Cytokine Expression at the Inoculation Site and Nearby Tissues in an Animal Model Infected with Metacyclic Trypomastigotes of *Trypanosoma cruzi*

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Abstract

Background. *Trypanosoma cruzi* infection is induced by triatomine contaminated feces containing metacyclic trypomastigotes acquired via small wounds in the skin. Natural unspecific and adaptive humoral and cellular immune responses play an important role in controlling primary infection.

Objective: To determine the cytokine profile at the inoculation site and nearby tissues such as draining lymph nodes, as well as the heart and serum, throughout the acute and chronic phases in mice infected with metacyclic *T. cruzi* trypomastigotes.

Material and methods. Balbc/c mice were intradermally inoculated with vector derived metacyclic trypomastigotes of *T. cruzi*. The RT-PCR technique was used to analyze the cytokine profile at the inoculation site, draining lymph nodes and heart. ELISA was used to determine cytokines in serum.

Results. A poor induction of cytokines within the two weeks after infection such as IL-12, IFN-gamma, IL-4, IL-10 and TGF-beta and undetectable IL-2 was observed at the inoculation site. On the other hand, in draining lymph nodes, cytokines such as IL-2, IL-10, IL-12, TGF-beta were detected from the first day after infection. In the heart, a mixed inflammatory and anti-inflammatory cytokine pattern was identified. In serum, all cytokines tested (IL-4, IL-10 IL-12 and IFN-gamma) were detected from the 5th day post-infection with the notorious exception of IL-2.

Conclusion. A poor and late induction of protective cytokines such as IFN-gamma and IL-12 was observed at the inoculation site, in spite of adequate immune responses in lymph nodes. In addition, the pattern of cytokine expression strongly depended on the kind of tissue.

Keywords: *Trypanosoma cruzi*, American trypanosomiasis, inoculation site, cytokines, Chagas disease

INTRODUCTION

*Trypanosoma cruzi* is the etiologic agent of Chagas disease. The metacyclic *T. cruzi* trypomastigote, which is the infective parasite phase for mammals, develops in the rectum of the reduviid bug vector. These are deposited on mucous membranes or through small wounds in the skin or even the puncture made by the of triatompine’s bite. [1-3]. It has been reported that a local inflammation is produced at the inoculation site; polymorphonuclear leukocytes appear as early as 1h post-infection and peak at 24h, while mononuclear cells are observed for the first time at 24h post-inoculation, reaching their maximum density at 15 days post-infection. [3;4]. This typical inflammatory reaction is induced with the use of any parasite phase, trypomastigotes derived from tissue culture [4], blood [5] or vector-derived trypomastigotes [3].

Cell invasion and intracellular replication are fundamental steps in the parasite’s life cycle; in this respect, it has been reported that metacyclic trypomastigotes can be disseminated via triatomine’s puncture as soon as 5 min after initial contact [5]. The immune mechanism triggered during the first days of *T. cruzi* infection seems to be essential for controlling the parasite’s dissemination, replication and the outcome of the disease. Macrophages and natural killer cells (NK), counted among the various cells involved in the immune responses, probably play the most crucial role in the early phase to control the parasites’ load.

Peritoneal macrophages obtained from *T. cruzi* infected mice showed a significantly increased ability to in-
hibit *T. cruzi* multiplication [7], while NK cells increase their cytotoxicity and represent the main source of IFN-γ. This cytokine is produced as early as 12 h after infection [8, 9].

The IFN-γ has been associated with host resistance and inhibits both in vivo and in vitro parasite replications through the induction of nitric oxide (NO) synthesis by IFN-γ-activated macrophages [10,11]. However, IFN-γ-primed macrophages infected in vitro with different *T. cruzi* developmental stages respond in a different manner. In this regard, tissue-culture trypomastigotes, bloodstream trypomastigotes and amastigotes can induce high-level production of NO by macrophages primed with IFN-γ, but none of the forms encountered in the triatomine vector such as epimastigotes and metacyclic trypomastigotes have the same ability to trigger NO synthesis [12].

When macrophages are infected with metacyclic culture-derived trypomastigotes, the IL-12 cytokine is poorly produced, whereas when macrophages are infected with tissue-culture tryomastigotes, this cytokine is produced at high levels [13;14].

