Review Article

Trypanosoma cruzi-Induced Central Nervous System Alterations: From the Entry of Inflammatory Cells to Potential Cognitive and Psychiatric Abnormalities

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Abstract *Trypanosoma cruzi*, a protozoan parasite and the causative agent of Chagas disease, is capable of inducing meningoencephalitis. Independent of the progression from acute to chronic myocarditis observed in immunocompetent *T. cruzi*-infected patients, inflammation of the central nervous system (CNS) self-resolves during acute infection. In contrast, in chronically infected immunocompromised Chagas disease patients, the CNS is a major site of reactivation, which can lead to severe and frequently fatal meningoencephalitis. More than one hundred years after the discovery of Chagas disease, many questions concerning the molecular mechanisms involved in the induction and resolution of *T. cruzi*-provoked meningoencephalitis remain unanswered. The study of murine models that reproduce crucial aspects of *T. cruzi*-elicited CNS inflammation has not only shed light on some of these questions, but it has also raised additional ones. Here, we discuss our results in the context of the current literature, questioning the involvement of CNS alterations caused by the inflammation and parasite in the behavioral abnormalities observed during *T. cruzi* infection.

Keywords *Trypanosoma cruzi*; central nervous system; inflammation; behavioral abnormalities

1 Chagas disease and the central nervous system

American trypanosomiasis, which was discovered by Carlos Chagas in 1909 [14] and is also known as Chagas disease, is caused by *Trypanosoma cruzi*, a protozoan parasite that is mainly transmitted via triatomine insect vectors. It is estimated that 40 million people are at risk for infection in a region that ranges from the south of the United States to the south of Argentina and Chile. Furthermore, epidemiological evidence indicates the following: 8–15 million people either are *T. cruzi* carriers or present the clinical manifestations of the infection; the incidence of vector transmission is greater than 40,000 new cases per year; the congenital transmission rate is estimated to be 14,000 cases per year; approximately, 21,000 deaths occur per year attributable of Chagas disease [21,58,100]. Although the success of efforts aimed at controlling the primary vector of the disease deserve recognition, sustaining such progress will depend on the control of autochthonous triatomines through permanent epidemiological and entomological surveillance. Furthermore, several challenges in the management of Chagas disease remain unresolved, including the improvement of accessibility to diagnostic tools, treatments, and medical care and of education aimed at both avoiding and managing infections. Moreover, Chagas disease is at risk of becoming a global health problem due to human migration [46,81].

In cases of acute *T. cruzi* infection, the observed prominent myocarditis is associated with intense parasitism and cardiac fiber injury [15,35,36]. The central nervous system (CNS) is considered an immunoprivileged site [30] into which the entrance of macromolecules and immune cells is restricted. However, this organ is a target for acute infection by viruses and parasites, including *T. cruzi*. This parasite has been observed in a variety of glial cells, including astrocytes and microglia, in addition to endothelial cells and CNS-invading macrophages; however, it is rarely observed in neurons [98,103]. In fact, Carlos Chagas was able to identify a malign form of the acute infection due to the presence of focal inflammatory regions in the CNS; such an infection would likely lead to a morbid condition, particularly in children. In 1911, Gaspar Vianna [103] demonstrated the presence of histopathological alterations in the CNS of
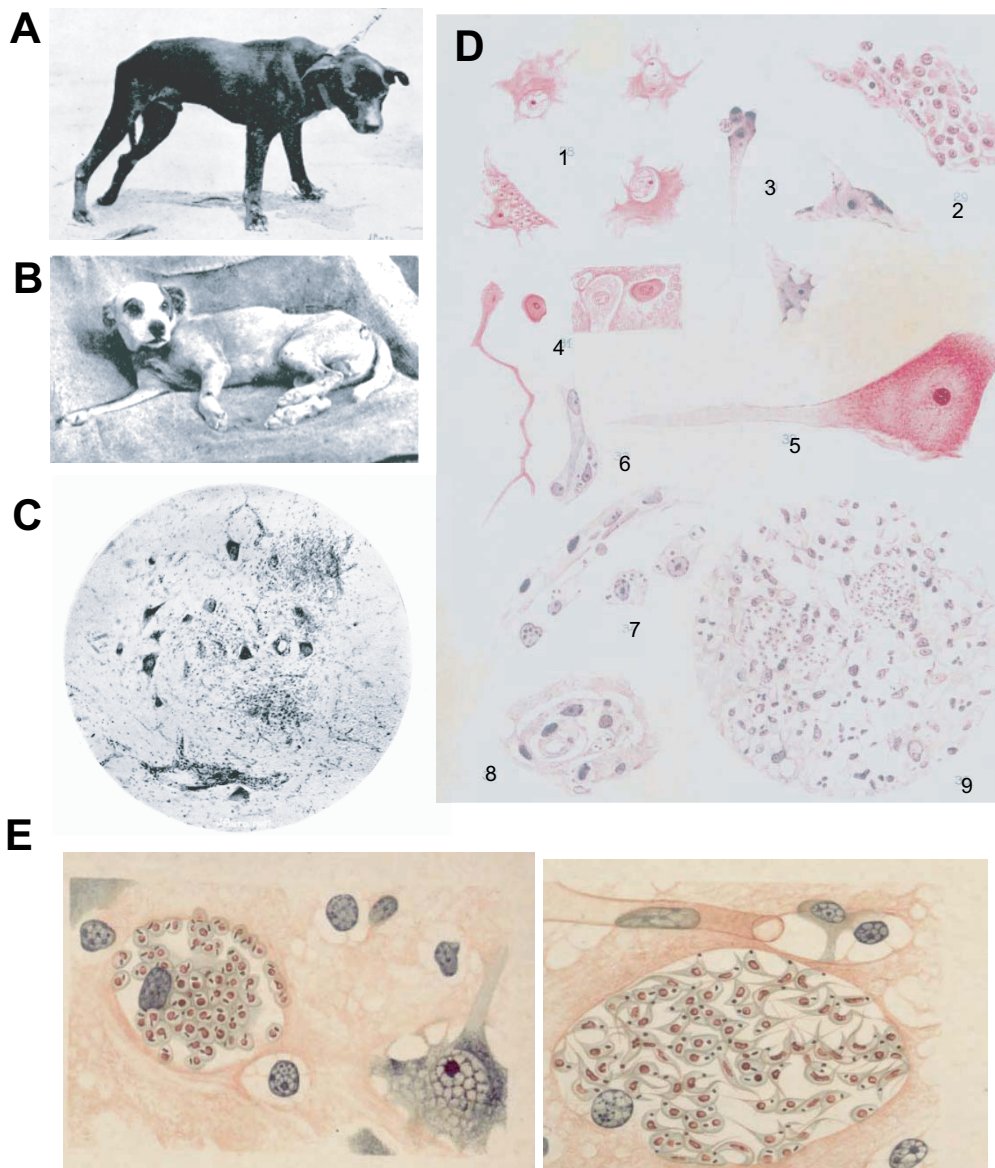
Figure 1: Motor and CNS abnormalities in dogs infected with trypomastigote forms of *T. cruzi* isolated from wild armadillo. A: paresis of the hind limbs 20 days postinfection. B: complete paresia. C: illustration of inflammatory infiltrates in spinal cord tissue. D: illustrations of CNS sections of *T. cruzi*-infected dogs showing (1) a focus of encephalomyelitis composed of macrophages/microglia, (2) Purkinje cells among macrophages in an inflammatory focus, (3) brain cortex cells, (4) Purkinje cells, (5) multipolar cells in the right anterior horn of the spinal cord, (6) and (7) amastigote-carrying cells near a capillary in the cerebellum, (8) parasite-carrying endothelial cells in the cerebellum, and (9) a focus of encephalitis surrounding *T. cruzi*-carrying cells in the brain E: illustrations of brain tissue from *T. cruzi*-infected dogs showing (right) amastigote and (left) trypomastigote forms inside glial cells in an inflammatory focus. Published by Vianna, 1911 [103] and Villela and Torres, 1926 [104]. Reproduced and modified (i.e., the addition of numbers in panel B) with the permission of the editor of the Memórias do Instituto Oswaldo Cruz (http://memorias.ioc.fiocruz.br).

