NCC models. However, Moura et al.<sup>15</sup>, studying an intraventricular experimental NCC model, reported that BALB/c mice presented with a predominance of a systemic mixed Th1/Th17 immune profile in the initial phases of the infection, while this gradually modified into a Th2 profile through the course of the infection.

Currently, there are five different experimental models used for NCC studies. The first one developed by Cardona et al.<sup>7</sup>, used BALB/c female mice infected with *Mesocestoides corti*. Subsequently, our group found that the intracranial inoculation of *T. crassiceps* cysticerci in both BALB/c and C57BL/6 mice lineages showed the susceptibility and resistance of different lineages<sup>16</sup>. Other examples are the inoculation of active *T. solium* oncospheres in rats<sup>17</sup> and the inoculation of active *T. solium* oncospheres in the subarachnoid space of young swine<sup>18</sup>. The most recent model described simulated the extraparenchymal racemose NCC in rats through the inoculation of *T. crassiceps* cysticerci<sup>19</sup>.

It is important to highlight that different mice lineages produce different inflammatory responses with different cellular and cytokine profiles<sup>20</sup>. The subcutaneous inoculation of *T. crassiceps* cysticerci allowed analysis of the kinetics of the infection in susceptible (BALB/c) and resistant (C57BL/6) mice lineages, showing a greater parasitic burden in the susceptible mice<sup>21</sup>.

Other studies of NCC models<sup>16</sup> and in a subcutaneous model<sup>22</sup> showed that BALB/c mice are less efficient in the precocious destruction of the parasite, allowing its proliferation with greater inflammatory intensity in the acute

phase of the infection, with a predominance of polymorphonuclear cells and macrophage infiltration. The C57BL/6 mice lineage is capable of inducing the parasite death with greater inflammatory intensity in the late phase of the infection with less tissue injury and a predominance of infiltration of mononuclear inflammatory cells. These findings confirmed the resistance profile of the C57BL/6 mice lineage in both experimental models.

To date, there is little description of the brain lesions found in the resistant mice lineage (C57BL/6) in experimental NCC, and no reports on the local immune response in this kind of infection. Therefore, the aim of this study was to describe the brain injuries and the *in situ* immune response in C57BL/6 mice inoculated intracranially with *T. crassiceps* cysticerci.

## METHODS

### Parasite maintenance

This study was approved by the Ethics Committee in Animal Use of the Federal University of Goiás, protocol number 065/2015. The maintenance of the *T. crassiceps* ORF strain was performed through successive intraperitoneal passages every 90 days in BALB/c female mice aged 8–12 weeks old<sup>14</sup>. The parasite is maintained in the animal facilities of the Tropical Pathology and Public Health Institute of the Federal University of Goiás.

### Infection and euthanasia of the mice

Wild type C57BL/6 female mice aged 8–12 weeks old, weighing between 20–30 grams were used. They were divided into a control (CT) group and an infected (INF) group. Mice from the INF group were weighed, intraperitoneally anesthetized with ketamine (100 mg/mL) and xylazine (20 mg/mL) in a ratio of 0.1 mL/10g. Afterwards, the animals were inoculated intracerebrally as described previously<sup>16</sup>. The CT group (n = 9) received an inoculation of physiologic solution (NaCl 0.9%) in a similar volume to the INF group (n = 15).

Ninety days after the inoculation, all the mice were euthanized and had their brains removed.

### Histopathology analysis

The histopathology analysis was performed in fragments of the brain tissue using a matrix for histological slices (Insight<sup>®</sup>), fixed in a solution of 4% paraformaldehyde and 70% alcohol, dehydrated with alcohol, diaphonized in xylol, and embedded in paraffin. The blocks were cut into 4 µm width slices. The fragments were captured with glass slides and stained with hematoxylin-eosin<sup>15,16</sup>.

The general pathologic processes were described and classified in a semi-quantitative analysis as follows: absent when there was no compromise of the host tissue,

score = 0; discrete with up to 25% of area commitment, score = 1; moderate from 26% to 50% of area commitment, score = 2; and accentuated with more than 50% of area commitment, score = 3<sup>15,16</sup>.

