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ORIGINAL PAPER

Trypanosoma cruzi: experimental parasitism in the central nervous system of albino mice

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Abstract *Trypanosoma cruzi* causes a pan-infection, Chagas disease, in American mammals through fecal transmission by triatomine insects, resulting in an acute phase parasitemia with intracellularity mainly in the myocells and cells of the central nervous system (CNS).The parasites, due to the immune response, then decrease in number, characteristic of the life-long chronicity of the disease. We infected a mouse model with isolates obtained from reservoirs and vectors from rural and urban endemic areas in Venezuela. Intracellular proliferation and differentiation of the parasite in astrocytes, microglia, neurons, endothelial cells of the piarachnoid, cells of the Purkinje layer, and spinal ganglion cells, as well as extracellularly in the neuropil, were evaluated during the acute phase. Damages were identified as meningoencephalitis, astrocytosis, reactive microglia, acute neuronal degeneration by central chromatolysis, endothelial cell hyperplasia, edema of the neuropil, and satellitosis. This is the first time that satellitosis has been reported from a mammal infected with *T. cruzi*. Intracellular *T. cruzi* and inflammatory infiltrates were found in cardiac and skeletal myocytes and liver cells. No parasitism or alterations to the CNS were observed in the chronic mice, although they did show myocarditis and myocitis with extensive infiltrates. Our results are discussed in relation to hypotheses that deny the importance of the presence of tissue parasites versus the direct relationship between these and the damages produced during the chronic phase of Chagas disease. We also review the mechanisms proposed as responsible for the nervous phase of this parasitosis.

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Introduction

American trypanosomiasis or Chagas disease, caused by the hemoflagellate *Trypanosoma (Schizotrypanum) cruzi* Chagas, 1909 (Kinetoplastida, Trypanosomatidae), is a metaxenic parasitosis whose principal mechanism of natural transmission to humans and other mammals is by contamination of the skin or mucous membranes with the feces or urine of obligatory hematophagous insect vectors (Hemiptera, Reduviidae and Triatominae). An estimated 12 million people are infected with the parasite and 80 million exposed to infection in 22 countries within the American continent, between the USA and Argentina and Chile (42° N to 49° S), this distribution being superimposed on the distribution of the triatomines (Zingales 2011).

Due to the low mortality rates (<5 %) during the acute phase of Chagas disease and the fact that it generally occurs in rural areas affecting the most unprotected and forgotten sectors of society, investigations into the effects of *T. cruzi* on the CNS during this phase have been, in most cases, based on old data. This, in spite of the fact that Vianna's (1911) pioneering research identified pronounced damages

to the brain, cerebellum and spinal cord accompanied by the multiplication and intracellular differentiation of the parasite in neuroglial cells in human patients. This author was also the first to interpret the pathogenesis of the process, which he characterized as a meningoencephalomyelitis. Chagas (1916), Villela and Torres (1926), Villela and Villela (1932), and others confirmed these results using experimental animals, as well as expanding our knowledge about several neuroglial cells as hosts for the parasite.

Following vector transmission, there is a 4–10-day incubation period (prepatency) during which one or two generations of *T. cruzi* intracellular amastigote develop preceding the evolution of the acute phase (patency). The *T. cruzi*–vertebrate host relationship at this stage is characterized by parasitemia, the invasion, proliferation, and intracellular differentiation of trypanosome stages, damages to parasitized tissues, the immune response, and inflammatory infiltrates that lead to cardio- and digestive problems, and, in many cases, damage to the CNS, these being the most common causes (10 %) of fatal acute cases. Histologically, the acute form of this trypanosomiasis is a systemic infection and the intracellular stages of the parasite can be found in all organs and cell types except non-nucleated erythrocytes and thrombocytes (Lenzi et al. 1996). A decrease in these characteristic alterations defines the transition to the chronic phase of the parasitosis (Pittella 1991; Dias 2000; Rassi et al. 2000).

From the literature, we have identified aspects that deserve further investigation into the behavior of *T. cruzi* in the central nervous system (CNS) such as: use of the same *T. cruzi* strain in different reports by the same author, use of metacyclic trypomastigotes obtained from sources different to those of natural transmission, very high inoculum levels, no identification of the parasitized or injured cells or those involved in the inflammatory reaction to the parasitic infection, and controversies over neuronal parasitism. In order to clarify some of these aspects, we carried out studies using isolates from reservoirs and vectors captured in northeastern Venezuela (Morocoima et al. 2010).

Material and methods

Experimental infections: isolation of *T. cruzi*

Reservoirs (opossums, *Didelphis marsupialis*, Didelphimorphia, Didelphidae) captured in rural and urban areas endemic for Chagas disease in the north of Anzoátegui State (Northeastern Venezuela) were xenodiagnosed using fourth instar nymphs of *Rhodnius prolixus*. After dissection of these triatomines, rectal contents were diluted in sterile isotonic solution 0.85 % (*w*/*v*) and examined microscopically (×400). After the determination of the number of metacyclic trypomastigotes (Brener 1962), aliquots were inoculated i.p. into young NMRI mice (five mice/isolate; mean weight, 12 g; 20 days old) using an infection does of (200 metacyclics/g). The flagellates were identified as *T. cruzi* according to Barretto (1979). Blood samples taken from the tail veins of the experimental animals were examined microscopically (×400) 3 days postinfection (dpi) and thereafter three times weekly, in order to determine the prepatent period, quantify the parasitemia according to Brener (1962) and specify the acute and chronic phases of the parasitosis. The symptoms of infection and mortality rates were noted daily.

Vectors (*R. prolixus* and *Triatoma maculata*) infected naturally with *T. cruzi* (Morocoima et al. 2010) were obtained from coconut palms (*Cocos nucifera* L.; Palmae) close to houses in the villages under study. Metacyclics were then isolated from these vectors and used to infect the mouse model as described above to obtain the *T. cruzi* isolates.

