MOUSE MODEL FOR CHAGAS DISEASE: IMMUNOHISTOCHEMICAL DISTRIBUTION OF DIFFERENT STAGES OF TRYPSANOSOMA CRUZI IN TISSUES THROUGHOUT INFECTION

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Abstract. Different stages of *Trypanosoma cruzi* are seen during mammalian infection. Histologic sections of infected hearts have shown amastigotes and, when using immunohistochemistry (IHC), parasite antigens; however, demonstration of trypomastigotes in these tissues has proven elusive. Using a mouse strain that develops chagasic cardiomyopathy (histologically similar to human infection) 70 days after injecting *T. cruzi*-Brazil strain, we studied the distribution of parasite stages and the extent of inflammation. All organs had varying amounts of mononuclear inflammation by day 10, which peaked between day 20 and day 30, and decreased by day 50. Amastigotes were detected in myocytes, histiocytes, acinar pancreatic cells, astrocytes and ependymal cells by day 10, and the number of amastigotes peaked on day 30. Immunohistochemistry demonstrated trypomastigotes in sinusoids, vessels and interstitial tissues of several organs between day 15 and 50. Abundant parasite antigens (granular staining) were detected in connective tissues throughout the infection. The burden of amastigotes and trypomastigotes during the acute phase seems to correlate with the degree of inflammation and granular staining in the chronic stage.

INTRODUCTION

*Trypanosoma cruzi* is a parasite endemic to many regions of Latin America, where an estimated 18 million people are infected. The chronic stage of infection starts 1 to 2 months after the end of the acute phase; however, symptomatic cardiomyopathy, megaesophagus or megacolon appear 10 to 30 years later in 30% of patients. The presence of *T. cruzi* trypomastigotes in histologic preparations has been difficult to demonstrate, although the presence of *T. cruzi* amastigotes has been demonstrated histologically in several tissues. In patients with chronic cardiomyopathy, inflammatory infiltrates and granular staining (representing either *T. cruzi* antigens and nucleic acids) have been reported using several techniques. In the present study, all parasite mammalian forms (amastigotes, trypomastigotes, and granular staining) were detected in a mouse model that mimics human chronic chagasic cardiomyopathy.

Immunohistochemistry studies of animal models can help elucidate why, during acute *T. cruzi* infection, very young children and patients with immune deficiencies may have heavy parasitemia and die. These studies may also assist in understanding some of the controversial pathogenesis issues related to chronic sequelae. For example, some investigators believe that only immunologic reactions play a fundamental role, since the composition of the lymphoid infiltrate is similar to that of heart transplant recipients, while others believe that the infiltrate is due to persistence of parasites in the tissues. Mouse models of Chagas disease using dogs, rabbits and mice have studied cardiac or central nervous system (CNS) pathology in both the acute and chronic phases. Mouse models have presented the advantage of producing varying degrees of chronic cardiomyopathy dependent on the strain of mouse and the strain of *T. cruzi* parasite. DBA/2 mice develop severe chronic chagasic cardiomyopathy which is similar to severe chronic Chagas disease in humans, thus being a valuable tool for the study of immune responses at different time points. For this particular mouse model, little data are available on distribution of the parasites in various tissues, or on the correlation of

![Figure 1a](image1.png)

**Figure 1a.** Right ventricular wall of a normal (non-infected) DBA/2 mouse heart; note the calcifications (arrow head) in the pericardium (H&E, magnification 200×).

![Figure 1b](image2.png)

**Figure 1b.** Right ventricular wall of a DBA/2 mouse heart 70 days after *T. cruzi* infection; note the chronic inflammatory infiltrate inside the myocardium, and the presence of fibrosis. Note the thinning of the ventricular wall compared with Figure 1 (H&E, magnification 200×).

![Figure 1c](image3.png)

**Figure 1c.** Skeletal muscle from a DBA/2 mouse 30 days after *T. cruzi* infection; note the presence of trypomastigotes (arrow head) in the interstitium, granular antigen staining in the inflammatory areas, and an amastigote pseudocyst (arrow) (IHC, magnification 630×).

![Figure 1d](image4.png)

**Figure 1d.** Pancreas from a DBA/2 mouse 30 days after *T. cruzi* infection; note the amastigote pseudocyst (red staining) in an acinar cell (IHC, magnification 630×).

![Figure 1e](image5.png)

**Figure 1e.** Central nervous system from a DBA/2 mouse 30 days after *T. cruzi* infection; note the calcifications (arrow head) in the pericardium (IHC, magnification 400×).