The above data support the notion that the parasite phase may influence the host immune response. If we assume that the infective phase for the mammalian host in nature is the metacyclic trypomastigote, then the use of a murine model mimicking this condition could offer an insight into of the mechanism of the innate immune response. If we assume that the infective phase for the mammalian host in nature is the metacyclic trypomastigote, then the use of a murine model mimicking this condition could offer an insight into of the mechanism of the innate immune response. If we assume that the infective phase for the mammalian host in nature is the metacyclic trypomastigote, then the use of a murine model mimicking this condition could offer an insight into of the mechanism of the innate immune response.

As far as we know, there are no studies describing the cytokine response induced at the inoculation site and nearby tissues using metacyclic insect-derived tryomastigotes.

**MATERIAL AND METHODS**

**Parasite**

Mexican *Trypanosoma cruzi* isolate called “Ninoa” obtained from a human acute phase was used in this work [15]. This isolate belongs to biodeme III and to a separate cluster of zymodeme as reported previously [3;16]. It was maintained by sequential culture in LIT medium, *Triatoma pallidipennis* infection, and murine passage. First-instar larvae were fed on *T. cruzi* infected mice to obtain metacyclic tryomastigotes; when these insects were adults, they were fed on rabbit. Infectious urine was collected and metacyclic tryomastigotes were adjusted with sterile PBS to 1 ⅛ 10⁵ / ml before inoculation.

**Infection to animals**

Inbred Balb/c mice (8-10 weeks-old) were obtained from the Institute’s Animal House Facility. These were inoculated by intradermal injection into the hind footpad with 1,000 metacyclic tryomastigotes. Control animals received trypomastigote- free triatoma urine under similar conditions.

All controled and experimental animals were sacrificed at different times post-inoculation: 1, 2, 5, 10, 20, 30, 90, 180 and 360 days. Skin (inoculation site), draining lymph node, heart and blood were removed. Tissues were used for RNA extraction in order to perform RT-PCR for cytokines.

**RNA isolation cDNA synthesis and PCR**

A pool of skin (inoculation site) from three hind foot pads of three different animals, lymph nodes and heart of infected and control animals were homogenized with Trizol (Life Technologies, Grand Island, NY), total RNA was prepared following the manufacturer’s recommendation, and 1 μg of total RNA was reversed transcribed in 20μl of total reaction.

**PCR for cytokines**

Samples of cDNA (1μl) were amplified in 50μl of total reaction, with gene-specific upstream and downstream primers for IL-2, IL-4, IL-10, IL-12, TGF-β and β-actin (Clontech, Palo Alto Ca, USA); and IFN-γ (5’-CTG GCT GTT ACT GCC ACG GCA CAG TC-3’; 5’- TCG GAT GAG CTC ATT GAA TGC TTG GCG CT-3’).

The amplification program consisted of an initial 3 min denaturation at 95 °C, followed by 30 repeated cycles of denaturation for 45s at 95 °C, and primer annealing for 45s at 60 °C and extension for 45s at 72 °C. For β-actin 25 repeated cycles was used instead. A negative control lacking cDNA and a commercial positive control for IL-2, IL-4, IL-10, IL-12, and IFN-γ (Clontech, Palo Alto Ca, USA) were included in each experiment. The PCR products were run on a 2% agarose gel and stained with ethidium bromide. Semiquantification of PCR products was performed by comparison of signals for each cytokine under study with respect to β-actin. The stained gel was scanned for densitometrical analysis. Cytokine expression data is represented as the ratio of each cytokine signal to the corresponding β-actin signal.

**Parasitemia.**

A 20μl blood sample from a group of 5 infected mice was obtained from the caudal vein. In the fresh preparation, parasites were counted as described elsewhere [17].

**Evaluation of cytokine production in serum**

Murine cytokines IL-2, IL-4, IL-10, IL-12 and IFN-γ were measured by specific two-site enzyme-linked immunoabsorbent assay (ELISA) according to the manufacturer’s specifications (BD PharMingen, USA) with reference standard curves, using known amounts of the respective murine recombinant cytokines. All samples were processed individually and assayed in duplicate. Plates were read at 405 nm.
RESULTS

Parasitemia

Parasitemia in infected mice peaked at around 30 days post-infection with 4.8 \( \times 10^5 \) parasites/ml; the stout form was predominant over the slender form. Parasites were detected for the first time at 15 days post-infection and disappeared around 65 days post-infection (Figure 1). All animals survived the period of experimentation. Our results are consistent with other studies using the same biodeme strain as Ninua. For example, the Colombian strain that belongs to biodeme III has a similar curve of parasitemia with a peak on the 27th day. [18].