Study of the behavior of T. cruzi isolates

Infected mice were killed during the period of highest parasitemia and in the chronic phase when this occurred. One mouse in each batch was killed, and samples of the brain and cerebellum were extracted; the spinal cord and the spinal ganglion were also extracted. The possible spread of the parasitism to other tissues was determined considering the heart, skeletal muscle, liver, lung, adipose tissue, and urinary bladder. All samples were immediately fixed in formalin (10 %), processed histologically, embedded in paraplast, sliced into 3-μm sections, and dyed with hematoxylin–eosin. Tissue parasitism and alterations were identified microscopically (×400 and ×1,000) and photographed using a digital camera Genius G-Shot P510. Photomicrographs were converted to gray scale and adjusted to 300 pixels.

Results

Five isolates from opossums, two from *R. prolixus* and three from *T. maculata*, gave the following results (means) in experimental mice (n=50) over the course of infection. Isolates from opossums and triatomines, respectively: prepatent period (dpi)=11.5–14; highest parasitemia (/ml)=3.25×10^5–4.25×10^5; time (dpi) to reach maximum parasitemia=24–22.5; duration of the parasitemia=36–41 days; mortality=80–100 %. Of these isolates, two from *D. marsupialis* (MDID/VE/2009/AM10); MDID/VE/2009/RC1), one from *R. prolixus* (TRPX/VE/2009/RP3), and another from *T. maculata* (TTMA/VE/2009/TMG1) invaded the CNS of the experimental animals, producing the following neurological symptoms 15 days (on average) after the onset of the acute phase: walking disorders (hesitation, incoordination, circular running with sideways tilt always towards

the same side), paraplegia in the hind limbs, ataxia up to the point of paralysis, and relaxation of the vesical sphincter. Loss of weight, reduced growth, abdominal swelling, and pronounced hair loss were also registered. These symptoms were extreme in chronically affected mice.

Overall, the CNS samples showed the following alterations in the brain (Fig. 1) and cerebellum (Fig. 2): meningoencephalitis of the cortex; edema of the neuropil, satellitosis, and astrocytosis in the white matter; reactive microglia; acute central neuronal degeneration by chromatolysis; random or focally distributed infiltrations with lymphocytic cells and polymorphonuclear leukocytes, sometimes near microvessels; and hyperplasia of endothelial cells.

Furthermore, an isolate from *D. marsupialis* (MDID/VE/2009/AM10), one from *R. prolixus* (TRPX/VE/2009/RP3), and another from *T. maculata* (TTMA/VE/2009/TMG1) produced pseudocysts in the brain with amastigotes or trypomastigotes of *T. cruzi* in the cytoplasm of astrocytes and in the neuropil of the white matter (Fig. 1). Likewise, intracellular proliferation of *T. cruzi* by isolates from opossums (MDID/VE/2009/RC1), *R. prolixus* (TRPX/VE/2009/RP3), and *T. maculata* (TTMA/VE/2009/TMG1) was found in the following areas of the cerebellum (Fig. 2): cortex (amastigote nests in astrocytes associated with edema of the neuropil); granular layer (amastigote and flagellate stages in the microglial cells with edema of the neuropil and satellitosis); piarachnoid (amastigotes in meningoendothelial cells); Purkinje layer cells; and white matter (pseudocysts with amastigote forms in astrocytes). Both intra- and extracellular parasitic pseudocysts were observed in the spinal cord and spinal ganglion (Fig. 3).

All isolates moderately invaded cardiac and skeletal myocytes and hepatocytes (Fig. 4); Other tissues were sparsely invaded.