![Figure 1f](image6.png)

**Figure 1f.** Skeletal muscle from a DBA/2 mouse 70 days after *T. cruzi* infection; note the single amastigotes (arrow) surrounded by inflammatory infiltrate (IHC, magnification 630×).

![Figure 1g](image7.png)

**Figure 1g.** Central nervous system from a DBA/2 mouse 70 days after *T. cruzi* infection; note single amastigotes (arrow) and granular staining (arrow head) in an area with inflammation. Also present are empty vacuolar spaces in the neuropil (IHC, magnification 400×).

![Figure 1h](image8.png)

**Figure 1h.** Perinephric adipose tissue from a DBA/2 mouse 30 days after *T. cruzi* infection; note the presence of multiple trypomastigotes (arrow) and granular antigen staining (arrow head) in the connective tissues, but not in the kidney parenchyma (IHC, magnification 400×).

![Figure 1i](image9.png)

**Figure 1i.** Liver from a DBA/2 mouse 30 days after *T. cruzi* infection. Note the trypomastigote in the sinusoid (IHC, magnification 630×).

![Figure 1j](image10.png)

**Figure 1j.** Lymph node from a DBA/2 mouse 70 days after *T. cruzi* infection. Note the granular antigen staining (red staining) in the sinusoidal macrophages (IHC, magnification 400×).
IMMUNOHISTOCHEMICAL DETECTION OF *T. CRUZI* IN TISSUES
parasite burden with inflammatory infiltrates. The current study documents the distribution of the different parasite forms in various tissues and their relationship with the inflammatory infiltrate in DBA/2 T. cruzi-infected mice, and discusses issues related to pathogenesis.

MATERIALS AND METHODS

Six- to eight-week-old male DBA/2 mice were purchased from Jackson Laboratory (Bar Harbor, Maine) and housed in the AAALAC-approved facilities at the Centers for Disease Control and Prevention (CDC). Mice were infected by an intraperitoneal injection of $2 \times 10^4$ trypanomastigotes of T. cruzi Brazil strain (routinely maintained by serial subinoculation in female C3HeBFe/J mice at 3-week intervals) suspended in 0.1 ml blood. Three to six DBA/2 mice were sacrificed at 0, 10, 15, 20, 25, 30, 50, and 70 days after infection. Fragments from the heart, skeletal muscle (quadriceps), gastrointestinal tract (lower esophagus and stomach), pancreas, lung, kidney, liver, spleen, lymph nodes, adipose tissue (surrounding these organs), and central nervous system (coronal section including parietal and temporal cortex, and basal nuclei) were fixed in 10% buffered formalin. Paraffin blocks were prepared, and three sections from each tissue were stained, one with hematoxylin and eosin stain (H&E), and 2 by IHC.

For IHC, 4-µm sections of the tissues were placed on Fisher Plus slides (Fisher Scientific Co., Pittsburgh, PA). These were deparaffinized and rehydrated in graded alcohol washes, and washed in water and then buffer. Tissue sections were placed in the DAKO autostainer (Dako Corporation, Carpinteria, CA), digested in 0.1 mg/ml Protease K (Boehringer Mannheim Biochemicals, Indianapolis, IN) in 0.6 M Tris (pH 7.5)/0.1% CaCl$_2$ for 15 minutes, and later blocked with swine anti-rabbit serum (Dako Corporation). A polyclonal rabbit antibody against T. cruzi (Division of Parasitic Diseases, CDC, Atlanta, GA) was applied to the tissue sections and allowed to incubate for 60 minutes. This was followed by sequential application of biotinylated anti-rabbit antibody, avidin alkaline phosphatase complex, and naphthol/fast red substrate (all four from Dako Corporation). Sections were then counterstained in Meyer’s hematoxylin (Fisher Scientific) and mounted with aqueous mounting medium (Lerner Laboratories, Pittsburg, PA). Sections of formalin-fixed, paraffin-embedded normal mouse tissues mixed with T. cruzi cultures were used as a positive control. Negative controls included non-infected mice, as well as adjacent tissue sections incubated with normal rabbit antiserum.