### Immunoenzymatic assay

After the euthanasia, a fragment of the brain tissue was frozen at -80°C for the enzyme-linked immunosorbent assay (ELISA) analysis. After this it was sonicated with a solution of Tris HCl, NP40 1% (SIGMA) and protease inhibitor<sup>23</sup>.

The interleukin (IL)-4, IFN-gamma and IL-10 cytokines were quantified in the brain tissue homogenate solution through sandwich ELISA according to the manufacturer's instructions (BD OptEIA™). The IL-4 and IL-10 dosages were performed with *Mouse* IL-4 ELISA (Lot: 26389) and *Mouse* IL-10 ELISA (Lot: 20169) BD OptEIA™ Sets (BD Biosciences, San Diego, CA). The IFN-gamma dosage was performed with ELISA high binding microplates sensitized with 80 µL of IFN-gamma monoclonal antibody (5µg/mL of clone XMG 1.2 in PBS). The optic density was detected through the Thermo/Labsystems microplate reader, using specific filters for each cytokine<sup>23</sup>.

### Statistical analysis

The statistical analysis of the histopathology was performed using the Sigma Stat 2.3 software. The Mann-Whitney test was used, followed by the Bonferroni post-test. Differences were considered significant when  $p < 0.05$ .

The statistical analysis of the *in situ* cytokine dosage was performed with the GraphPadPrism 5.0 software, and after the establishment of distribution and variance of the samples, the T-test was used. Differences were considered significant when  $p < 0.05$ .

## RESULTS

The histopathology analysis allowed the evaluation of the CNS infection of C57BL/6 mice with viable *T. crassiceps* cysticerci. The general pathologic processes found are described in the Table. The INF group had a greater degree of meningitis, parenchymal edema, mononuclear inflammatory cell infiltration and hemisphere compression ( $p < 0.05$ ) than the CT group (Figures 1 and 2).

The compression of the brain hemispheres with consequent dislodgement of the medial line due to the installation and growth of the cysticerci was observed in the INF group, both in the corticomeningeal region as well as in the lateral ventricle regions (Figure 2). These injuries were not observed in the control group.

The results of the *in situ* cytokine analyses are shown in Figure 3. It is possible to observe a significant increase in the IL-4 and IL-10 dosages in the INF group when compared with

**Table.** General pathologic processes found in C57BL/6 mice inoculated intracranially with viable *Taenia crassiceps* cysticerci.

Pathologic processes	CT group Median (min.-max.)	INF group Median (min.-max.)	p
Meningitis	0 (0-0)	0 (0-1)	0.012*
Parenchymal edema	0 (0-0)	1 (0-3)	0.011*
Hyperemia	0 (0-0)	0 (0-1)	0.176
Perivascularitis	0 (0-0)	0 (0-2)	0.177
Inflammatory infiltration (MN)	0 (0-0)	0 (0-2)	0.008*
Microgliosis	0 (0-0)	0 (0-2)	0.106
Perivascular edema	0 (0-0)	0 (0-1)	0.491
Ventriculomegaly	0 (0-0)	0 (0-3)	0.177
Hippocampus alterations	0 (0-0)	0 (0-3)	0.291
Choroiditis	0 (0-0)	0 (0-0)	1
Ependymitis	0 (0-0)	0 (0-0)	1
Tissue loss	0 (0-0)	0 (0-3)	0.108
Hemorrhage	0 (0-0)	0 (0-0)	1
Hemisphere compression	0 (0-0)	2 (0-3)	0.006*

CT: control group; INF: infected group; MN: mononuclear cells; \*statistically significant differences ( $p < 0.05$ ).