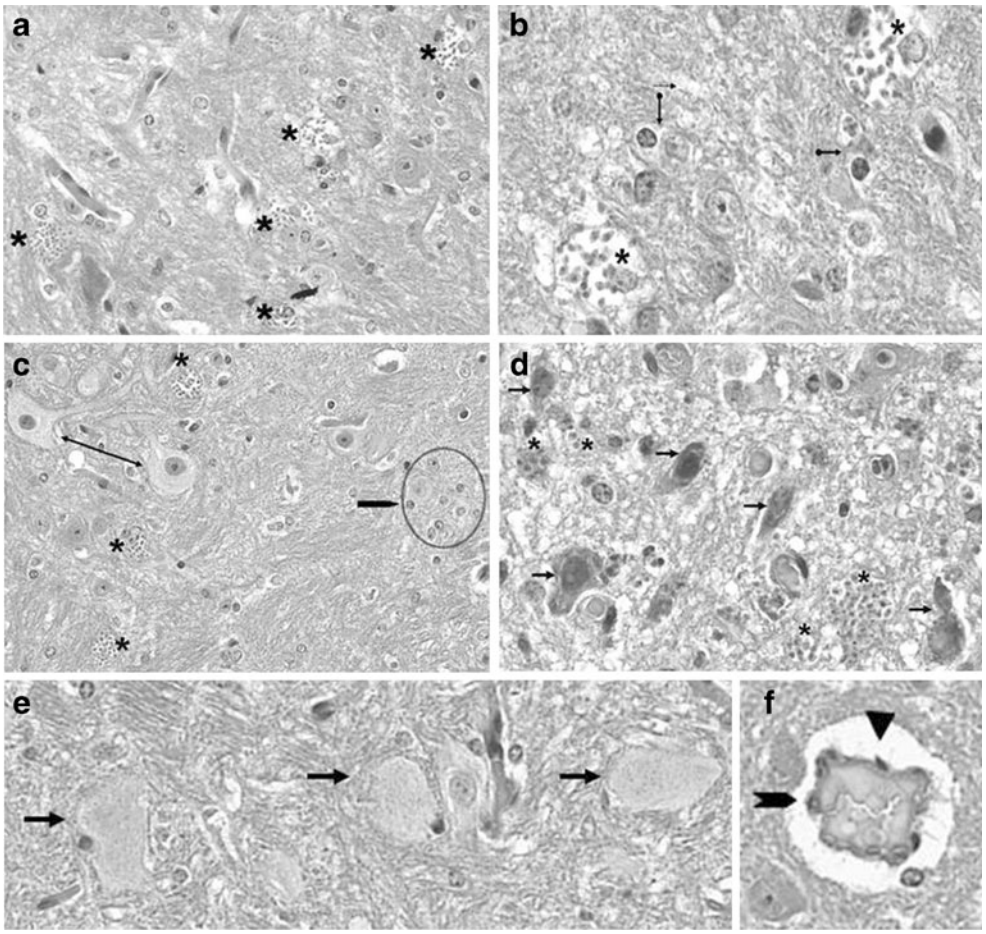

Fig. 1 Photomicrographs of stained histological sections of the brain of experimentally infected mice. **a** White matter. Scattered pseudocysts with amastigote forms of *Trypanosoma cruzi* in astrocytes; several adjacent to blood vessels (H–E; ×400; isolate MDID/VE/2009/AM10). **b** Highly developed nests with numerous *T. cruzi* amastigotes, intermediate and flagellate stages in astrocytes; observe satellitosis and edema of the neuropil (H–E; ×1,000; isolate TRPX/VE/2009/RP3). **c** Pseudocysts with amastigote forms of *T. cruzi* in astrocytes; adjacent neurons not parasitized; astrocytosis (H–E; ×400; isolate MDID/VE/2009/AM10). **d** Extracellular amastigote stages of *T. cruzi*; several neurons with acute degeneration; edematous neuropil (H–E; ×1,000; isolate MDID/VE/2009/AM10). **e** Several neurons degenerated by central chromatolysis (H–E; ×400; isolate TRPX/VE/2009/RP3). **f** Hyperplasia of the endothelial cells; artifact of perivascular clearance by tissue autolysis (H–E; ×400; isolate TRPX/VE/2009/RP3)