Using routine histopathology and IHC, we studied and graded the following parameters: 1) Type of inflammatory infiltrate: acute (predominant polymorphonuclear cells) or chronic (predominant mononuclear cells); 2) Distribution of inflammation: diffuse or focal pattern; 3) Amount of inflammation: rare (single inflammatory cell in a particular tissue), mild, moderate or severe; 4) Other pathologic changes: calcifications, necrosis, fibrosis, and vascular thickening; 5) Parasite forms: amastigotes (seen with H&E and IHC), trypanomastigotes (seen with IHC) and granular antigen staining (seen with IHC); 6) Distribution of parasites; tissue, cell type, and relationship with inflammatory infiltrate; and 7) Amount of parasite forms: absent, rare (one parasite), scattered (few parasites), and abundant (multiple clusters).

All these parameters were used to compare infected tissues at each time point with uninfected DBA/2 mice at times 0 and 70 days.

RESULTS

No difference in histopathology was observed between non-infected DBA/2 mice at day 0 and day 70. Histopathology of all tissues examined was essentially unremarkable except for cardiac tissue, where a band of pericardial calcifications in the right ventricle accompanied by minimal lymphohistiocytic infiltrate (Figure 1a) was observed.

The histopathologic changes of T. cruzi-infected DBA/2 mice at different time points in various tissues are shown in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Histopathology of different tissues during the time course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>Feature</td>
</tr>
<tr>
<td>Heart</td>
<td>inflammation</td>
</tr>
<tr>
<td></td>
<td>necrosis</td>
</tr>
<tr>
<td></td>
<td>calcifications</td>
</tr>
<tr>
<td></td>
<td>right ventricular thinning</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>inflammation</td>
</tr>
<tr>
<td></td>
<td>necrosis</td>
</tr>
<tr>
<td></td>
<td>calcifications</td>
</tr>
<tr>
<td>GI tract*</td>
<td>inflammation</td>
</tr>
<tr>
<td>CNS*</td>
<td>perivascular inflammation</td>
</tr>
<tr>
<td>Lung</td>
<td>patchy inflammation</td>
</tr>
<tr>
<td>Pancreas</td>
<td>focal inflammation</td>
</tr>
<tr>
<td>Spleen/LN</td>
<td>active germinal centers</td>
</tr>
<tr>
<td></td>
<td>sinus histiocytes</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>inflammation</td>
</tr>
<tr>
<td></td>
<td>necrosis</td>
</tr>
</tbody>
</table>

*GI tract = gastrointestinal tract including esophagus and stomach; CNS = coronal section of the central nervous system including parietal and temporal cortex and basal nuclei; LN = lymph nodes.
IMMUNOHISTOCHEMICAL DETECTION OF *T. CRUZI* IN TISSUES

Table 1. Blood vessels did not demonstrate significant differences between the two groups. The most important difference between infected and control mice was the presence of inflammatory foci in multiple organs. The predominant inflammatory infiltrate observed was mononuclear (lymphocytes and histiocytes with abundant foamy cytoplasm), with occasional polymorphonuclear cells in areas of marked inflammation. Eosinophils were rarely seen. In all tissues the inflammatory infiltrate was present by day 10 after infection, peaked at days 20 to 30, and was undetectable by day 50. Myocyte necrosis and dystrophic calcifications in the heart and skeletal muscle were also seen during the acute (days 20 to 30) phase. During this phase, lymph nodes and spleen had active germinal centers and epithelioid histiocytes, expanding sinusoids, and red pulp. The chronic sequelae were present by day 50 and included marked thinning, fibrosis, and focal moderate inflammation in the right ventricular wall of the heart (Figure 1b). In the chronic phase, the CNS demonstrated vacuolar degeneration accompanied by focal mild chronic inflammation.

The tissues where amastigotes were identified with either IHC or H&E are shown in Figure 2. Amastigotes were present by day 10 and peaked on day 30. During the acute phase, this parasite form was seen inside myocytes (in heart, skeletal muscle [Figure 1c], esophagus, and stomach), histiocytes (of liver, lymph nodes and spleen), acinar cells (in pancreas [Figure 1d]), astrocytes [Figure 1e], and ependimal cells (the latter two in the CNS). Amastigotes were mostly present intracellularly in nests (pseudocysts); usually no inflammation was present around them. Occasionally, single amastigotes were seen extracellularly; these were usually surrounded by a mononuclear inflammatory infiltrate (Figure 1f). During the chronic phase (day 50 to 70), rare amastigotes were identified as intracellular nests in myocytes (in heart, skeletal muscle, esophagus, stomach and vessels), in acinar cells (in the pancreas), and in astrocytes (in CNS [Figure 1g]), and were seldom surrounded by inflammation.