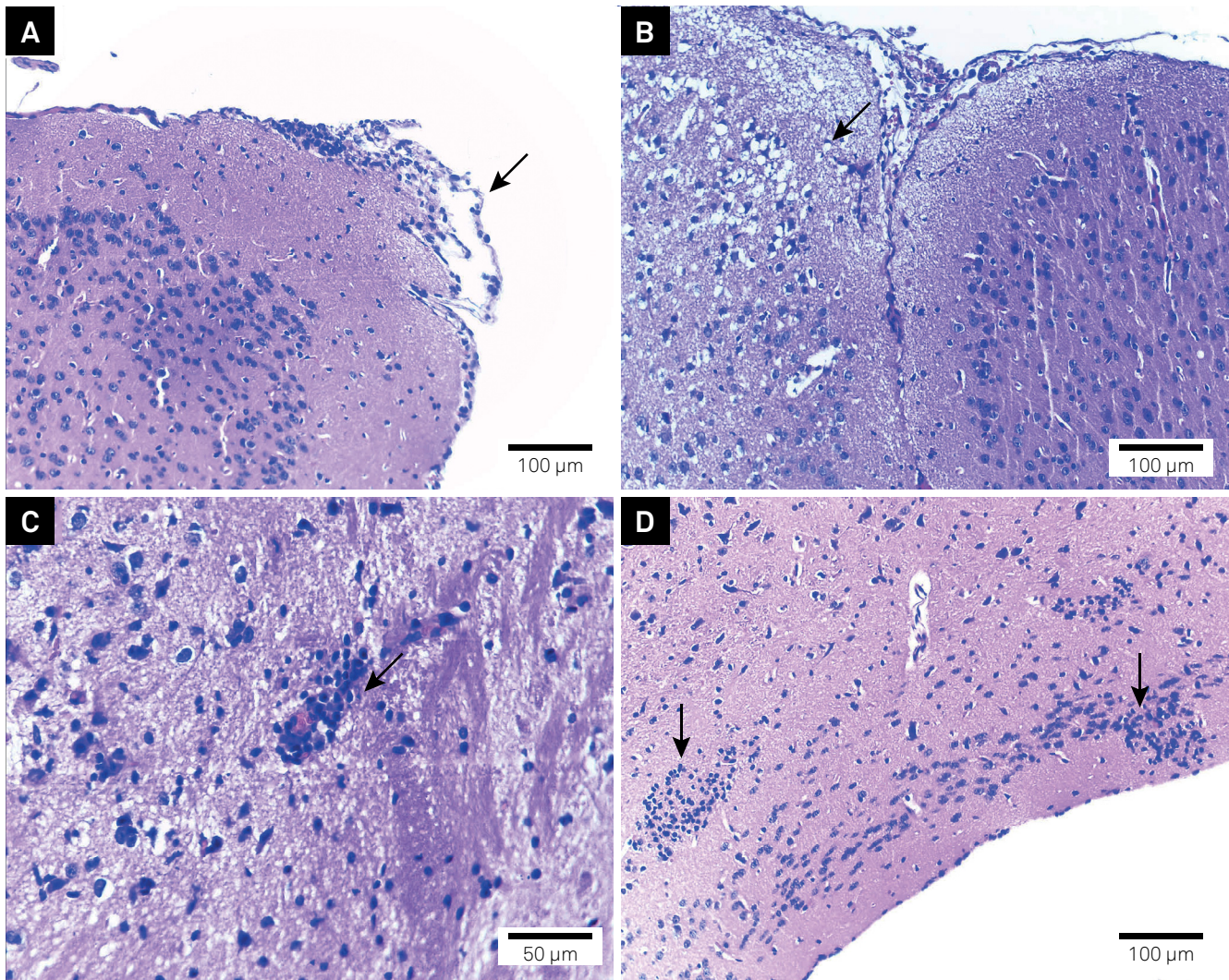
the CT group ( $p < 0.05$ ). There were no differences in the IFN-gamma dosages between the two groups.

## DISCUSSION

This study showed the *in situ* histopathology and immune response of a resistant mice lineage (C57BL/6) given an intracerebral inoculation of *T. crassiceps* cysticerci. The mechanisms in which the brain injuries are induced by the presence of cysticerci depend on a combination of several factors, such as the site of implantation of the parasite and the host immune-inflammatory response<sup>6,11</sup>, which is mediated by cytokines produced by resident and infiltrated cells, which in turn are activated by the cysticerci antigens<sup>4,24</sup>.

