Trypanosoma cruzi

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Increased Susceptibility of Stat4-Deficient and Enhanced Resistance in Stat6-Deficient Mice to Infection with *Trypanosoma cruzi*¹

Rick L. Tarleton,²,³ Michael J. Grusby, † and Lei Zhang ³*

Although Th1-type responses tend to be associated with resistance to *Trypanosoma cruzi* infection, mixed Th1 and Th2 cytokine responses are generally observed in both resistant and susceptible mice. To help clarify the role of type 1 and type 2 cytokine mechanisms, including activation of macrophages, high level production of type 1 cytokines, and downregulation of type 2 cytokines, were investigated in the immune response to *Trypanosoma cruzi* infection. A mixed type 1-type 2 cytokine response is observed in resistant mice, while a type 2 response is observed in susceptible mice. The results of this study suggest that a balanced type 1/type 2 cytokine response may be beneficial in host immunity to *Trypanosoma cruzi*.

*Trypanosoma cruzi* is an intracellular protozoan and the agent of Chagas disease, the leading cause of heart disease and one of the major causes of morbidity and mortality in Latin America. Current methods for treatment of the infection are of limited efficacy, and effective vaccines are nonexistent.

Mouse models of *T. cruzi* infection have been crucial for defining the mechanisms important in immune control of this parasite. Experiments employing mouse strains with null mutations in genes encoding proteins of immunological function have been particularly insightful. Among other findings, these studies have revealed that immune control of *T. cruzi* requires multiple immune effector mechanisms, including activation of macrophages, high level production of Abs, and stimulation of CD8⁺ T cells (1–6). The role of cytokines in the immune control of *T. cruzi* has also been addressed in a variety of murine model systems, demonstrating that the overproduction of type 2 cytokines or blockage of type 1 cytokine production correlates with increased susceptibility to lethal infection (7–15). However, mice that are resistant to *T. cruzi* infection exhibit a mixed type 1/type 2 pattern of cytokine production in both the lymphoid compartment and in organs that are the targets of parasite infection (e.g., heart and skeletal muscle) (16, 17). This mixed pattern of cytokine production in resistant mice, along with the requirement for strong Ab responses for protection, suggests the possibility that a balanced type 1/type 2 cytokine response may be beneficial in host immunity to *T. cruzi*.

To test this hypothesis, infection with *T. cruzi* was monitored in mice with induced defects in the Stat4 or Stat6 genes. STATs are transcriptional regulators that are responsible for transduction of cytokine signals from cell surface cytokine receptors to the nucleus (18). Stat4 is activated in response to IL-12 and provides the signals necessary to drive Th cells along a Th1 lineage. Stat6 is activated by IL-4 and IL-13 binding, and provides the alternative signal for progression along a Th2 lineage. Specific disruption of the Stat4 or Stat6 genes results in animals that are able to generate only Th2 or Th1 responses, respectively, providing an excellent experimental system for analysis of the importance of these responses during infection (19–23). The results of the present study establish that type 2 cytokine responses are not only not required for control of *T. cruzi* infection, but that their presence contributes to enhanced parasite persistence and an increase in the severity of disease in the chronic phase of the infection.

**Materials and Methods**

**Mice and parasites**

Stat4⁻⁻ and Stat6⁻⁻ deficient mice, produced as previously described (20, 23), were of a mixed BALB/c × 129Sv genetic background. Homozygous Stat4⁻⁻ and Stat6⁻⁻ deficient mice and their control wild-type littermates were bred in the facilities of the Harvard School of Public Health (Boston, MA) under specific pathogen-free conditions and were transferred to the University of Georgia (Athens, GA). Female mice (6–12 wk of age) were infected by the i.p. route with blood-form trypomastigotes of the Brazil strain of *T. cruzi*. Parasitemia levels were determined by hemacytometer counting of diluted tail vein blood, and mice were monitored daily for deaths.

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positive cells; 2
and by in situ immunocytochemistry for cytokine-producing cells. Mice infected for 16 and 25 days were analyzed; data are shown for animals at day 25 postinfection. Inflammatory foci are evident in the wild-type and Stat6-deficient mice (A and B), but not in the Stat4-deficient mice, in which parasite-infected cells are abundant (arrows in C and D).

In situ PCR

In situ PCR for detection of kinetoplast DNA (kDNA)\(^4\) was performed as previously described in detail (25, 26). PCR was performed in a GeneAmp In Situ PCR System1000 (Perkin-Elmer, Foster City, CA), according to manufacturer’s instructions. Primers specific for a conserved region of kDNA (forward primer, 5'-GGTTCGATTGGGGTTGGTGTAATATA-3'; reverse, biotinylated primer, 5'-biotin-CCAAAAATTITGAACCGCCCTCCCCTCCCAA-3') of T. cruzi were used for the amplification reactions. Upon completion of the PCR reaction, slides were washed and incubated in avidin-peroxidase (Vector Laboratories, Burlingame, CA) before detection with the peroxidase substrate 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) and counterstaining with hematoxylin.