a three-month-old child that had succumbed to an acute infection. The meningoencephalomyelitis was characterized by multiple inflammatory foci distributed in the cerebral tissue. In addition, histopathological study performed on experimentally *T. cruzi*-infected dogs has revealed that lesions occur more frequently in the central medulla and white brain matter, although scattered lesions are observed throughout the CNS tissue without an apparent preferential localization [98] (Figure 1). In such cases, glial cell hypertrophy, the presence of plasma cells, and clusters of amastigote forms near the encephalitic foci have been described. In 1926, Villela and Torres [104] showed that *T. cruzi*-infected dogs presented clinical signs, such as astasia, paralysis, and convulsion; although, at that time, the pathognomonic causes remained unclear. In 1964, Deolindo Couto [22] confirmed these data and concluded that in

the acute phase of Chagas disease, the damaging effect on the leptomeninges and nervous tissues was undeniable. Furthermore, a prospective study of infected children revealed that between 5% and 10% of those who did not receive treatment died during the acute phase of infection due to severe cardiac failure and/or encephalomyelitis [2]. However, questions regarding the delayed effects that result from T. cruzi infection of the CNS remain unanswered.

During the chronic phase of T. cruzi infection, parasites are scarce, and Chagas disease is characterized mainly by cardiac symptoms; between 30% to 40% of infected patients exhibit myocarditis associated with prominent fibrosis and organ dysfunction 10 to 30 years postinfection [24, 36, 69]. It has been suggested that the observed cardiac injuries result from an imbalance in effector immune responses due to persistent parasite infection [9, 36, 40, 95]. In fact, therapies designed to control the inflammatory reaction without interfering with the clearance of parasites have proved effective in inhibiting cardiac fibrosis and heart dysfunction in cases of acute and chronic T. cruzi infections [45, 52, 54]. Chagas described various neurological manifestations related to the presence of T. cruzi in the CNS during the chronic phase of infection [15]; however, Chagas and other investigators were unable to characterize the chronic nervous form of the disease. Nevertheless, those studies did not consider important variables such as alcoholism, the presence of other infections or noninfectious diseases, and the nutritional status of the patients; further those studies suffer of lack of appropriate control groups. During the 1960s, the majority of researchers agreed that the CNS was not affected in cases of chronic infection because Chagas disease patients exhibited either no or small inflammatory processes with a sparse number of parasites within the nervous tissue [2, 22, 45]. In fact, most Chagas disease patients responded normally to neurological exams, and the apparent lack of both neurological alterations and complaints from patients was not favorable for the diagnosis of neurological syndromes in patients with Chagas disease [68]. While there is no anatomical or histological basis for a characterization of the nervous form of Chagas disease, a study of 35 patients using a P300-evoked potential and quantitative electroencephalography (EEG) analysis by Prost et al. [70] suggested an electrophysiological involvement of the CNS in cases of chronic T. cruzi infection. More recently, a study of 19 patients with mild cardiac Chagas disease by Wackermann et al. [105] described limited clinical, EEG and MRI alterations, and a focal nervous system dysfunction. These alterations were likely associated with grey matter focal lesions and possible white matter damage, although they were insufficiently severe to interfere with the patients' daily activities. These unique results could be explained by the small size of the patient group studied, and therefore, the presence of

a chronic nervous syndrome in Chagas disease remains subject of debate.