The inoculated cysticerci were located in the ventricles or in the extraparenchymal/meningocortical region and triggered a discrete inflammatory response with little mononuclear inflammatory cell infiltration. As well, when the parasite installed in the surroundings of the meninx, it was capable of inducing discrete meningitis. These findings are in accordance with other reports on the location and inflammatory response of the *T. crassiceps* experimental model<sup>7,9,16</sup> as well as the human NCC inflammatory response<sup>5,11</sup>.

The histopathology findings described in this study are in accordance with Matos-Silva et al.<sup>16</sup>, who induced an experimental intraventricular NCC with *T. crassiceps* cysticerci in C57BL/6 mice and described discrete mononuclear inflammatory cell infiltration, parenchymal edema and meningitis. The findings described by Matos-Silva et al.<sup>16</sup> are similar to the those described in this study regarding the lineage, age, weight and sex of the animals and the



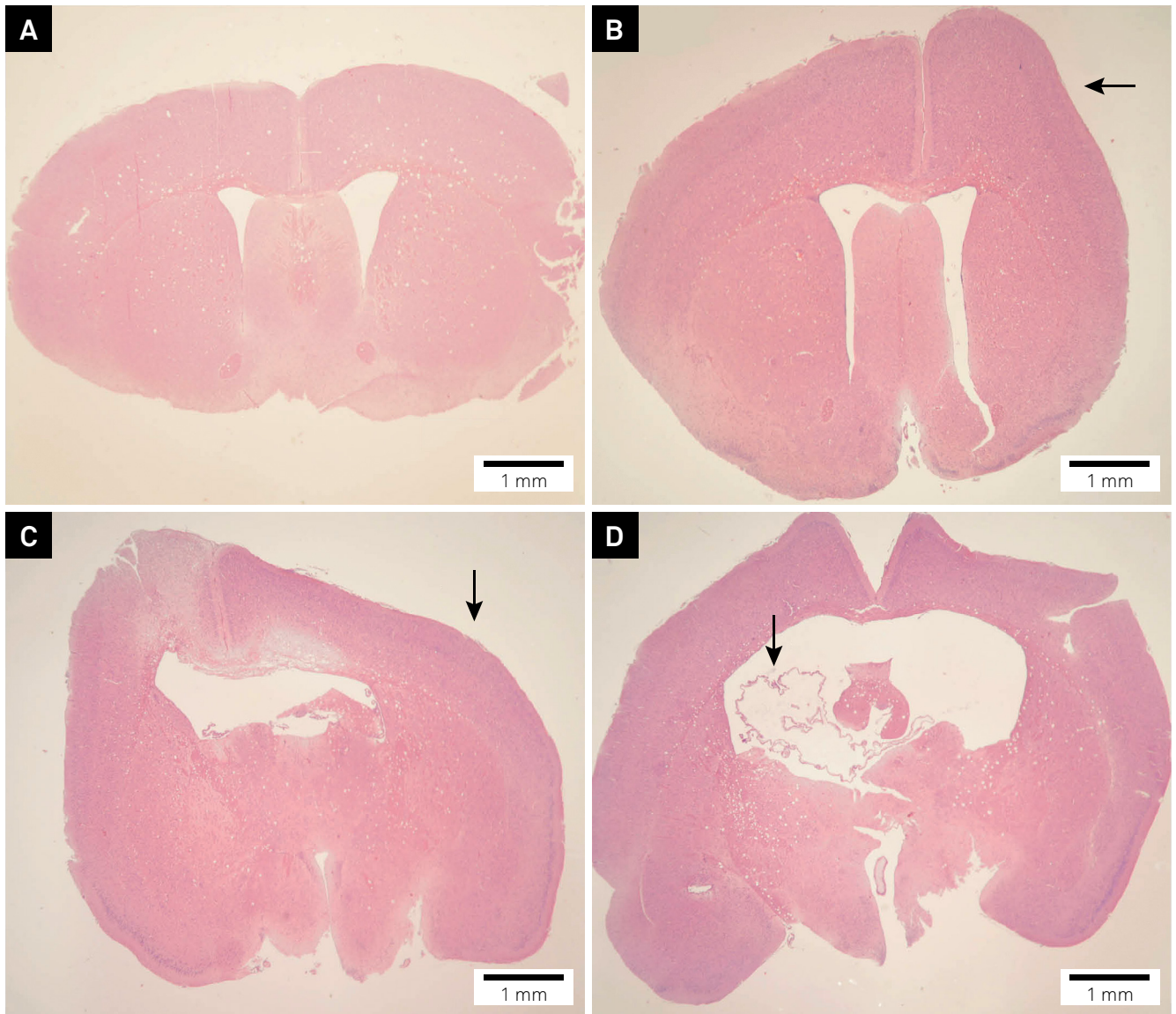
**Figure 1.** Photomicrograph of the general pathologic processes found in C57BL/6 mice inoculated intracranially with viable *Taenia crassiceps* cysticerci after 90 days. A. Discrete meningitis in the infected (INF) group (arrow) (scale = 100 µm); B. Accentuated parenchymal edema in the INF group (arrow) (scale = 100 µm); C. Moderate perivascularitis in the INF group (arrow) (scale = 50 µm); D. Moderate microgliosis in the INF group (arrows) (scale = 100 µm). H&E.