Profile of cytokine message at the inoculation site

Skin tissue samples from the inoculation site covering the acute phase of infection from day 1 to day 30 showed a very poor cytokine expression in animals infected with metacyclic trypomastigotes. No expression of cytokines was detected in the first week; however, constitutive \( \beta \)-actin messenger was readily detected, thus ruling out the possibility of methodological failure. Until the second week of infection the first cytokines IL-10 and IL-12 appeared, the former being the stronger signal. In the third week post-infection, the cytokines IL-4, TGF-\( \beta \) and IFN-\( \gamma \) were observed, and in this case the stronger signal was for TGF-\( \beta \) (Table 1 and Figures 2 and 3). This finding may suggest that metacyclic trypomastigote phase is a poor inducer of IFN-\( \gamma \) at the inoculation site, favoring their spread. No signals of cytokine induction were observed in control animals inoculated with triatomine parasite-free urine.

![Figure 1. Parasitemia](image)

Balb/c mice were intradermally inoculated with *Trypanosoma cruzi* metacyclic trypomastigotes. Parasitemia was followed by direct observation of fresh blood samples from five mice. Stout and slender forms of the parasite are shown.

![Figure 2. Representative image of IL-12 amplified PCR products at the inoculation site](image)

Table 1. Cytokine expression profile at the inoculation site (skin) of infected Balb/c mice with metacyclic trypomastigotes

<table>
<thead>
<tr>
<th>Days</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>IL-12</th>
<th>IFN-( \gamma )</th>
<th>TGF-( \beta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neg*</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>2</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>10</td>
<td>Neg</td>
<td>Neg</td>
<td><strong>0.6</strong></td>
<td><strong>0.1</strong></td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>20</td>
<td>Neg</td>
<td>Neg</td>
<td><strong>0.6</strong></td>
<td><strong>0.06</strong></td>
<td><strong>0.5</strong></td>
<td><strong>0.6</strong></td>
</tr>
<tr>
<td>30</td>
<td>Neg</td>
<td><strong>0.4</strong></td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td><strong>0.6</strong></td>
</tr>
</tbody>
</table>

Balb/c mice were intradermally inoculated with 1,000 *Trypanosoma cruzi* metacyclic trypomastigotes. RT-PCR assays were done at different times after inoculation. \( \beta \)-actin was used to normalize cytokine expression. Results are expressed as cytokine/\( \beta \)-actin ratio. Values are representative of two independent experiments.

*no amplification.*

![Figure 3. Representative image of INF-\( \gamma \) amplified PCR products at the inoculation site](image)

Figure 2. Representative image of IL-12 amplified PCR products at the inoculation site: Line 1 Molecular markers (ladder 100bp). Lines 3, 5, 7 and 9 constitutive \( \beta \)-actin. Lines 2, 4, 6 experimental times (1, 10, 20 days respectively). Line 8 IL-12 positive control.

Figure 3. Representative image of INF-\( \gamma \) amplified PCR products at the inoculation site: Line: 1 Molecular markers (ladder 100bp). Lines: 3, 5, 7 and 9 constitutive \( \beta \)-actin. Lines: 2, 4, 6 experimental times (1, 10, 20 days respectively). Line 8 INF-\( \gamma \) positive control.
Cytokine expression profile in draining lymph nodes

Tissue samples of draining lymph nodes collected from the early acute phase through the chronic phase were analyzed. In contrast to the inoculation site, cytokine expression in lymph nodes near the inoculation site was observed for IL-2, IL-10, IL-12 and TGF-β from the first day post-inoculation. Among these, IL-2 message was present from the first and second day but suddenly disappeared during the entire acute phase, reappearing briefly in the chronic phase. However, strong and consistent expression of TGF-β and IL-12 was observed during the acute phase, while IFN-γ expression only appeared later at the end of the first week post-infection, in spite of the fact that IL-12 induction was observed from the first day post-inoculation. It is a well known fact that IL-12 is an excellent IFN-γ inducer.

In the chronic phase of infection, IL-2, IL-4, and TGF-β were seldomly expressed whereas IL-12 was the cytokine most consistently induced, indicating at least macrophage activation.