The CNS is the primary tissue that is injured during the reactivation of T. cruzi infection [51, 53, 74, 80]. The common use of corticosteroids, immunosuppressors, and cytostatic agents, increased numbers of organ transplants, and, in particular, cases of HIV/T. cruzi coinfection have increased the occurrence of infection reactivation in chronic Chagas disease [31]. Common clinical manifestations of this disease include headaches, focal neurological deficits, fever, seizures, altered mental status, and cardiac involvement. The survival of HIV/T. cruzi coinfected patients is dependent on early diagnosis, which should be performed via cerebrospinal fluid examination. The presence of parasites can often be detected in cerebrospinal fluid, although they can also be detected in blood [20, 31, 102]. Cranial MRI studies have revealed a right temporoparietal mass lesion with surrounding edema [74, 80]. The histopathological picture is distinct from acute self-resolving meningoencephalitis, displaying necrotizing aspects, and numerous amastigotes that are frequently tumoral or pseudotumoral in form (termed brain "chagomas") [67] and are usually located in the white matter. If a timely diagnosis is achieved, the reactivation of T. cruzi infection can be successfully treated using benznidazol [6]. The amastigotes are observed in glial fibrillary acid protein (GFAP)-positive cells that resemble astrocytes and in microglial cells, but they are rarely detected in neurons. Sofroniew [86] suggested that astrocyte dysfunction in patients infected by HIV could represent a potential human correlate of the AIDS-dementia complex. In this article, Chagas disease reactivation has been considered as a differential diagnosis of meningoencephalitis in HIV patients with low CD4$^+$ T cell counts in endemic areas and Latin American immigrants [20, 81, 101].

2 Experimental *Trypanosoma cruzi*-induced meningoencephalitis

Studies investigating the status of the CNS during T. cruzi infection have been performed in a number of animal models, including monkeys, dogs, cats, rats, and mice. In initial studies, dogs and cats inoculated with T. cruzi reproduced the neurological alterations observed in carriers of Chagas disease by Carlos Chagas et al. [15, 98, 104]. However, the genetic and immunological status, the age of the host, and the size of the parasite inoculum must be considered when establishing appropriate models to reproduce one or more aspects of Chagas disease [3, 13, 44, 76, 96, 98, 99, 104]. Furthermore, in attempts to reproduce specific aspects of this infection, it is important to consider the genetic diversity of T. cruzi [63]. However, despite the

diversity of various strains and inoculums of the parasite and the diversity of mouse lineages, the murine model reproduces most of the clinical and histopathological aspects of the acute and chronic phases of *T. cruzi* infection observed in Chagas disease carriers [4,5]. Similar to the findings described by Moncada et al. in children [57] and Villela & Torres in young dogs [104], newborn (i.e., 10 days after birth) C3H/He (H-2^k) mice exhibited higher levels of susceptibility with increased parasitemia (Figure 2A) and greater numbers of amastigote-positive areas in the CNS in comparison to young (3–4 weeks old) adult animals infected with the Colombian strain of *T. cruzi* (Figures 2B and 2C). The presentation of acute meningoencephalitis appears to be dependent on the maturation of the blood brain barrier (BBB); infected suckling rats are significantly more affected and the numbers of nests and glial nodules vary with the inoculum size, as compared with juvenile rats [23]. Additionally, when infected with the Colombian strain of *T. cruzi*, male C3H/He mice are more susceptible than are females (data not shown); in both males and females, F4/80$^+$ and CD8$^+$ cells are the predominant infiltrating inflammatory cells in the CNS (Figure 2D), corroborating previous data [83].

With respect to specific mouse lineages, C3H/He (H-2^k) mice infected with low inoculums of the Colombian *T. cruzi* strain display elevated levels of parasitemia in comparison to C57BL/6 (H-2^b) mice; however, roughly 70% of the animals of both lineages survive the acute infection and develop chronic disease [45,83,85,93]. Interestingly, in C3H/He mice, the elevated parasitemia peak observed at 42 days postinfection is paralleled by an intense CNS parasitism associated with macrophages and CD8-enriched meningoencephalitis [75,83]. In contrast, *T. cruzi*-infected C57BL/6 mice rarely exhibit macrophages and CD8$^+$ cells in the CNS perivascular spaces during acute infection [75]. In contrast with the acute CD8-enriched myocarditis detected in C3H/He and C57BL/6 *T. cruzi*-infected mice, which evolves to a chronic cardiomyopathy [25,54,85], inflammation of the CNS is not observed in cases of chronic infection in either mouse lineage [75].

Antibodies that recognize neurons, glial cells, peripheral nervous tissues, myelin, and myelin basic protein (MBP, a molecule that forms the myelin sheath of axons and is involved in synapse formation) have been described in carriers of Chagas disease and in animal models of *T. cruzi* infection [1,39,73,79,88]. In fact, C3H/He mice acutely infected with the Colombian strain of *T. cruzi* present increased levels of circulating anti-MBP antibodies prior to the peak of parasitemia that decline during parasitemia apices but remain elevated during the chronic infection. However, anti-MBP antibodies are not observed in C57BL/6 mice during the acute infection, and low anti-MBP levels are observed in cases of chronic infection (Figure 3B).