parasite used. However, in our study, the localization of the parasite within the brain was mainly in the meningo-cortical region, differing from the previously-cited study. This difference is important in the description of the host response to the parasite, due to the fact that parenchymal NCC is associated with seizures and focal neurological signs and is the form that presents with greater inflammation when treated<sup>26</sup>. The human extraparenchymal NCC is associated with high mortality rates due to the increase in intracranial pressure<sup>6</sup>.

The hemispheric compression caused by the presence and growth of the cysticerci was accentuated in the mice used in this study. The main consequence of this process is the increase in the intracranial pressure which may lead to obstruction of CSF and edema in different degrees of severity<sup>11,24</sup>. The presence of parenchymal edema in the INF group of this study, even at 90 days after the inoculation, may have been due to this compression process.

The *in situ* cytokine dosage results found in this study suggest the predominance of a Th2 immune profile at 90 days after the inoculation of *T. crassiceps* cysticerci. This may be inferred by the significantly higher concentrations of IL-4 in the INF group when compared with the CT group and also because of the lack of difference in the IFN-gamma concentrations. It is important to highlight that, within the brain, the inflammatory and immune processes occur in a unique form, which may alter the expected order of inflammation, tissue injury, damage to the host, death of the parasite and resolution of the infection<sup>24,25</sup>.

The brain, as an immunoprivileged organ, attenuates the inflammation in its tissue because it can threaten the brain's integrity and function<sup>11,24</sup>. This protective mechanism is due to passive factors, such as the presence of the blood brain barrier and the intracerebral circulation, as well as active factors such as immunosuppressive cytokine production,