The tissues where trypomastigotes were present are shown in Figure 3. This parasite form was only evident when using IHC. By day 15, extracellular trypomastigotes were observed inside sinusoids of liver (Figure 1i), spleen, cardiac chambers, and blood and lymphoid vessels of several organs. At the same time point, trypomastigotes were observed in connective tissues of kidney (Figure 1h), skeletal muscle (Figure 1c), lung, and pancreas. The number of trypomastigotes peaked at day 30. Occasional trypomastigotes were seen at these sites on day 50. This parasite form was not immediately associated with an inflammatory infiltrate. However, in the chronic stage of infection, inflammation and granular staining were observed in the areas where trypomastigotes had been detected at earlier time points.

The distribution of granular antigen staining in the tissues is shown in Figure 4. Granular staining could only be demonstrated with IHC, and was seen both intracellularly and extracellularly. During the acute phase (day 30), granular staining was present in all the tissues studied, although stain-
Tissues where trypomastigotes were present

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Peak day</th>
<th>Amount</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>15 to 30</td>
<td>scattered</td>
<td>cavities and vessels</td>
</tr>
<tr>
<td>Muscle</td>
<td>25 to 30</td>
<td>abundant</td>
<td>surrounding soft tissues</td>
</tr>
<tr>
<td>GI tract</td>
<td>30</td>
<td>scattered</td>
<td>vessels</td>
</tr>
<tr>
<td>Lung</td>
<td>30</td>
<td>abundant</td>
<td>vessels and alveoli</td>
</tr>
<tr>
<td>Pancreas</td>
<td>30 to 40</td>
<td>abundant</td>
<td>surrounding soft tissues</td>
</tr>
<tr>
<td>Liver</td>
<td>15 to 30</td>
<td>abundant</td>
<td>sinusoids and central veins</td>
</tr>
<tr>
<td>LN/spleen</td>
<td>20 to 30</td>
<td>scattered</td>
<td>sinusoids</td>
</tr>
<tr>
<td>Kidney</td>
<td>30</td>
<td>scattered</td>
<td>perinephric soft tissues</td>
</tr>
</tbody>
</table>

Note: Muscle= refers to skeletal muscle only
LN= lymph node
GI=gastrointestinal (esophagus and stomach

**DISCUSSION**

The distribution of parasite stages and associated inflammation in both the acute and chronic phases of *T. cruzi* infection in the DBA/2 mouse model has not been described previously. This model permits temporal analysis of infection that is not possible in humans. Since this mouse model shows a cardiomyopathy similar to that seen in the more severe human cases, it may help address questions relating to tissue reservoirs of the parasite at different stages, as well as the possible causes of the chronic sequelae associated with *T. cruzi* infection. We used routine histopathology and IHC to describe the parasite forms in different tissues and the associated inflammatory reaction.

In the current study, amastigotes were seen throughout the infection, mostly intracellularly, and appeared to have a distinct tropism for muscle cells (cardiac, smooth and skeletal). Amastigotes were also found in macrophages (of lymph nodes and spleen), acinar pancreatic cells and astrocytes. Previous reports of mouse models have documented the presence of *T. cruzi* pseudocysts in the heart, CNS, liver, spleen, skeletal muscle, lymph nodes and colon. Immunohistochemistry detected amastigotes in cells where they have been difficult to detect by H&E, such as in acinar pancreatic cells. With IHC, we were able to demonstrate single amastigotes extracellularly, usually surrounded by inflammation and accompanied by granular staining, as if they were in the process of being destroyed by the host immune system. Individual parasites dispersed among inflammatory cells probably come from recently ruptured host cells containing pseudocysts.

Trypomastigotes cannot be seen in routine histologic preparations. By using IHC, parasitemia was documented in blood and lymphoid vessels of most organs during the acute stage. Trypomastigotes were also present extracellularly in loose connective and adipose tissues surrounding the heart, skeletal muscle, kidney, pancreas and lymph nodes. Other investigators, using different animal models, were able to recover parasites from loose connective tissue and fatty tissues from infected hamsters. The presence of trypomastigotes in connective and adipose tissues likely explains the abundant granular staining and associated inflammatory in-
IMMUNOHISTOCHEMICAL DETECTION OF T. CRUZI IN TISSUES