In contrast with C57BL/6 mice, C3H/He infected mice display elevated levels of circulating total IgG during the acute phase of infection (28 days postinfection) that persist during the chronic phase. However, the levels of circulating anti-MBP are not affected by the hyperproduction of IgG. C3H/He mice, similar to C57BL/6 mice, exhibit elevated anti-*T cruzi* antibody levels that parallel a decline in parasitemia. No correlation has been found between the levels of anti-MBP antibodies in the plasma and meningoencephalitis because these antibodies remain elevated even after the meningoencephalitis is resolved during the chronic phase of *T. cruzi* infection in C3H/He mice. Similarly, elevated intrathecal myelin oligodendrocyte glycoprotein antibodies were detected in multiple sclerosis [41]. The biological role of anti-MBP antibodies during *T. cruzi* infection in both human and experimental models remains unclear. The access of inflammatory mononuclear cells to the brain is dependent on disruption of the BBB and alterations in the expression levels of cell adhesion molecules (CAMs) and attractant cytokines [29]. Identification of the molecules that are essential for the establishment of inflammatory CNS disease might reveal novel therapeutic targets, particularly in the case of chronic degenerative diseases. Considering the dynamics of *T. cruzi*-elicited meningoencephalitis in C3H/He mice, we adopted this murine model in our investigation of the molecular mechanisms involved in the progression and resolution of meningoencephalitis. The *T. cruzi*-elicited inflammatory process in the CNS of C3H/He mice is dispersed throughout the cerebral cortex, hippocampus, cerebellum, and choroid plexus, which suggests that the main point of entrance is the perivascular space of blood vessels [83]. In studies investigating the participation of *T. cruzi*-elicited encephalitis formation, we found that activated peripheral blood mononuclear cells (PBMCs) are adhered *ex vivo* to VCAM-1$^+$ CNS blood vessels of *T. cruzi*-infected mice. This adhesion was abrogated by anti-VLA-4 antibodies, which also inhibited the migration of cells into the CNS of infected mice. Moreover, encephalitis reactivation in immunosuppressive drug-treated chronically infected mice was paralleled by an upregulation of VCAM-1. Therefore, we hypothesized that the VLA-4/VCAM-1 pathway plays a pivotal role in the formation of *T. cruzi*-elicited encephalitis [75,82]. These data are in accordance with the results obtained for other inflammatory brain diseases. In experimental allergic encephalomyelitis (EAE), a model of multiple sclerosis induced by immunization with myelin antigens, the VLA-4/VCAM-1-mediated interaction is well documented and raises the possibility that this pathway of interactions between inflammatory and endothelial cells might represent a target for the therapeutic modulation of inflammation [8,43,87]. However, although several therapeutic tools (i.e.,
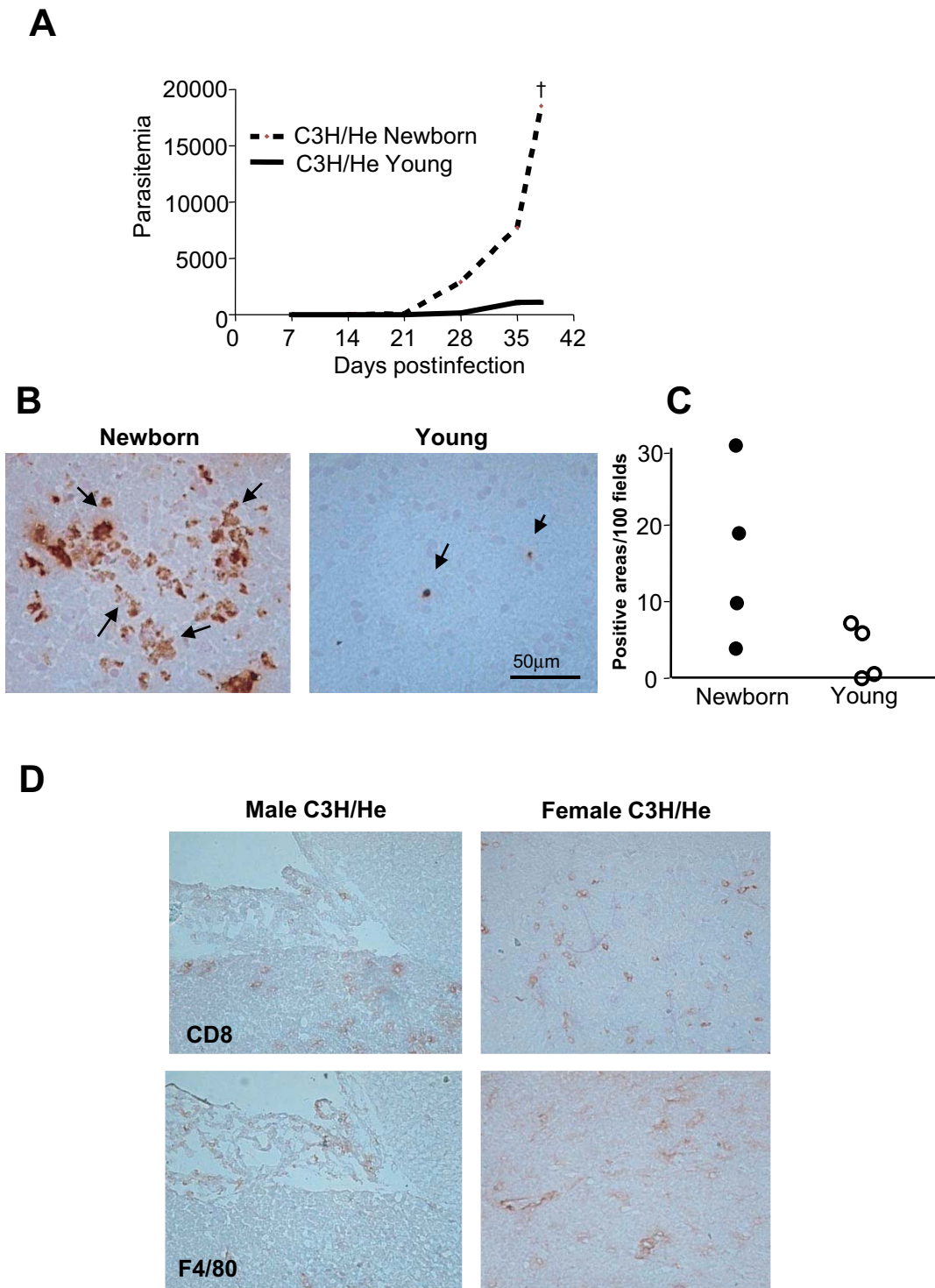
Figure 2: Experimental *T. cruzi* infection in C3H/He (H-2^k) mice. A: parasitemia curves obtained for newborn (10 days old) and young (7 to 8 weeks old) C3H/He mice after infection with the Colombian strain of *T. cruzi*. The line indicates a mean of 5–8 animals per group. The figure is representative of two independent experiments. B: presence of *T. cruzi* antigens in brain sections or cerebral cortex areas obtained from newborn (10 days old) and young (7 to 8 weeks old) C3H/He mice. In these experiments, an anti-*T. cruzi* polyclonal antibody was used. The figure is representative of three independent experiments. C: quantification of *T. cruzi* antigen-positive areas in the brain of both newborn and young C3H/He mice. Each circle indicates one animal. The figure is representative of two independent experiments. D: inflammatory infiltrates composed of CD8^{+} cells and macrophages (F4/80^{+}) in the encephalons of *T. cruzi*-infected male and female C3H/He mice.
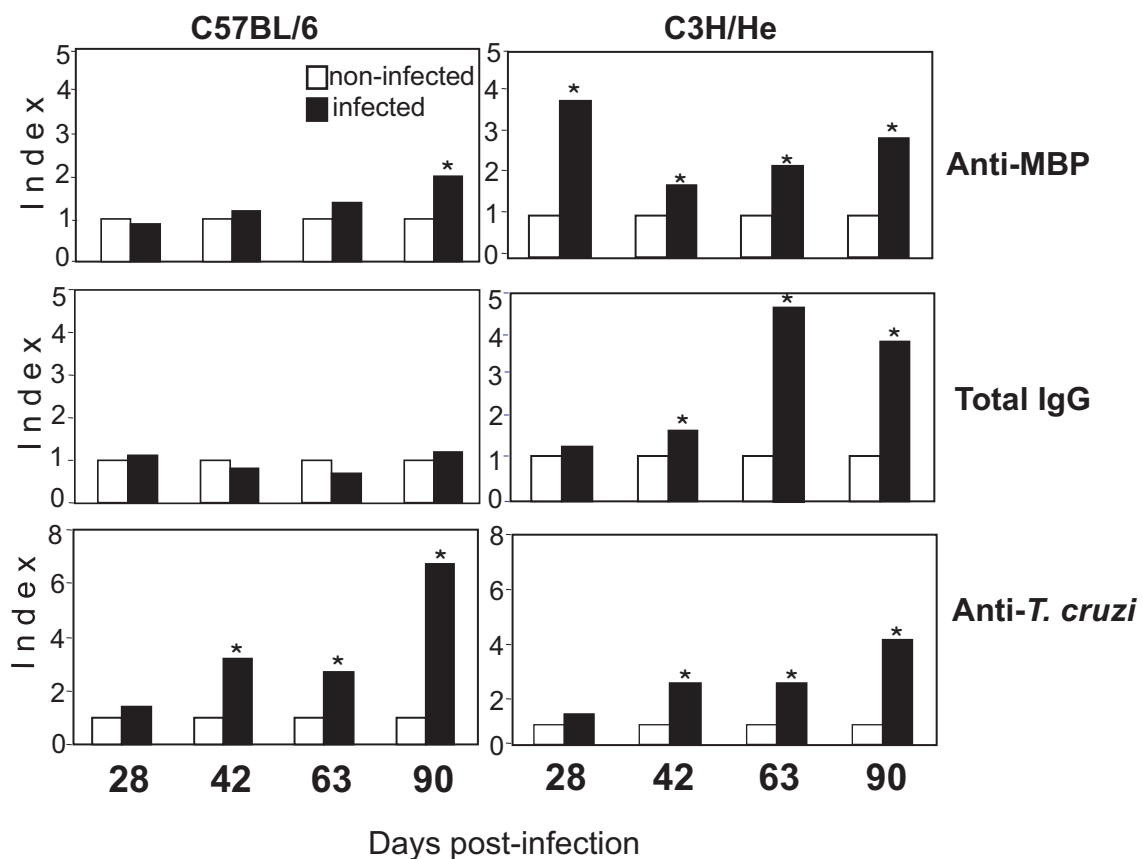
Figure 3: Experimental *T. cruzi* infection of C3H/He (H-2^k) and C57BL/6 (H-2^d) mice. Circulating antimyelin basic protein (MBP) antibodies, total IgG, and anti-*T. cruzi* antibodies. The results are expressed as index, representing the fold increase observed in infected animals compared to noninfected mice referred as 1. The figures are representative of three to five independent experiments $P < 0.05$.