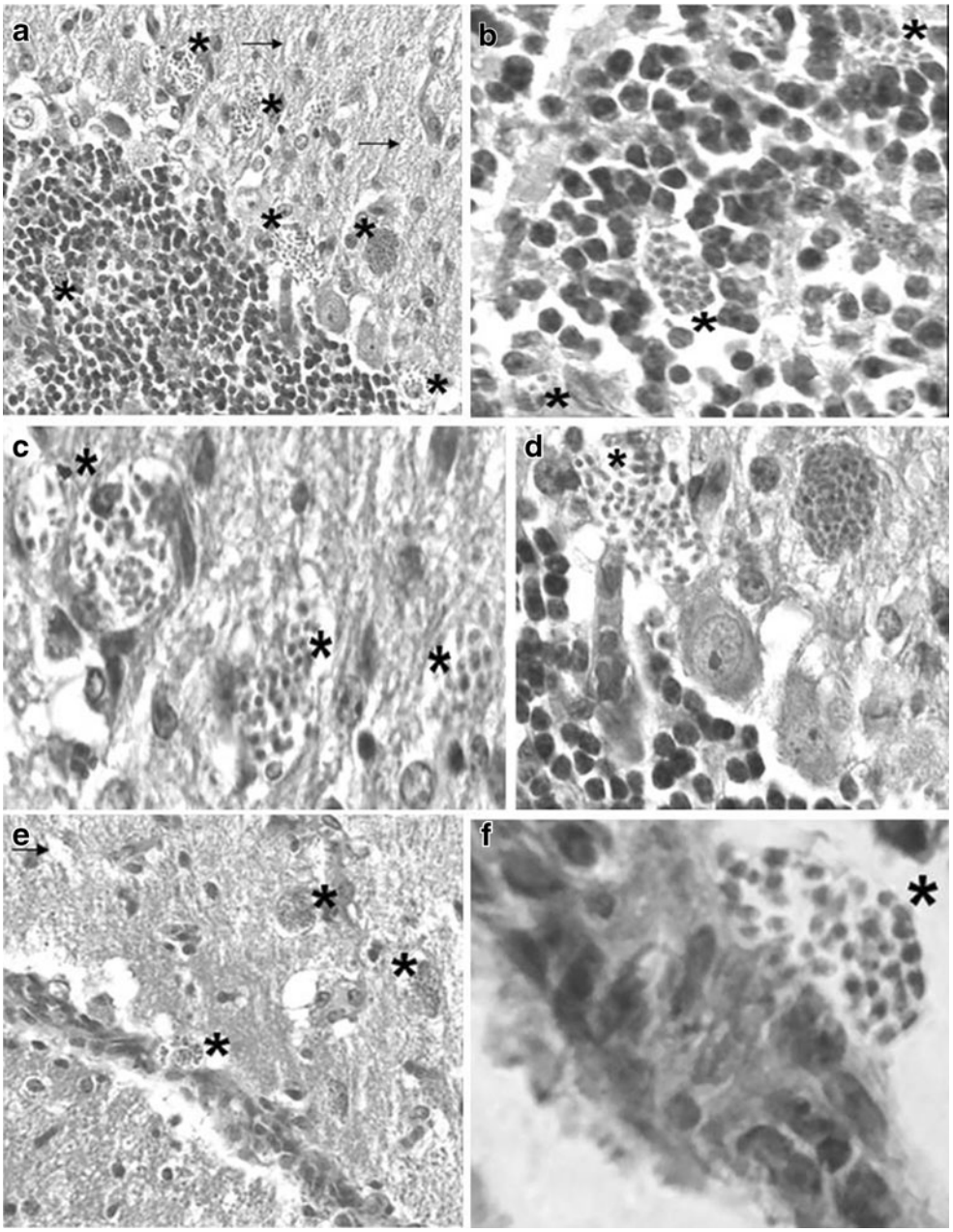

Fig. 2 Photomicrographs of stained histological sections of the cerebellum of experimentally infected mice. **a** Several focalized *T. cruzi* amastigote nests in: the microglial cells of the white matter, the Purkinje layer and the granular layer associated with edema of the neuropil (H–E; ×400; isolate MDID/VE/2009/RC1). **b** Pseudocysts with amastigotes in microglial cells of the granular layer (H–E; ×1,000; isolate MDID/VE/2009/RC1). **c** Several focalized nests with *T. cruzi* amastigote stages in the microglial cells of the white matter (H–E; ×1,000; isolate MDID/VE/2009/RC1). **d** Purkinje layer cells with *T. cruzi* amastigote pseudocysts (H–E; ×1,000; isolate MDID/VE/2009/RC1). **e** *T. cruzi* amastigote nest in a meningoendothelial cell of the piarachnoid coat and in astrocytes, associated with edema of the neuropil (H–E; ×400; isolate MDID/VE/2009/RC1). **f** *T. cruzi* amastigote pseudocyst in a meningoendothelial cell of the piarachnoid coat (H–E; ×1,000; isolate MDID/VE/2009/RC1)

Finally, four mice inoculated with two of the *T. maculata* isolates (TTMA/VE/2009/TMG1; TTMA/VE/2009/TMG2) survived until the chronic phase (90 dpi). All individuals showed the most extreme nervous symptoms accompanied by myocarditis and skeletal myositis with extensive inflammatory infiltrates. No parasites were, however, observed (Fig. 4).

Discussion

According to Andrade (2000), the study of the experimental behavior of *T. cruzi* in infected mammals requires prior knowledge of the susceptibility of the animal model to the trypanosome. The animals can thus be infected with the inoculum of parasites that causes them to develop an immunologically controllable acute phase, which enables the manifestations of the chronic phase to evolve naturally. The susceptibility of mice in general, despite their genetic diversity, is sufficiently similar (and well known) to give reproducible results as regards the development of the phases of Chagas disease. It is for this reason that we used NMRI mice as a model to investigate *T. cruzi* tissue parasitism in different cells and organs (Sampson-Ward and Urdaneta-Morales 1988; Herrera and Urdaneta-Morales 1992; Scorza et al. 1989).

In addition, we used metacyclic trypomastigote stages harvested from triatomines infected with *T. cruzi* to

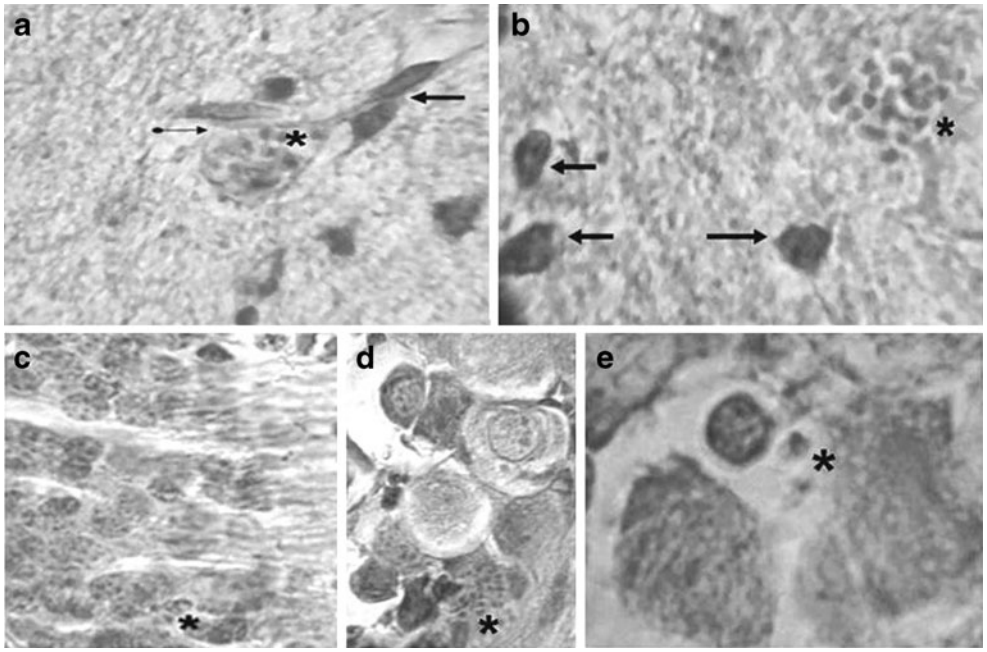

Fig. 3 Photomicrographs of stained histological sections of the spinal cord and spinal ganglion of experimentally infected mice. **a** Spinal cord: Neuronal satellitosis with amastigote nest in the edematous neuropil, acute neuronal degeneration (H–E; ×1,000; isolate MDID/VE/2009/RC1). **b** *T. cruzi* pseudocyst in edematous neuropil, acute neuronal degeneration (H–E; ×1,000; isolate MDID/VE/2009/RC1). **c** Amastigote nest in gray matter with edema of the neuropil (H–E; ×400; isolate MDID/VE/2009/RC1). **d** Spinal ganglion with amastigote nest in capsule cell (H–E; ×400; isolate MDID/VE/2009/RC1). **e** Spinal ganglion: Intracellular *T. cruzi* amastigote nest in ganglion cell (H–E; ×1,000; isolate MDID/VE/2009/RC1)

inoculate the mice since these are the parasite stages that are naturally transmitted and also because they have proven to be highly infective compared to blood or culture media parasites (Zeledón et al. 1977).