Results

Wild-type 129J and BALB/c × 129J mice are normally resistant to infection with moderate doses (<10\(^3\)) of the Brazil strain of T. cruzi and survive for as long as 2 yr postinfection. However, compromising the ability of these mice to mount CD4\(^+\) or CD8\(^+\) T cell responses (5, 6) or to produce Abs (4) results in their failure to control the infection and in death during the acute phase of infection. To determine the contribution of type 1 and type 2 cytokine

![Figure 1](http://www.jimmunol.org/)

**Figure 1.** Stat4- and Stat6-deficient mice and their wild-type littermates were infected with 5 × 10\(^3\) blood-form trypomastigotes of the Brazil strain of T. cruzi. Parasitemia levels were determined by hemacytometer counting of diluted tail vein blood, and mice were monitored daily for deaths (n = 10 for wild-type mice, and 7 each for Stat4- and Stat6-deficient groups, respectively).

**Table I.** Histological, parasitological, and immunocytochemical analysis of Stat4- and Stat6-deficient mice infected with T. cruzi\(^2\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>I.S.</th>
<th>Parasite Load</th>
<th>CD8 cells</th>
<th>IFN-γ</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
<th>TGF-β</th>
<th>TNF-α</th>
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<td>NA</td>
<td>+++</td>
<td>+/+</td>
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<td>+/−</td>
<td>+++</td>
<td>+++</td>
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<tr>
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<td>NA</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>+/−</td>
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<td>NA</td>
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<td>Stat4(^+/+)</td>
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<td>Stat4(^−/−)</td>
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<td>+</td>
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</table>

\(^2\) Spleen and heart sections were analyzed by hematoxylin staining for the presence of inflammatory cells (I.S., inflammation score), T. cruzi-infected cells (parasite load), and by in situ immunocytochemistry for cytokine-producing cells. Mice infected for 16 and 25 days were analyzed; data are shown for animals at day 25 postinfection. +, No positive cells; +/−, 1–5 mildly positive cells; +, 1–10 strongly positive cells; ++, 11–25 strongly positive cells; ++++, 25–50 strongly positive cells; and ++++, >50 strongly positive cells per 25 noncontiguous ×25 microscopic fields.

\(^4\) Abbreviation used in this paper: kDNA, kinetoplast DNA.
responses to control of T. cruzi infection, we infected mice with induced deficiencies in the Stat4 or Stat6 genes with 5 × 10³ of the Brazil strain of T. cruzi. As expected, Stat4-deficient mice exhibit high parasitemias and die relatively early in the infection (Fig. 1) at time points close to that previously observed for mice lacking CD4⁺ and/or CD8⁺ T cells (5, 6). However, Stat6-deficient mice were indistinguishable from wild-type mice in both parasitemia and in longevity and survived the acute phase of the infection. Thus, mice lacking the ability to amplify type 1 cytokine responses fail to generate the responses necessary to control T. cruzi infection, but the absence of type 2 cytokine responses has no apparent adverse effect on the generation of protective immunity.

Histological analysis of heart tissue from STAT-deficient mice confirmed the results of the parasitism and longevity studies. Wild-type and Stat6-deficient mice display multiple inflammatory foci, but very low parasite load in the heart at 25 days postinfection (Fig. 2). In contrast, Stat4-deficient mice exhibit a poor inflammatory response and large numbers of heavily infected cardiocytes, indicative of the failure to generate a productive cell-mediated immune response.

Table I summarizes the histological, parasitological, and immunological characteristics of heart and spleen in wild-type and STAT-deficient mice at 25 days postinfection. In addition to the relatively low inflammatory score and the high tissue parasite load, Stat4-deficient mice also have very few IFN-γ-producing cells in the spleen or heart. In addition, IFN-γ was not detected in the serum of T. cruzi-infected Stat4-deficient mice (data not shown). In contrast, wild-type and Stat6-deficient mice have large numbers of IFN-γ-producing cells, particularly in the spleen. Consistent with previous reports (16, 17), few cells producing the type 2 cytokines IL-4 and IL-5 are present in mice at these early time points in the infection; no IL-4- or IL-5-producing cells were observed in Stat6-deficient mice. The number of cells producing TGF-β or TNF-α appeared to be unaltered by the lack of expression of either Stat4 or Stat6.

Ab responses to T. cruzi were consistent with the status of Stat4 and Stat6 expression in the deficient strains (Fig. 3). Levels of anti-T. cruzi Abs were substantial in both Stat4-deficient and Stat6-deficient mice. Stat4-deficient mice show a significant increase in IgG1 production, and Stat6-deficient mice exhibit decreased IgG1 levels and increased IgM, and IgG2b levels relative to wild-type littersmates. No anti-T. cruzi IgE Abs were detected in any of the groups. Thus, based upon Ab subclasses and cytokine production patterns, Stat4-deficient and Stat6-deficient mice continue to exhibit the respective phenotypes of Th1- and Th2-deficient mice when infected with T. cruzi. Furthermore, the absence of Th2 cells does not compromise the overall capacity of mice to produce Abs to T. cruzi.