antibodies and antagonists) have been designed, developed, and tested in clinical trials, current treatments that target VLA-4/VCAM-1-mediated interactions are not completely effective [28, 92].

Other attractive targets for controlling the recruitment of mononuclear inflammatory cells to the extravascular space during chronic autoimmune and infective CNS inflammation processes are chemoattractant cytokines, named chemokines, and their receptors. Rolling leukocytes interact with chemokines that are immobilized on the membranes of endothelial cells and bind to receptors on leukocytes. The elevated expression of chemokines and their receptors in inflammatory cells or blood vessel endothelial cells of the CNS has been associated with chronic degenerative diseases [27, 28]. In our models of *T. cruzi* infection, C3H/He mice infected with the Colombian strain exhibited increased numbers of CCR5$^+$ PBMCs and splenocytes during the acute and chronic phases of infection [52, 84]. Our previous data support the idea that CD8-enriched *T. cruzi*-elicited acute and chronic myocarditis formation involve CCR1/CCR5-mediated cell migration [52, 54]. These results led us to test whether these

CC-chemokine receptors participate in the development of *T. cruzi*-induced CNS inflammation or not. In the CNS, enhanced mRNA expression levels of CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES (all of which are CCR5 ligands) were restricted to the acute infection and paralleled inflammation. Elevated expression levels of the CCR5 receptor in circulating CD8$^+$ T cells were associated with the expression of VLA-4, particularly with the activated form of the $\beta1$ integrin chain, thus demonstrating their enhanced migration potential [54]. In fact, the PBMCs of acutely infected mice selectively migrated towards CCL4/MIP-1β and CCL5/RANTES *in vitro*; furthermore, this migration was partially inhibited by Met-RANTES, a selective CCR1/CCR5 antagonist [84]. In contrast, the treatment of C3H/He-infected mice with Met-RANTES *in vivo* resulted in a partial blockade of *T. cruzi*-induced acute myocarditis [52] but did not alter the parasitism or CNS inflammation [84]. Thus, in contrast with myocarditis [52, 54], *T. cruzi*-elicited meningoencephalitis is a CCR1/CCR5 independent process [84].