Furthermore, given the high heterogeneity of subpopulations of *T. cruzi* (Yeo et al. 2005; Zingales et al. 2009), we used 10 isolates from reservoirs and vectors captured in endemic rural and urban areas. Regarding this, we must re-emphasize that Lenzi et al. (1996) did not observe either parasitism or inflammatory infiltrates in the CNS when he used the CL strain that parasitizes cells of three embryonic strata, despite infecting the peripheral nervous system and

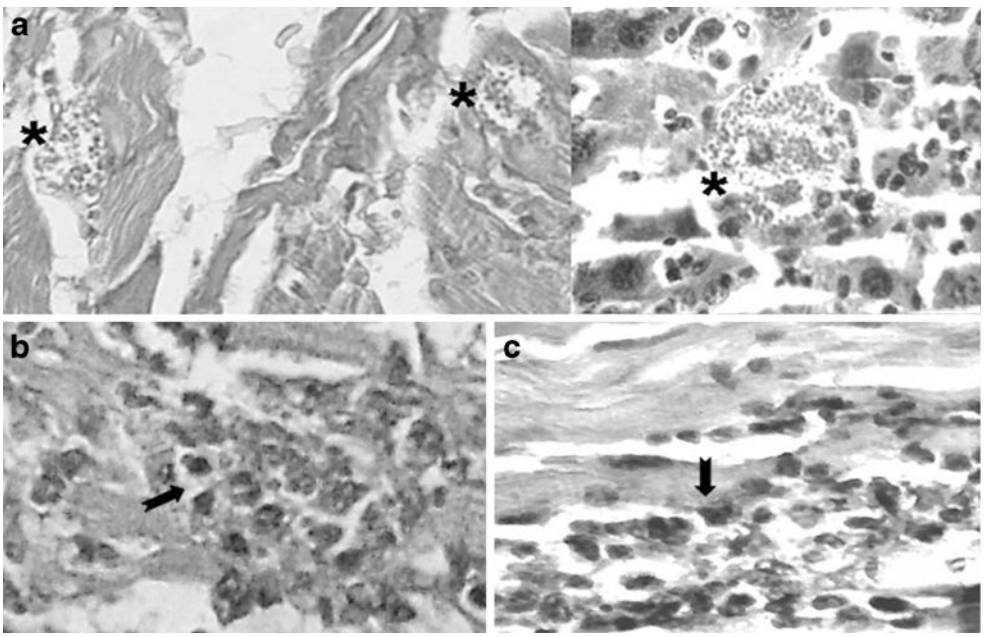

Fig. 4 Photomicrographs of stained histological sections of other tissues of experimentally infected mice: skeletal muscle fibers (**a**) and liver cells (**b**) showing pseudocysts with *T. cruzi* amastigotes in the acute phase of Chagas´ disease (H–E; ×400; isolate MDID/VE/2009/AM10); skeletal muscle fibers (**c**) and cardiac muscle (**d**) of the chronic phase showing diffuse inflammatory infiltrates and loss of muscle fibers (H–E; ×1,000; isolate TTMA/VE/2009/TMG1)

autonomic ganglia. Neither did Silva et al. (1999) find amastigotes in the CNS of mice infected with the Colombian strain notwithstanding the high parasitemias produced. This highlights the complex histotropism of *T. cruzi* that produces, among other characteristics, the aforementioned heterogeneity underlining the need to use parasite isolates from different sources when undertaking investigations such as this.

The CNS cells parasitized or damaged by *T. cruzi* have not been completely identified, as a detailed review of the available scientific information revealed. In this study, we identified the presence of *T. cruzi* amastigote, intermediate and trypomastigote stages, either localized or scattered, in astrocytes, microglia, meningoendothelial cells of the piarachnoid coat, Purkinje cells, brain and cerebellar cortex cells, and cells of the spinal cord and spinal ganglion. Astrocytes and microglia proved to be more frequent and appropriate environments for the parasites, thus explaining why they are the main targets for invasion and proliferation of *T. cruzi* in the CNS, regardless of the origin of the parasite subpopulation used. In addition, these cells were often found adjacent to vascular structures, which could facilitate parasite dispersion.

We would like to highlight our observations of the damages to the CNS produced by *T. cruzi*, such as: meningoencephalitis, edema of the neuropil, astrocytosis, hyperplasia of endothelial cells, satellitosis, and acute degeneration of neurons by central chromatolysis. Although chromatolysis has been previously reported (Villela and Villela 1932), it has been scarcely documented elsewhere and has even been debated by some authors. This is the first time, as far as we are aware, that satellitosis has been reported from a mammal infected with *T. cruzi*.

Our results lead us to suggest that the different aspects we have discussed regarding the *T. cruzi*–mammal–host relationship should be further investigated in order to try to explain the genesis of the tissular lesions observed during the chronic phase of Chagas disease. Teixeira et al. (2011), after discussing the persistence of the parasites, autoimmunity, and the neurogenic hypothesis, accepted the cryptic persistence of *T. cruzi* in chronic patients with cardiac disease, megacolon, and megaesophagous, even though they judged that these alterations were not supported by their findings.

Conversely, the fact that parasites have been found in blood cultures or by xenodiagnosis and that parts of their genome have been detected by molecular biology tools in chronically infected patients suggests the ubiquitous and persistent presence of pseudocysts with amastigotes encrypted in the tissues that could explain the intricate pathogenesis of chronic Chagas disease. This behavior of *T. cruzi* could be facilitated by regional differences in the dynamics of the immune response against the parasite in specific sites such as cardiac, skeletal, intestinal, and nervous tissues (Zhang and Tarleton 1999; Tarleton 2001; Higuchi et al. 2003; Marinho et al. 2004; Benvenuti et al. 2008; Marcon et al. 2011). According to these authors, the presence of *T. cruzi* and/or metabolites is absolutely correlated with the severity of the chronic phase of the disease, and, although the number of amastigotes is relatively low, their pathogenicity is comparable to that of the acute phase (Añez et al. 1999). Furthermore, intracellular amastigotes and trypomastigotes have been identified in preadipocytes and mature adipocytes of mice in the acute phase and *T. cruzi* has been detected by PCR in adipose tissue up to 300 dpi (Combs et al. 2005; Herrera et al. 2005; Nagajyothi et al. 2009). In addition, amastigotes have been observed in in the gingiva of patients and fibroblasts in the cornea and placental stroma of experimental animals (Moreno et al. 2005; Herrera et al. 2007; Añez et al. 2011). Thus, the many and diverse tissue microhabitats available to the parasites could act as long-term reservoirs during the chronic phase, from which infection relapses could occur.