An interesting observation was the coincidental induction of IFN-γ, which is a key cytokine in parasite control, and the appearance of circulating parasite in blood with the persistence of TGF-β expression throughout the parasitic phase, which is a regulatory cytokine of T cell activation (Table 2).

Table 2. Cytokine expression profile in draining lymph node of infected Balb/c mice with metacyclic trypomastigotes

<table>
<thead>
<tr>
<th>Days</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>IL-12</th>
<th>IFN-γ</th>
<th>TGF-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12</td>
<td>Neg</td>
<td>0.70</td>
<td>0.42</td>
<td>Neg</td>
<td>0.53</td>
</tr>
<tr>
<td>2</td>
<td>0.14</td>
<td>Neg</td>
<td>0.58</td>
<td>0.48</td>
<td>Neg</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>Neg</td>
<td>Neg</td>
<td>0.53</td>
<td>0.10</td>
<td>0.14</td>
<td>0.51</td>
</tr>
<tr>
<td>10</td>
<td>Neg</td>
<td>0.14</td>
<td>0.43</td>
<td>0.41</td>
<td>0.15</td>
<td>0.48</td>
</tr>
<tr>
<td>30</td>
<td>Neg</td>
<td>0.12</td>
<td>0.44</td>
<td>0.63</td>
<td>Neg</td>
<td>0.44</td>
</tr>
<tr>
<td>90</td>
<td>Neg</td>
<td>0.11</td>
<td>0.44</td>
<td>0.32</td>
<td>0.41</td>
<td>Neg</td>
</tr>
<tr>
<td>180</td>
<td>0.11</td>
<td>0.11</td>
<td>Neg</td>
<td>0.53</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>360</td>
<td>Neg</td>
<td>0.11</td>
<td>Neg</td>
<td>0.30</td>
<td>0.50</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Balb/c mice were intradermally inoculated with 1,000 Trypanosoma cruzi metacyclic trypomastigotes. RT-PCR assays were done at different times after inoculation. β-actin was used to normalize cytokine expression. Results are expressed as cytokine/β-actin ratio. Values are representative of two independent experiments.

At the peak of parasitemia around 30 days post-infection, consistent expression of IL-4, IL-10 IL-12 and TGF-β with the notorious absence of IFN-γ was observed, suggesting a delicate balance between proinflammatory and anti-inflammatory responses. Control animals inoculated with triatomine parasite-free urine only showed IL-10 and TGF-β expression at day 5 post-inoculation (data not shown).

Cytokine expression profile in the heart

The first heart sample analyzed was at day 20 post-infection. At this time, infiltration of mononuclear cells and pseudocyst of amastigotes was evident, as previously reported [3]. In this parasitic phase, the cytokines expressed in the heart were IL-12, IFN-γ, IL-10 and TGF-β, IL-10 and TGF-β being more strongly expressed than IL-12 and IFN-γ.

In the chronic phase of infection after 90 days post-infection, only IL-4, IL-10 and IL-12 messages persisted. The lack of expression of IL-2 was a clear-cut finding in this tissue (Table 3). Again, in this tissue a balance between proinflammatory and anti-inflammatory cytokines is observed. No cytokine expression was detected in the heart of control animals (data not shown).

Table 3. Cytokine expression profile in heart of infected Balb/c mice with metacyclic trypomastigotes

<table>
<thead>
<tr>
<th>Days</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>IL-12</th>
<th>IFN-γ</th>
<th>TGF-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Neg</td>
<td>Neg</td>
<td>0.61</td>
<td>0.40</td>
<td>0.55</td>
<td>0.71</td>
</tr>
<tr>
<td>30</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>90</td>
<td>Neg</td>
<td>Neg</td>
<td>0.20</td>
<td>0.42</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>180</td>
<td>Neg</td>
<td>Neg</td>
<td>0.45</td>
<td>0.33</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>360</td>
<td>Neg</td>
<td>0.10</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Balb/c mice were intradermally inoculated with 1,000 Trypanosoma cruzi metacyclic trypomastigotes. RT-PCR assays were done at different times after inoculation. β-actin was used to normalize cytokine expression. Results are expressed as cytokine/β-actin ratio. Values are representative of two independent experiments.