Other chemokines are involved in the entrance of inflammatory cells into the CNS. Previous studies have

shown that the knockdown of CCL2/MCP-1 and CCR2 led to a reduction of monocyte infiltration and recruitment during EAE but did not inhibit the onset of clinical signs [26, 33]. In cases of murine *Toxoplasma* encephalitis, astrocytes are the major source of interferon (IFN-γ)-inducible protein 10 (CRG-2/IP-10) and CCL2/MCP-1, and microglia express CCL5/RANTES, monokine induced by IFN-γ (MuMIG), and, occasionally, CRG-2/IP-10 mRNA. Only astrocytes and microglia that are confined to inflammatory infiltrates express chemokine genes [91]. Recently, we demonstrated that CCL2$^{-/-}$ mice infected with a highly virulent strain of *T. cruzi* developed higher levels of parasitemia and died earlier compared to C5BL/6 wild-type mice. Clinical signs of a systemic inflammatory response and amastigote nests were more frequent in the hearts and livers of infected CCL2$^{-/-}$ compared to wild-type mice. Our results also demonstrated that CCL2 contributed to a reduction of parasite growth by controlling the distribution, cellular composition, and state of activation of inflammatory infiltrates during acute *T. cruzi* infection [62]. However, the role of CCL2/MCP-1 and other chemokines in the control of inflammation and parasite burden as well as their sources in the CNS during *T. cruzi* infection require further investigation. Presently, we are investigating the role of glial cells (i.e., astrocytes and microglia) in the control of parasite burden using models that are either susceptible to *T. cruzi*-elicited acute CNS inflammation (C3H/He mice) or resistant to *T. cruzi*-elicited meningoencephalitis (C57BL/6 mice). Our preliminary data show that GFAP$^+$ cells from C3H/He mice presenting more parasite-positive areas in the CNS are also more prone to *T. cruzi* infection *in vitro* compared to astrocytes isolated from C57BL/6 mice (Silva et al., in preparation). Interestingly, in cases of acute meningoencephalitis, both astrocytes and microglia are targeted by the parasite [15,23]. Furthermore, *T. cruzi*-infected astrocytes and microglia express MHC-II (our unpublished data). Via a mechanism similar to that observed in cases of *Toxoplasma*-induced encephalitis, these glial cells might play a key role in protective meningoencephalitis in cases of *T. cruzi* infection. In fact, *T. gondii*-triggered regulatory mechanisms include prostaglandin E2 secretion by astrocytes and cAMP-dependent IL-10 secretion by microglia; these signaling molecules may reduce host tissue inflammation, thus avoiding neuronal damage that could occur during an established Th1 protective immune response [77]. Additionally, in the presence of transforming growth factor (TGF)-β1-neutralizing antibodies, the beneficial effect of the parasite on neurons was abrogated, and nitric oxide production reverted to levels similar to those observed in IFN-γ-activated uninfected cocultures. Together, these data may explain the neuroprotective pattern observed during immunocompetent host infection that is dependent on *T. gondii*-triggered TGF-β1 secretion by

infected microglia [78]. IL-12$^{-/-}$ mice infected with *T. cruzi* exhibit a partial abrogation of IFN-γ production, which suggests that this cytokine is a major determinant in the reactivation of *T. cruzi* infection in immunocompromised hosts [55]. The role of glial cells and cytokine networks in the establishment and resolution of inflammation and in the control of *T. cruzi* infection in the CNS remains an important topic of investigation.

T. cruzi infects both glial and neuronal cells and provokes CNS inflammation during the acute phase of infection that self-resolves during the chronic phase of infection [75,83,103,104]. However, the biological role of parasite molecules in these processes is unclear. Interestingly, proteins from the parasite synergize with cytokine ciliary neurotrophic factor to prevent the apoptosis of neuronal cells via activation of the TrkA nerve growth factor receptor [17–19]. Furthermore, the parasites can mediate the survival of neuronal and Schwann cells by activation of TrkC receptors [106]. It is possible that during *T. cruzi* infection, glial cells such as astrocytes and microglia play a protective role to maintain CNS homeostasis; however, this theory requires further investigation.

Whereas many studies have investigated the pathology and pathogenesis of *T. cruzi*-induced myocarditis, few have addressed the pathogenesis and the exact causative molecular mechanism and effect of acute self-resolving meningoencephalitis. Questions concerning the participation of glial cells and their role in parasite control or in the neuroimmune response remain unanswered. It is possible that the resolution of acute meningoencephalitis is related to parasite control; however, the occurrence of delayed effects during the chronic phase of infection complicates this interpretation, as discussed in Section 3.

3 *Trypanosoma cruzi* as a potential inducer of neurocognitive abnormalities

Carlos Chagas hypothesized the existence of a nervous form of American trypanosomiasis based on the observation of abnormalities in motor control, intelligence, and language in infected patients, particularly in children [15]. Furthermore, Chagas affirmed that these abnormalities resulted from the presence of parasites in the CNS and that delayed symptoms resulted from acute *T. cruzi* infection elicited CNS alterations [16]. However, characterization of the chronic nervous form of this illness has remained a challenge.