The CNS is considered an immunoprivileged site since the entrance of macromolecules and immune cells is restricted. However, this system is a target for acute infections by viruses and parasites including *T. cruzi*, depending on the state of the blood–brain barrier that is formed during the fetal stage and develops gradually from then onwards to days to weeks after birth (Xu and Ling 1994). Thus, one of the reasons why the parasites were able to use different types of nervous cells as hosts, their wide proliferation, and the severity and extent of the injuries observed in the CNS could be that the mice we used were juveniles and consequently had an undeveloped blood–brain barrier (Da Mata et al. 2000).

Some parasites may manage to infiltrate and infect the CNS by avoiding its unique immune response. Examples of CNS-invasive parasites are *Acanthamoeba culbertsoni*, *Naegleria fowleri*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Angiostrongylus cantonensis*, *Strongyloides stercoralis*, *Toxocara canis*, *Trichinella spiralis*, *Paragonimus westermani*, *Taenia solium*, *Histoplasma capsulatum*, and *Paracoccidiodes brasiliensis* (Syed and Syed 2010).

As has already been mentioned, histopathological alterations in the CNS during the acute phase of Chagas disease have been demonstrated by Vianna (1911), Chagas (1916), Villela and Torres (1926), and Villela and Villela (1932), among others. Chagas (1916) emphasized the possibility of a nervous form of the disease, based on motor, intelligence, and language impairments in children. Nevertheless, the effects of the parasite on the CNS during the chronic phase as well as the behavioral changes it may produce after invasion of the host brain require further study.

Because of this, and the fact that in our study the symptoms shown by the animals infected with *T. cruzi* were unquestionably neurological, we would like to discuss recent

investigations about the Apicomplexa parasite *T. gondii*. *T. gondii* is a ubiquitous intracellular obligatory of cells of the CNS (astrocytes, microglia, and neurons) and myocytes, among others and causes neurological damage in the acute phase of the infection as well as changes in the natural behavior of the host in the chronic phase. This parasite has been held responsible for behavioral disturbances in its intermediate hosts (rodents, lagomorphs, insectivores, carnivores, and primates, including man), such as learning retardation and memory performance, decreased climbing in novel and familiar environments, and an increase in the number of movements (sometimes with circular movements towards one side). An increase in the predation of rodents and in the transmission of the parasite to its definitive hosts (felines, especially the domestic cat) has also been reported due to a decrease in exploratory activity of the prey necessary for obtaining basic information about their environment. This has been linked to infections of the rodents by slow growing bradyzoite-containing cysts, localized mainly in the CNS, muscle and eye tissues. These cysts pass into the ileum, enterocytes, and excyst, then differentiate into fecal oocytes, and are shed into the environment, where they contaminate the food and water supply, infecting per os additional felines as well as intermediate hosts (Witting 1979; Hay et al. 1985; Silva and Langoni 2009; Gatkowska et al. 2012).

Bradyzoites present in intermediate hosts eaten by felines may also differentiate into rapidly growing tachyzoites, thus repeating the cycle. In addition, *T. gondii* possesses the unique ability to bypass reproduction within the feline and directly infect intermediate hosts by oral ingestion. A similar cycle may be produced in humans on eating raw or undercooked meat. The molecular mechanisms by the interconversion of tachyzoites and bradyzoites is not well defined (Sullivan et al. 2009; Kamerkar and Davis 2012).

Immunosuppressed humans by tissue transplants or chemotherapy for cancer or infection by AIDS are particularly susceptible to these types of infection. The tachyzoites proliferate indiscriminately and differentiate into bradyzoites, which have particular cardiac and CNS tissue tropism, leading to severe cardiovascular and neurological complications. Recent results have demonstrated the tight relationship between neurosychiatric disorders (epilepsy, schizophrenia, and Parkinson' disease) and infection by *T. gondii.* These are produced when the humoral and cellular immune response becomes compromised, mainly affecting the cerebral hemisphere, cerebellum, and basal ganglia. Further studies are necessary in order to establish the role of *T. gondii* in the etiopathogenesis of these disorders (Yolken et al. 2009; da Silva and Langoni 2009; Miman et al. 2010; Contreras-Ochoa et al. 2012).

In a similar way to toxoplasmosis, the reactivation of the chronic phase with the CNS as a primary target is produced in immunosuppressed humans infected with *T. cruzi* with numerous amastigotes found in the astrocytes and microglia of white matter in the brain and blood (Mattosinho-Franca et al. 1969; Rocha et al. 1994; Córdova et al. 2010).

The elevated expression of chemokines and receptors in inflammatory cells or blood vessel endothelial cells of the CNS associated with degenerative chronic diseases has been studied by Silva et al. (2010) in relation to Chagas disease. These authors also suggest the need to determine whether glial cells control the parasites or the neuroimmune response during chronic meningoencephalitis.

Our suggestion of the presence of many and ubiquitous microniches that lodge parasites, thus acting as reservoirs during the chronic phase and producing infection relapses, should be tested with regard to Pitella (2009) who studied patients with chronic heart disease and cerebral infarctions and Prost et al. (2000) who demonstrate electrophysiologically cerebral envelopment during chronic Chagas´disease.

Mangone et al. (1994) and Py et al. (2009) showed an association between Chagas disease and cognitive dysfunction as white matter brain disease, as well as between parasympathetic system alterations and white matter brain lesions, respectively. Silva et al. (2010) observed neurological signals in experimental mice similar to those found in our investigation and in studies on *T. gondii* (Gatkowska et al. 2012). These authors are at present undertaking experiments to determine the possible influence of parasite infection and the immune response of the CNS in behavioral abnormalities.

These findings, following the suggestion made by Coura (2010) that new approaches to the study of Chagas disease should be taken, indicate that it would be interesting to establish whether certain neurotropic *T. cruzi* strains behave in the CNS in a similar way to that of the aforementioned findings.