Concentration of cytokines in serum

We assessed cytokine concentration in serum at different times of the early acute phase and chronic phase by ELISA. IL-2 was undetectable at all the experimental times. The first detectable cytokines were IL-4, IL-10, IL-12 and IFN-γ at day 5 post-infection. Among these, IL-4 was present at a lower concentration during the acute parasitic phase, but once parasitemia dropped it reached its maxi-

Table 4. Cytokine levels in serum

<table>
<thead>
<tr>
<th>Days</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>IL-12</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>187</td>
<td>254</td>
<td>8084</td>
<td>533</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>190</td>
<td>200</td>
<td>4828</td>
<td>403</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>286</td>
<td>188</td>
<td>11706</td>
<td>2543</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>1874</td>
<td>7164</td>
<td>4161</td>
<td>16119</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>1993</td>
<td>435</td>
<td>9711</td>
<td>38516</td>
</tr>
<tr>
<td>360</td>
<td>0</td>
<td>5128</td>
<td>83</td>
<td>3494</td>
<td>40804</td>
</tr>
</tbody>
</table>

Balb/c mice were intradermally inoculated with Trypanosoma cruzi metacyclic trypomastigotes. Serum samples were collected and cytokine concentration was assayed by ELISA. Animals inoculated with parasite-free urine showed undetectable levels of all cytokines in serum.

*Concentration is expressed as pg/ml.
pomastigotes do not elicit such proinflammatory responses. and NO production by epithelial cells, while metacyclic try-

tion site, lymph nodes and heart.

immune response differs from that observed at the inocula-

tion site. A similar pattern was observed for IL-10, but it dropped to its lower level in the late chronic phase. In con-

trast, higher levels of IL-12 were reached from the early acute phase of infection and ran in parallel with parasitemia, but the IL-12 concentration only dropped slightly in the late chronic phase even though parasitemia and tissue parasitism were undetectable. A discrete amount of IFN-γ was circulating in the serum during the first week and gradually increased until reaching its highest level in the late chronic phase (Table 4). From this data, it is clear that the systemic immune response differs from that observed at the inoculation site, lymph nodes and heart.

In control animals inoculated with tryptomastigote-free urine, IL-12 was detected from day 1 to day 5 but at levels under 3,000 pg/ml (data not shown).

DISCUSSION

In a previous work, we reported that infection with metacyclic tryptomastigotes induces an inflammatory reaction at the inoculation site characterized by the presence of polymorphonuclear cells as early as 4 h post-inoculation and peaking 24 h later, while mononuclear cell infiltrates appear for the first time at 24 h post-inoculation, reaching their maximal level 15 days later [3]. In the present study, we extended our observation and found that induction of cytokine expression at the inoculation site is undetectable by RT-PCR during the first week after inoculation, in spite of the mononuclear infiltration. These undetectable levels of cytokine do not necessarily rule out the possibility that other cytokines or chemokines could be produced as recruitment factors. In very recently published work, it was demonstrated at the molecular level that tissue culture tryptomastigotes generate proteolytic kinins depending on CXCR2 and CXC, KC and MIP-2 production by macrophages activated via Toll-like 2 receptors, providing an interface between the mechanism involved in pathogen sensing by innate sentinel cells and the kinin system. Thus, activation of TLR2/neutrophils at very early stages of infection promotes rapid accumulation of plasma proteins, including kininogens, thereby establishing conditions for the proteolytic release of proinflammatory peptides. However epimastigotes and metacyclic tryptomastigotes fail to trigger such response [19]. Of further interest in this context is the report by Eikhoff et al [20] that bloodstream tryptomastigotes stimulate IL-8, growth-related oncogene-α, MCP-1, and NO production by epithelial cells, while metacyclic tryptomastigotes do not elicit such proinflammatory responses.

In our work, the first sign of induction of cytokine expression was the appearance of IL-10 and IL-12 messages at day 10 after inoculation, the former being stronger. The other cytokines expressed were IL-4 and TGF-β, and IFN-γ but these appeared late in the infection. These findings suggest that *T. cruzi* could ensure their dissemination and establishment into the host by impairing protective IFN-γ and IL-12 cytokine expression at the site of entrance. As shown earlier, the first cytokines expressed do so only a week after infection. Moreover, the cytokine consistently induced at this time is IL-10, which has been involved in susceptibility rather than in parasite resistance [21].

The role of IL-12 in resistance to *T. cruzi* infection has previously been reported in *in vitro* studies with tryptomastigotes infected macrophages. Its production was detected as early as 12 h after *in vitro* infection [14]. Resistance is also mediated by NK cell activity and IFN-γ production [8,9,22].