Cardiac involvement of Chagas disease increases the risk for ischemic strokes. Although cerebrovascular complications in patients with Chagas disease have not been previously described, postmortem studies have shown that roughly 9% to 36% of patients with chronic Chagas cardiomyopathy show evidence of cerebral infarctions [12, 61,66,67,72]. After demonstrating that 81.6% of Chagas

disease patients exhibit vascular risk factors, Carod-Artal et al. [11] suggested that Chagas disease should be included in the differential diagnosis of stroke in patients of South American origin. The association between Chagas disease, stroke, and the risk for vascular dementia has not been properly investigated. Some works have investigated the possible alterations present in the CNS during the chronic phase of infection and attributed them to the presence of parasites in nervous tissue or to secondary consequences of heart lesions [38, 61, 70, 105]. In most patients with the symptomatic acute form of the disease, all clinical manifestations, including neurological signs and symptoms, disappear spontaneously without apparent delayed effects [68]. It is possible that the nervous form of the disease can be attributed to delayed effects on the CNS that result from acute lesions or parasite persistence [16]. Prost et al. [70] studied the P300-evoked potentials and quantified EEGs (qEEGs) of 35 Chagas disease patients in comparison to an equal number of control subjects. The authors described that multivariate analysis showed three subpopulations: (i) a normal one, (ii) pathological one with higher alpha power, and (iii) pathological with alpha decrement and delta-theta increment. Furthermore, the pathological findings represented 20% for the qEEG and 11.43% for cognitive potentials. The authors concluded that cardiac and neurological symptoms were not correlated, and they provided electrophysiological evidence of cerebral involvement in chronic Chagas disease. Recently, 1,449 individuals, aged $\geq$ 60 years from a community-based sample of older adults living in an endemic area in Brazil, were subjected to a mini-mental state examination (MMSE) and examined for *T. cruzi* infection, Chagas disease-related electrocardiographic abnormalities and the use of digoxin, a digitalis-based medication [48]. In this article, a graded and independent association between infection and the MMSE score (adjusted odds ratios estimated by ordinal logistic regression = 1.99; 95% CI 1.43–2.76) was described. No significant associations between the MMSE score and EEG abnormalities or the use of digoxin medication were found. These results reproduced the findings reported by Mangone et al. [50], who demonstrated an association between Chagas disease and cognitive dysfunction that was suggestive of a brain white matter disease. Recently, a study of 23 chronic Chagas disease patients without cardiac dysfunction demonstrated a significant association between parasympatic system alterations and the presence of brain white matter lesions [71].

More recently, we investigated whether motor/exploratory and psychological/cognitive are a result of delayed effects of *T. cruzi* infection or whether they require the development of *T. cruzi*-elicited inflammation. Interestingly, clinical signs, including (i) a tendency to fall or to adopt a circular running pattern, always directed to the same side, (ii) a humpback walk, (iii) loss of tail tonus, and (iv) paralysis that affects mainly the hind limbs, were observed during the progression from acute to chronic phase of infection, although CNS damage occurs predominantly during the acute phase [83]. Thus, our first hypothesis was that the cognitive alterations were caused by delayed effects of acute *T. cruzi*-induced meningoencephalitis. Initially, we performed the open field test with the C57BL/6 mouse strain, which is resistant to acute meningoencephalitis, to investigate effects on locomotor/explorative activity during experimental *T. cruzi* infection. Our preliminary data showed that independent of the infection phase (i.e., either acute or chronic), these animals exhibited a decrease in locomotor/explorative activity when compared to noninfected control animals (Figure 4). Additionally, the study of the C3H/He strain, which is susceptible to acute meningoencephalitis [75], revealed no locomotor/explorative abnormalities during the acute and chronic infections (data not shown). Therefore, our data suggest that in the experimental model of *T. cruzi* infection, locomotor/exploratory impairment is not associated with CNS inflammation but instead is a direct result of *T. cruzi* infection. However, susceptible animals that survived the acute infection with the Tulahuén *T. cruzi* strain showed that brain invasion, mainly in basal ganglia by the parasite and a strong inflammatory response did not trigger neurodegeneration and did not result in motor alterations [10]. At present, our laboratory is performing studies designed to enhance our understanding of the influence of parasite infection and the resulting immune response within the CNS in behavioral abnormalities observed during *T. cruzi* infection. We also intend to contribute with the evaluation of additional cognitive aspects such as anxiety.