### Table: Tissues where antigen (granular) staining was present

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Peak day</th>
<th>Amount</th>
<th>Localization</th>
</tr>
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<tbody>
<tr>
<td>Heart</td>
<td>15 to 30</td>
<td>abundant</td>
<td>in inflammatory areas and perimyisium</td>
</tr>
<tr>
<td>Muscle</td>
<td>25 to 30</td>
<td>abundant</td>
<td>in inflammatory areas and perimyisium</td>
</tr>
<tr>
<td>GI tract</td>
<td>30</td>
<td>scattered</td>
<td>in muscle layer by inflammatory foci</td>
</tr>
<tr>
<td>CNS</td>
<td>30 to 50</td>
<td>abundant</td>
<td>in areas of inflammation</td>
</tr>
<tr>
<td>Lung</td>
<td>30 to 40</td>
<td>abundant</td>
<td>in muscular vessels</td>
</tr>
<tr>
<td>Pancreas</td>
<td>30 to 50</td>
<td>abundant</td>
<td>in inflammatory areas and surrounding soft tissues</td>
</tr>
<tr>
<td>LN/spleen</td>
<td>30 to 50</td>
<td>scattered</td>
<td>in histiocytes of sinusoids</td>
</tr>
<tr>
<td>Adipose</td>
<td>15 to 50</td>
<td>abundant</td>
<td>in spindle cells and inflammatory areas</td>
</tr>
<tr>
<td>Kidney</td>
<td>30 to 50</td>
<td>abundant</td>
<td>in perinephric soft tissues</td>
</tr>
</tbody>
</table>

Note: Muscle refers to skeletal muscle only
LN= lymph node
GI=gastrointestinal (esophagus and stomach)
CNS= central nervous system

**Figure 4.** Schematic representation of the level of granular antigen staining in different tissues over time.

filtrate in the pericardium and perimyisium during the chronic stages.

Granular or antigen staining and parasite nucleic acids have been documented in human and mouse chagasic myocarditis. Granular antigen staining was usually associated with inflammation in both acute and chronic stages, and antigens were detected inside macrophages and in extracellular spaces. The level of granular staining in each tissue varied, but paralleled the parasite burden (amastigotes and trypomastigotes) observed during the acute stage. The relationship between parasitemia and chronic sequelae has not been completely established because the techniques used previously detected mostly amastigotes. Immunohistochemistry has the advantage of demonstrating all parasite forms and parasite breakdown products. Our data suggest that there is a correlation between parasite burden (amastigotes, trypomastigotes, and granular antigen staining) and chronic inflammatory infiltrate. Previous studies demonstrated a correlation between nucleic acids and the inflammatory infiltrate. These persistent antigens and nucleic acids are possibly responsible for localized sustained immune reactions that occur throughout the infection, and may play a major role in the chronic stages.

In this mouse model, the most prominent chronic sequelae were in the heart and the CNS. The chagasic cardiomyopathy found in this mouse model at day 70 is similar to that described in infected humans. In addition, uninfected DBA/2 mice appear to have pericardial calcifications at a very young age. In the heart, pathologic changes found in the chronic stages of disease include focal inflammatory infiltrates of lymphocytes and histiocytes, fibrosis and marked thinning of the ventricular wall. By IHC, granular antigen staining was prominent in the perimyisium, where trypomastigotes were detected in the acute stage. Occasionally, an amastigote pseudocyst in intact myocytes was also detected. In the brain, pathologic changes during the chronic stage included persistent inflammatory foci associated with granular staining. The neuropil surrounding these inflammatory foci appeared thinned and vacuolated (microcystic change), as can be seen in post infectious processes. From these observations, it appears that in the chronic stages of Chagas disease, mononuclear inflammation is frequently associated with persistent antigens and nucleic acids, but only occasionally with intact parasite tissue forms.

This study has at least two limitations: mice were only followed for what can be considered the early chronic phase (70 days). However, at this time point, the histopathologic features in the heart are similar to those found in human cases with severe cardiomyopathy. Previous studies from our laboratory compared heart sections from the same mouse strain at 70, 100, and 200 days post infection with T. cruzi Brazil strain, and did not identify significant histopathological differences. Another limitation is that IHC cannot document parasite viability. Immunohistochemistry, similar to
molecular assays, can detect parasite forms in tissues, but cannot determine if a single amastigote or trypomastigote surrounded by inflammation is viable and capable of evading the immune response, thus maintaining a persistent infection.

In summary, this study presents a detailed histopathological description of different tissues in acute and chronic *T. cruzi* infection of a mouse strain prone to chagasic cardiomyopathy. Furthermore, IHC can demonstrate different parasite forms in tissues even when they were not visible with routine H&E. Immunohistochemical and pathology studies of pathomimetic mouse models of chagasic myocarditis provide critical baseline knowledge that should allow a better understanding of the pathogenesis of acute and chronic *T. cruzi* infection in humans.

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