In this context, *in vitro* studies using metacyclic trypomastigotes infected macrophages have shown impaired ability to release IL-12 but not IL-1 [13]. Furthermore, amastigotes and trypomastigotes (vertebrate host-derived stages) but not metacyclic tryptomastigotes are capable of triggering the synthesis of nitric oxide by macrophages primed with IFN-γ [12]. This suppression or impairment of IL-12 production has also been observed in *Leishmania mexicana* amastigotes infected macrophages [23].

Our *in vivo* findings support the above *in vitro* data, where IL-12 and IFN-γ are poorly and belatedly induced at the inoculation site.

The immune response at regional lymph nodes was somewhat different to that observed at the inoculation site. In the former, an immediate and strong response of IL-12, IL-10, TGF-β and IL-2 was detected from the first day after inoculation. The stronger signal at day 1 post-infection corresponded to IL-10 and the weaker to IL-2. Although IL-12 was expressed throughout the first week post-inoculation, IFN-γ, a cytokine implicated in parasite resistance, was not detected until the fifth day. This delayed expression may influence or allow for the spreading of parasites from the inoculation site to the periphery. Likewise, delayed and weak IFN-γ expression could be associated with the strong expression of TGF-β observed during the first days post-infection. It is known that type 1 helper T cell requires IL-12 for enhanced production of IFN-γ and, on the contrary, that TGF-β exerts a strong inhibitory action on the production of IFN-γ [24]. Moreover, IL-10 was more consistently expressed in regional lymph nodes than IFN-γ, which was undetectable in the early and chronic phases of the infection.

It has been reported that immune responses in secondary tissues such as lymph nodes and spleen could be different. Our results show that IFN-γ is expressed in lymph nodes solely at the beginning of the parasite load corre-
responding to days 5 to 10 post-infection, but it has been reported in the spleen that IFN-γ remained expressed throughout acute and chronic infection [25]. This difference may be due in part to the possibility that blood trypomastigotes themselves act as immune activators and are eliminated preferentially in the spleen or liver rather than the lymph nodes, making the spleen cells the main source of IFN-γ even in the chronic phase, as well as to intrinsic differences in organs as suggested in previous works [26,27].

In the heart, a mixed inflammatory and anti-inflammatory cytokine profile was observed, with a delicate balance between hyperactivity and protective immune responses. In the parasitic acute phase, the cytokines expressed in the heart were IL-12, IFN-γ, IL-10 and TGF-β. As mentioned above, IL-12 and IFN-γ play an important role in the control of parasite load, and IL-12 is essential to the induction and maintenance of IFN-γ production by CD4 + T cells and cell-mediated immunity. However, it has been reported that IL-12 also plays a critical role in the development of autoimmune myocarditis, supporting Th1 type autoreactive cells, whereas IFN-γ has a protective rather than destructive role [28]. Since one hypothesis to explain the pathology in Chagas disease is autoimmunity, it is plausible that the outcome of the disease development is established in the acute phase. On one hand IFN-γ favors the destruction of parasites, but on the other IL-12 may support autoreactive cells. In this intricate spectrum there are inflammatory (IL-12 and IFN-γ) and anti-inflammatory cytokines (IL-10 and TGF-β). Moreover, TGF-β is also implicated in the generation of extracellular matrix and consequently fibrosis. Similarly, there is data that implicates TGF-β in Chagas disease myocardiopathy [29]. In the chronic phase of infection after 90 days post-infection, only IL-4, IL-10 and IL-12 messages persisted, supporting the notions regarding autoimmunity mentioned above, but at the same time an active suppression is mediated by IL-4 and IL-10 [30-32].

Differences in cytokine expression patterns among the tissues tested (skin from inoculation site, lymph node, and heart) are not at all surprising given the different cellular composition of these tissues as well as the difference in composition of infiltrating cells.

Serum cytokine concentrations seem to provide a clear example of the immune compartmentalization of the immune response. In this case, practically all cytokines assayed were detected, except for IL-2, a finding that is well documented [33,34].

It is important to point out that, while IL-12 and IFN-γ are strongly and consistently detected in serum throughout infection, they are only weakly and briefly expressed at the inoculation site and only IL-12 was consistently detected in lymph nodes.

In conclusion, the results presented here may contribute to the hypothesis that metacyclic trypomastigotes survive and spread from the inoculation site because of a delay in local protective immune responses.

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