The neurocognitive impairments observed during *T. cruzi* infection might be due to the immune response that develops within the CNS microenvironment; alternatively, they could be an indirect result of a more general systemic immune response. Interestingly, in depressed patients, T-cell abnormalities, such as (i) the increase of Fas (CD95) expression by circulating T CD4$^+$ cells, (ii) the inhibition of T-cell functions by glucocorticoids, (iii) the decreased expression of beta adrenergic receptors on circulating mononuclear cells, and (iv) the disruption of T-cell functions induced by inflammatory cytokines such as tumor necrosis factor (TNF) have been reported [56]. In this vein, in *T. cruzi* infections, the presence of elevated glucocorticoid levels in the plasma and the immunosuppression of T-cells induced by parasite antigens have been documented [47, 97]. Furthermore, some studies have shown that *T. cruzi* proteins recognize and adhere to host cells through parasite surface molecules that not only have an affinity for beta-adrenergic receptors on target organs but also
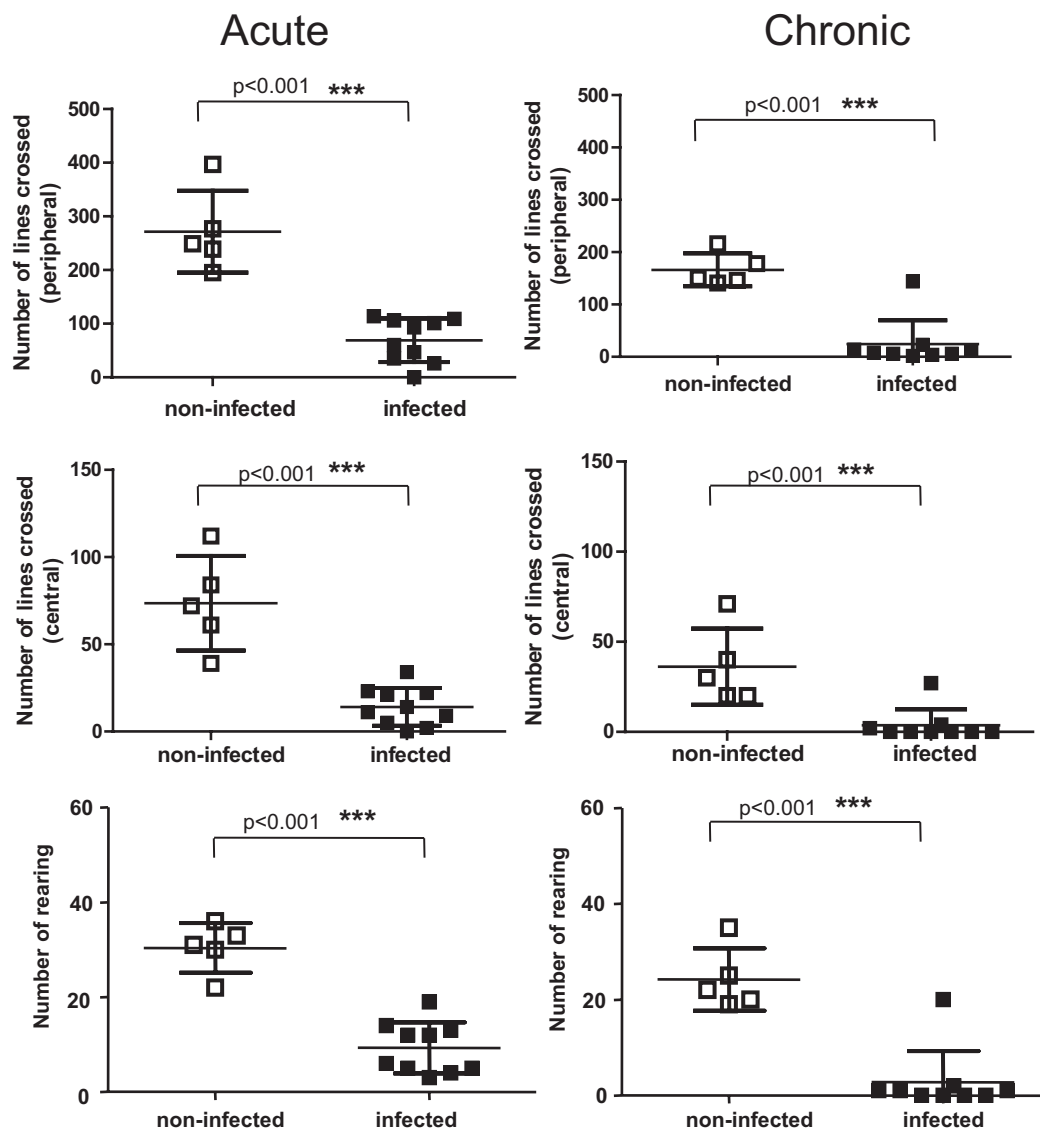
Figure 4: Locomotor and exploratory activities of C57BL/6 mice (strain H-2^d, resistant to meningoencephalitis) during experimental *T. cruzi* infection. The number of peripheral and central lines crossed, and the rearing performed by C57BL/6 during acute (30 dpi) and chronic (90 dpi) phases of infection. The horizontal and vertical lines indicate the mean $\pm$ SD of the analyzed group. Each animal is represented by a square. The figures are representative of three independent experiments: $P < .05$.

display beta-agonist like activity [34]. The cardiac beta-adrenergic system is severely compromised during acute and chronic stages of experimental *T. cruzi* infection [37, 49,90]. Interestingly, TNF levels are correlated with the severity of cardiomyopathy in Chagas disease patients [32, 64,94], and elevated levels of TNF are also detected in mice infected with the CL or the Colombian *T. cruzi* strains, during both acute and chronic infections [42,89]; however, the biological role of this cytokine in the nervous tissue during *T. cruzi* infection remains unexplored. Therefore, *T. cruzi* infection might present additional conditions that could induce behavioral abnormalities. Accordingly, Arankowaky-Sandoval et al. [7] demonstrated both sleep

dysfunction and memory impairment in *T. cruzi*-infected rats, although these conditions might not have been caused by CNS lesions alone. Importantly, memory deficits, lower quality of life, and depression have been reported in children and young adults during the chronic phase of Chagas disease [38,57], although the causative trigger of these conditions has not been considered. Recently, Mosovich and et al. [59] proposed the study of Chagas disease as a model to investigate the relationship between cardiac disease and depression. Accordingly, the authors suggested that the stress induced by chronic stimulation leads to neurocognitive and cardiovascular diseases. In fact, a positive diagnosis of Chagas disease provokes a social

uncertainty that has a strong mental impact on patients, particularly because there is no cure for this disease [65]. Living with the knowledge of being a Chagas disease carrier can elicit psychological disturbances such as stress and other psychological symptoms [60]. However, whether the parasite or its molecules directly trigger the psychological alterations observed in Chagas disease carriers remains to be investigated.

In conclusion, *T. cruzi* infection is a parasitic disease that might lead to the development of behavioral disorders. However, the direct or indirect participation of the parasite, the cellular, and molecular mechanisms of the immune response that take place in the CNS and the systemic influence of neuro-immune-endocrine factors in these processes deserve to be properly explored.

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