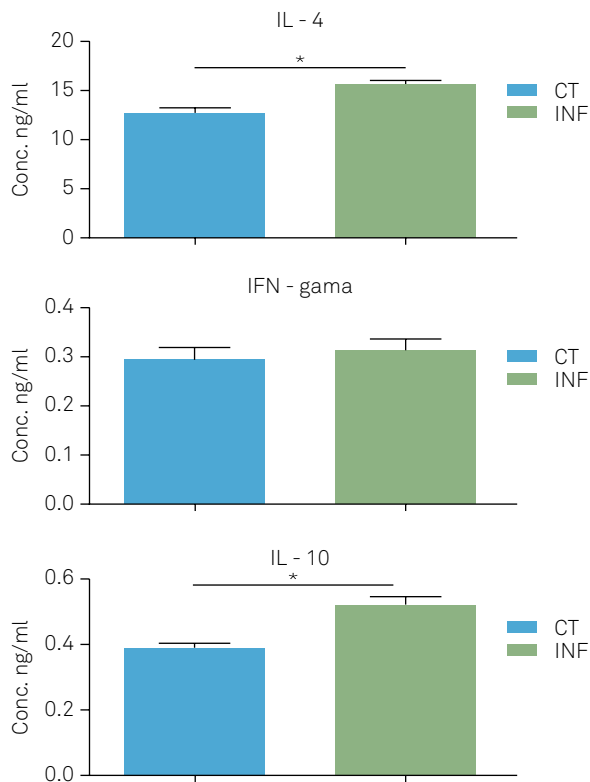


**Figure 2.** Mesoscopic image of the brains from C57BL/6 mice. A. Absence of hemisphere compression in a mouse from the control (CT) group. B, C and D. Hemisphere compression, dislodgement of the medial line, hippocampus and lateral ventricle in mice inoculated intracranially with *Taenia crassiceps* cysticerci (INF group). In D, the arrow shows the presence of a viable cysticercus in the lateral ventricle. Scale bar = 1 mm. H&E.

neuropeptides, limitation in the MHC class I and II expression, complement regulatory proteins and NK cell inhibitors, which also contribute to the immune privilege<sup>27,28</sup>. These factors act as a protective factor regarding NCC, preventing damage to the brain tissue and favoring the extension of the parasite's life within the brain<sup>29</sup>.

Moura et al.<sup>15</sup> reported that BALB/c mice, experimentally inoculated intracranially with *T. crassiceps* cysticerci, did not show significant differences in the *in situ* dosage of cytokines in spite of higher concentrations of IFN-gamma than IL-4, confirming a Th1 immune profile, allowing the parasite survival. In our study, the C57BL/6 mice inoculated with the same parasite showed a predominance of the Th2 immune profile as an attempt to contain the parasite growth.

It is important to highlight the immunomodulation capacity of the cysticerci, which is also responsible for the immune profile observed in C57BL/6 mice. The parasite's antigens stimulate the production of antibodies and the release of chemical mediators that are unable to destroy the cysticercus. This happens because the parasite simultaneously modulates the action of these immune components and becomes viable for a longer period of time<sup>30</sup>. Thus, it sways the immune profile from a Th1 response to a Th2 response, which protects the tissue from damage, activates macrophages with lower microbicide potential, and generates the activation and expansion of plasmocytes. As well, the IgE secretion, presence of eosinophils, mastocytes and basophils are unable to destroy the parasite<sup>6</sup>.



CT: control group; INF: infected group. \*statistical difference between groups ( $p < 0.05$ ), T Test.

**Figure 3.** *In situ* cytokine dosage from C57BL/6 mice intracranially inoculated with *Taenia crassiceps* cysticerci, 90 days after the inoculation.

The IL-10 dosage detected in this study shows that the presence of this cytokine within the brain tissue modulates the Th1 and Th2 immune responses. Interleukin-10 is a potent inhibitor of the antigen presentation, and of the mediators and pro-inflammatory cytokine production by macrophages, dendritic cells and TH1 cells<sup>30</sup>. It has an important regulatory role on the host inflammatory response during the infection. The inflammation is essential at the beginning of the response against the pathogen, but this must be controlled so it does not result in severe inflammatory complications<sup>31</sup>. It is also capable of regulatory T cell lymphocyte activation, which decreases the tissue damage<sup>30</sup>. Therefore, the statistically higher concentration of IL-10 in the infected group in our study demonstrates that it is regulating the immune response in the mice, swaying the immune response towards the Th2 immune profile, which decreases the parenchymal damage and contributes to the parasite survival.

Therefore, when the immune aspects are correlated with the histopathology described in this study, it is possible to conclude that the infection of *T. crassiceps* in C57BL/6 mice triggered an inflammatory response with a predominant Th2 and Treg *in situ* response. These facts are reflected in the control of the intensity of the general pathologic processes, such as meningitis, microgliosis, mononuclear inflammatory cell infiltration and compression of the brain hemispheres.

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