Descriptors of photomicrographs

Photomicrographs of hematoxylin–eosin (H–E) stained histological sections of parasitism and tissue alterations produced by *Trypanosoma cruzi* in the brain (Fig. 1), cerebellum (Fig. 2), spinal cord and spinal ganglion (Fig. 3), and other tissues (Fig. 4) of mice experimentally infected with isolates from opossums and triatomines. The symbols used in the figures are as follows:

*	Intra- or extra-cellular pseudocysts
→	Edema of the neuropil
●→	Satellitosis
←→	Normal neurons
⇒	Damaged neurons
▬	Astrocytosis
⋙	Hyperplasia of endothelial cells
▼	Artifact of perivascular clearance
⇛	Diffuse inflammatory infiltrates and loss of muscle fibers

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References

Andrade SG (2000) Patologia experimental da doença de Chagas. In: Brener Z, Andrade Z, Barral-Netto M (eds) *Trypanosoma cruzi* e doença de Chagas, 2nd edn. Guanabara Koogan, Rio de Janeiro, pp 177–200

Añez N, Carrasco H, Parada H, Crisante G, Rojas A, Fuenmayor C, Gonzalez N, Percoco G, Borges R, Guevara P, Ramirez JL (1999) Myocardial parasite persistence in chronic chagasic patients. Am J Trop Med Hyg 60:726–732

Añez N, Crisante G, Caraballo F et al (2011) *Trypanosoma cruzi* persistence at oral inflammatory foci in chronic chagasic patients. Acta Trop 117:207–211

Barretto MP (1979) Epidemiologia. In: Brener Z, Andrade Z (eds) *Trypanosoma cruzi* e doença de Chagas, 1st edn. Guanabara Koogan, Rio de Janeiro, pp 89–151

Benvenuti LA, Roggerio A, Freitas HF et al (2008) Chronic American trypanosomiasis: parasite persistence in endomyocardial biopsies is associated with high-grade myocarditis. Ann Trop Med Parasitol 102:481–487

Brener Z (1962) Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi*. Rev Inst Med Trop Sao Paulo 4:389–396

Chagas C (1916) Tripanosomiase americana. Forma aguda da molestia. Mem Inst Oswaldo Cruz 8:37–59

Combs TP, Mukheriee S, Almeida CJ et al (2005) The adipocyte as an important target cell for *Trypanosoma cruzi*. J Biol Chem 280:24085–24094

Contreras-Ochoa C, Lagunas- Martínez A, Belkind J et al (2012) *Toxoplasma gondii* invasion and replication in astrocyte primary cultures and astrocytoma cells lines: systematic review of the literature. Parasitol Res 110:2089–2094

Córdova E, Maiolo E, Corti M et al (2010) Neurological manifestation of Chagas' disease. Neurol Res 32:238–244

Coura JR (2010) Chagas disease: a new worldwide challenge. Nature 465:S6–S7

Da Mata JR, Camargos E, Chiari E et al (2000) *Trypanosoma cruzi* infection and the rat central nervous system: proliferation of parasites in astrocytes and the brain reaction to parasitism. Brain Res Bull 53:153–162

da Silva RC, Langoni H (2009) *Toxoplasma gondii*: host–parasite interaction and behavior manipulation. Parasitol Res 105:893–898

Dias JCP (2000) Epidemiologia. In: Brener Z, Andrade Z, Barral-Netto M (eds) *Trypanosoma cruzi* e doença de Chagas, 2nd edn. Guanabara Koogan, Rio de Janeiro, pp 48–74

Gatkowska J, Wieczorek M, Dziadek B et al (2012) Behavioral changes in mice caused by *Toxoplasma gondii* invasion of brain. Parasitol Res 111:53–58

Hay J, Aitken PP, Arnott MA (1985) The influence of congenital *Toxoplasma* infection on the spontaneous running activity of mice. Z Parasitenkd 71:459–462

Herrera L, Urdaneta-Morales S (1992) *Didelphis marsupialis*: a primary reservoir of *Trypanosoma cruzi* in urban areas of Caracas, Venezuela. Ann Trop Med Parasitol 86:607–612

Herrera L, Morocoima A, Aguilar CM, Urdaneta-Morales S (2005) *Trypanosoma cruzi*: parasitismo del tejido conectivo adiposo. Rev Cient FCV-LUZ 15:210–216

Herrera L, Martínez C, Carrasco H, Jansen AM, Urdaneta-Morales S (2007) Cornea as a tissue reservoir of *Trypanosoma cruzi*. Parasitol Res 100: 1395–1399

Higuchi ML, Benvenuti LA, Martins RM et al (2003) Pathophysiology of the heart in Chagas' disease: current status and new developments. Cardiovasc Res 60:96–107

Kamerkar S, Davis PH (2012) *Toxoplasma* on the brain: understanding host-pathogen interactions in chronic CNS infection. Parasitol Res 2012:589295. doi:10.1155/2012/589295

Lenzi H, Oliveira D, Lima M et al (1996) *Trypanosoma cruzi*: paninfectivity of CL strain during murine acute infection. Exp Parasitol 84:16–27

Mangone CA, Sica RE, Pereyra S et al (1994) Cognitive impairment in human chronic Chagas'disease. Arq Neuropsiquiatr 52:200–203

Marcon GE, Albuquerque DM, Batista AM et al (2011) *Trypanosoma cruzi*: parasite persistence in tissues in chronic chagasic Brazilian patients. Mem Inst Oswaldo Cruz 106:85–91

Marinho CR, Bucci DZ, Dagli ML (2004) Pathology affects different organs in two mouse strains chronically infected by a *Trypanosoma cruzi* clone: a model for genetic studies of Chagas' disease. Infect Immun 72:2350–2357