Wild-type and Stat6-deficient mice appeared equally resistant to infection with moderate doses of the Brazil strain of T. cruzi (Fig. 1). In an attempt to reveal a differential resistance of these strains, two experiments were conducted. First, wild-type and Stat6-deficient mice were infected with higher doses of T. cruzi (5 × 10³ Brazil strain parasites). However, as with the lower dose infections, wild-type and Stat6-deficient mice had similar parasitemia levels and longevity (Fig. 4). Second, we examined the severity of chronic disease and the presence of tissue parasites in wild-type and Stat6-deficient mice. Previous experiments have shown that a variety of conditions that enhance or suppress immune responses in T. cruzi-infected mice also alleviate or exacerbate, respectively, disease in the chronic phase of the infection previously reviewed (27). Qualitatively, Stat6-deficient mice exhibit substantially less inflammatory disease in the skeletal muscle at 5.5–6.5 mo postinfection (Table II and Fig. 5). More importantly, we were unable to detect parasites or parasite kDaNA in the tissues of Stat6-deficient mice, despite the use of a highly sensitive in situ PCR technique (26). Despite the apparent lack of parasites in tissues from chronically infected Stat6-deficient mice, these mice remain infected, as demonstrated by the exacerbation of parasite load following sublethal irradiation (data not shown). Nevertheless, these results strongly suggest that the lack of a type 2 cytokine response is not only not deleterious in T. cruzi infection, but it allows for the development of more efficient immune control of the infection.
Parasitemia and mortality in wild-type and Stat6-deficient mice infected with 5 x 10^6 trypomastigotes of the Brazil strain of T. cruzi (n = 5 mice per group).

FIGURE 5. In situ PCR analysis for the presence of T. cruzi kDaNA in skeletal muscle tissue from wild-type (A) and Stat6-deficient (B) mice 6 mo postinfection with T. cruzi. The brown precipitate in interstitial spaces and in inflammatory foci in A is indicative of the presence of T. cruzi kDaNA.

Table II. Inflammation and parasite load in wild-type and Stat6-deficient mice infected with T. cruzi

<table>
<thead>
<tr>
<th>Strain</th>
<th>Heart</th>
<th>Skeletal Muscle</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>I.S.</td>
<td>In situ PCR</td>
</tr>
<tr>
<td>Stat6^+/+</td>
<td>+/+</td>
<td>++</td>
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<tr>
<td></td>
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<td>Stat6^−/−</td>
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* I.S., inflammatory score, see Materials and Methods. The results of the analysis on an individual animal is indicated on each line.
have argued that type 2 cytokine responses may increase the severity of disease in acute and chronic T. cruzi infection (40, 42–44), or alternatively may reduce the severity of disease by modulating a potentially overactive type 1 cytokine response (10, 38, 45). The most dramatic example of exacerbation of inflammatory disease resulting from manipulation of cytokine production patterns in T. cruzi infection comes from studies with IL-10−/− mice infected with T. cruzi. These animals show a marked enhancement of production of type 1 cytokines and an earlier time to death, demonstrating a clear requirement for IL-10 in modulating disease severity (46).

The ability of Stat6-deficient mice to survive the acute infection provided the opportunity to study the course of disease in the presence of a strongly biased type 1 cytokine response. Although the T. cruzi-infected Stat6-deficient mice appear similar to wild-type mice in terms of parasitaemia and longevity, close examination of tissues from chronically infected mice revealed an important difference: relative to wild-type mice, Stat6-deficient mice exhibited fewer parasites and less inflammatory disease. Thus, animals that can generate only one type 1 cytokine response do not exhibit increased inflammatory disease. Indeed it appears that the presence of type 2 responses in wild-type mice contributes to the persistence of T. cruzi and potentially increases the severity of disease. These data thus support the hypothesis that the severity of disease in chronic T. cruzi infection depends on the quality and quantity of the immune response and the ability of this response to limit tissue parasite load (reviewed in Ref. 27). In the absence of a type 2 cytokine response, there is more efficient clearance of parasites from infected tissues and a resultant decrease in signs of disease (inflammation).

The type 1 cytokine response does not appear to be totally unchecked in the absence of type 2 cytokine production in the Stat6-deficient mice. Immunohistochemical analysis demonstrates that the number of IFN-γ- and TNF-α-producing cells is not significantly increased in the T. cruzi-infected Stat6-deficient mice. In addition, it is likely that TGF-β- and IL-10-producing cells (although we did not measure the latter) contribute to the IL-10-deficient mice. Thus, a strongly Th1-biased response in T. cruzi infection does not appear to be deleterious as long as IL-10 and (perhaps) TGF-β production is unimpeached.

It is also important to note the apparent absence of disease in the Stat6-deficient mice, despite the lack of complete clearance of T. cruzi. This observation is further evidence that parasite clearance may not be required to prevent the development of disease in chronic T. cruzi infection. It will be interesting to see whether humans with chronic T. cruzi infection, but without signs of Chagas disease, also exhibit a strongly biased type 1 cytokine response in association with modest parasite levels (47–49).

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References