Mattosinho-Franca LC, Fleury R, Ramos AJ et al (1969) Molestia de chagas cronica associada a leucemia linfatica: ocorrencia de encefalite aguda como alteraçao do estado imunitario. Arq Neuropsiquiatr 27:59–66

Miman O, Kusbeci OY, Aktepe OC et al (2010) The probable relation between *Toxoplasma gondii* and Parkinson's disease. Neurosci Lett 475:129–131

Moreno E, Mendez M, Alarcon M et al (2005) Reactivación de la infección chagasica en ratas Wistar gestantes. Kasmera 33:51–63

Morocoima A, Chique J, Zavala-Jaspe R et al (2010) Commercial coconut palm as an ecotope of Chagas disease vectors in northeastern Venezuela. J Vector Borne Dis 47:76–84

Nagajyothi F, Desruisseaux M, Weiss LM et al (2009) Chagas disease, adipose tissue and the metabolic syndrome. Mem Inst Oswaldo Cruz 104(Suppl 1):219–225

Pitella JEH (2009) Central nervous system involvement in Chagas disease: a hundred-year-old history. Trans R Soc Trop Med Hyg 103:973–978

Pittella JEH (1991) Central nervous system involvement in experimental *Trypanosomiasis cruzi*. Mem Inst Oswaldo Cruz 86:141–145

Prost JO, Romero VH, Morikone AM et al (2000) Evidence of cerebral involvement in the chronic stage of Chagas disease obtained using the P300 potential and quantified electroencephalography. Arq Neuropsiquiatr 58:262–271

Py M, Pedrosa R, Silveira J et al (2009) Neurological manifestations in Chagas disease without cardiac dysfunction: correlation between dysfunction of the parasympathetic nervous system and white matter lesions in the brain. J Neuroimaging 19:332–336

Rassi A, Rassi Junior A, Rassi GG (2000) Fase aguda. In: Brener Z, Andrade Z, Barral-Netto M (eds) *Trypanosoma cruzi* e doença de Chagas, 2nd edn. Guanabara-Koogan, Rio de Janeiro

Rocha A, Meneses AC, da Silva AM et al (1994) Pathology of patients with Chagas' disease and acquired immunodeficiency syndrome. AmJTrop Med Hyg 50:261–268

Sampson-Ward L, Urdaneta-Morales S (1988) Urban *Trypanosoma cruzi*: biological characterization of isolates from *Panstrongylus geniculatus*. Ann Soc belge Méd trop 68:95–106

Scorza C, Urdaneta-Morales S, Sampson-Ward L (1989) Urban *Trypanosoma cruzi*: pathology in white mice of isolates from *Panstrongylus geniculatus*. Ann Soc belge Méd trop 69:283–289

Silva RC, Langoni H (2009) *Toxoplasma gondii*: host–parasite interaction and behavior manipulation. Parasitol Res 105:893–898

Silva AA, Roffé E, Marino AP et al (1999) Chagas´disease encephalitis: Intense $CD8^+$ lymphocytic infiltrate is restricted to the acute phase, but is not related to the presence of *Trypanosoma cruzi* antigens. Clin Immunol 92:56–66

Silva AA, Pereira GV, Souza AS et al (2010) *Trypanosoma cruzi*-induced central nervous system alterations: from the entry of inflammatory cells to potential cognitive and psychiatric abnormalities. J Neuroparasitol 1:N100901. doi:10.4303/jnp/N100901

Sullivan WJ, Smith AT, Joyce BR (2009) Understanding mechanisms and the role of differentiation in pathogenesis of *Toxoplasma gondii*—a review. Mem Inst Oswaldo Cruz 104:155–161

Syed R, Syed A (2010) How CNS-invasive parasites evade the brain immune system http://www.stanford/edu/group/parasites/ParaSites2010/Adnan_and_Rehan_Syed/Parasites. Stanford University

Tarleton RL (2001) Parasite persistence in the etiology of Chagas'disease. Int J Parasitol 31:549–553

Teixeira ARL, Hecht MM, Guimaro MC, Sousa AO, Nitz N (2011) Pathogenesis of Chagas' Disease: parasite persistence and autoimmunity. Clin Microbiol Rev 24:592–630

Vianna G (1911) Contribuiçao para o estudo da anatomia patolojica da "Molestia de Carlos Chagas". Mem Inst Oswaldo Cruz 3:276–294

Villela E, Torres CM (1926) Estudo histopathologico do systema nervoso central em paralysia experimental determinada pelo *Schizotrypanum cruzi*. Mem Inst Oswaldo Cruz 19:175–198

Villela E, Villela E (1932) Elementos do sistema nervoso central parasitados pelo *Trypanosoma cruzi*. Mem Inst Oswaldo Cruz 26:77–79

Witting PA (1979) Learning capacity and memory of normal and *Toxoplasma*-infected laboratory rats and mice. Z Parasitenkd 61:29–51

Xu J, Ling EA (1994) Studies of the ultrastructure and permeability of the blood–brain barrier in the developing corpus callosum in postnatal rat brain using electron dense tracers. J Anat 184:227–237

Yeo M, Acosta N, Llewellyn M (2005) Origins of Chagas disease: *Didelphis* species are -natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *T. cruzi* II, including hybrids. Int J Parasitol 35:225–233

Yolken RH, Dickerson FB, Torrey EF (2009) *Toxoplasma* and schizophrenia. Parasite Immunol 31:706–715

Zeledón R, Trejos M, Chinchilla M (1977) Experimental infection of mice with blood, culture and insect forms of *Trypanosoma cruzi* by different routes. Protozoology 3:95–101

Zhang L, Tarleton RL (1999) Parasite persistence correlates with disease severity and localization in chronic Chaga´s disease. J Infect Dis 180:480–486

Zingales B (2011) Trypanosoma cruzi: um parasita, dois parasites ou vários parasitas da doença de Chagas? Rev Biol 6b:44–48

Zingales B, Andrade SG, Briones MRS et al (2009) A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz 104:1051–1054