

Short Report: Assessment of CD8⁺ T Cell Differentiation in *Trypanosoma cruzi*-Infected Children

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Abstract. We previously reported that the T cell compartment in chronically *Trypanosoma cruzi*-infected adult subjects display functional and phenotypic signs of immune senescence. This study aimed to investigate the differentiation and the senescent profile of the overall CD8⁺ T cell compartment in *T. cruzi*-infected children at the early stage of the disease. We found a lower percentage of naive (CD27⁺CD28⁺CD45RA⁺) and early antigen-experienced (CD45RA⁺CD27⁺CD28⁺), and higher percentages of late differentiated antigen-experienced (CD45RA⁺CD27⁺CD28⁺) CD8⁺ T cells in *T. cruzi*-infected children as compared with age-matched uninfected controls. The expression of the interleukin (IL)-7R is also decreased on naive and on antigen-experienced total CD8⁺ T cells with various degrees of differentiation. Conversely, the expression of HLA-DR, caspase-3, and CD57 did not vary on the total CD8⁺ T cell compartment. These findings suggest that the duration of the infection is relevant in the process of immune senescence that this parasite can induce.

Chagas disease caused by the intracellular protozoa *Trypanosoma cruzi* is one of the major human health problems in Latin America. The disease evolves through an acute to a chronic phase, where in subjects may be clinically asymptomatic or show progressive heart disease leading to an end-stage dilated cardiomyopathy in 20–30% of the infected individuals. It is estimated that approximately 4 million chagasic individuals have developed heart disease, making Chagas disease the most frequent cause of infectious cardiomyopathy in the world.^{1,2}

Immune control of *T. cruzi* is complex, requiring the generation of a substantial antibody response and the activation of both CD4 and CD8 T cell responses. Even in cases in which such responses are stimulated sufficiently to control the acute infection, *T. cruzi* is not completely cleared but instead, persists in infected hosts for decades.³

The peripheral T cell repertoire is in a constant state of flux as these cells see numerous environmental signals that are continuously varying.⁴ After a first encounter with cognate Ag, naive T cells proliferate and acquire effector function. As infection is controlled, the majority of T cells mediating the primary response die and a small population remains to form the memory population.^{5,6} A different situation occurs if the pathogen is not eliminated, where immune T cells are recurrently stimulated by these pathogens and T cell clonal exhaustion might occur.^{6,7} Moreover, the environment of a persistent infection may lead to a situation of general immune activation that also drives T cells not specific for the persisting pathogen to a differentiated state with impaired proliferation and interleukin (IL)-2 production in response to antigen or agonist T cell receptor (TCR)-specific antibodies, and variable reduced secretion of interferon-gamma (IFN- γ) that will ultimately result in exhaustion.^{8,9}

We have shown that humans with long-term (> 20 years) infection with *T. cruzi* have relatively modest CD4⁺ and CD8⁺ T cell responses to *T. cruzi* proteins/peptides and that the whole CD4⁺ and CD8⁺ T cell population in these subjects show signs of exhaustion and senescence, consistent with the persistence of infection in these individuals.^{10–13} In the face of persistence stimulating antigen, we hypothesize that the *T. cruzi*-specific

and the overall T cell compartments are eventually driven to exhaustion and exhibit a low frequency of competent parasite-specific T cells. One prediction of this hypothesis is that individuals with shorter-term infections would have fewer senescent/exhausted T cells. Herein, we studied the differentiation and the senescent phenotypic profile of the overall CD8⁺ T cell compartment in *T. cruzi*-infected children at the early stage of the disease.

Eight to 14-year-old children were enrolled at the Instituto Nacional de Parasitología “Dr M Fátala Chaben” (INP), the reference center for the diagnosis in Argentina, where the *T. cruzi* infection status was determined by a combination of indirect immunofluorescence assay, hemagglutination, and enzyme-linked immunosorbent assay (ELISA) tests. All mothers from the children enrolled in the study had positive serology for *T. cruzi*. Infected subjects positive on at least two of these tests were considered to be infected. All the infected children were in the asymptomatic phase of the infection and had not received etiological treatment at the time of this study. This protocol was approved by the Institutional Review Board of the Centro Nacional de Genética, Administración Nacional de Laboratorios e Institutos de Salud, Dr. Carlos G. Malbrán, Buenos Aires, Argentina and informed consent was obtained from the parents of all children.

Approximately 10 mL of blood were drawn by venipuncture into heparinized tubes (Vacutainer, Becton-Dickinson, San Jose, CA). Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation on lymphocyte separation medium (ICN, Aurora, OH) and were stained with anti-CD8 peridinin chlorophyll protein (PerCp), anti-CD28 allophycocyanin (APC), anti-CD45RA phycoerythrin-Cy5 (PE-Cy5), anti-CD27 phycoerythrin (PE), anti-IL-7R (CD127) PE, or anti-CD57 fluorescein isothiocyanate (FITC). After incubation, the cells were permeabilized with Cytotfix/Cytoperm solution (Pharmingen, San Diego, CA) and then stained with anti-caspase-3 FITC. Data were acquired on a FACScalibur (Becton-Dickinson) and analyzed with Flowjo (version 4.2, Tree Star, San Carlos, CA) software. Differences between groups were evaluated by the Mann-Whitney test. Differences were considered statistically significant when $P \leq 0.05$.

To evaluate the effects of persistent *T. cruzi* infection on total CD8⁺ T cells, the naive, memory, and effector profile was characterized in 41 chronically *T. cruzi*-infected children (age $\pm$ SD = 10.2 $\pm$ 2 y) and 34 age-matched uninfected controls (age $\pm$ SD = 11.2 $\pm$ 1.9 y) based on the expression of CD45RA, CD27, and CD28. The percentage of total CD8⁺ T lymphocytes

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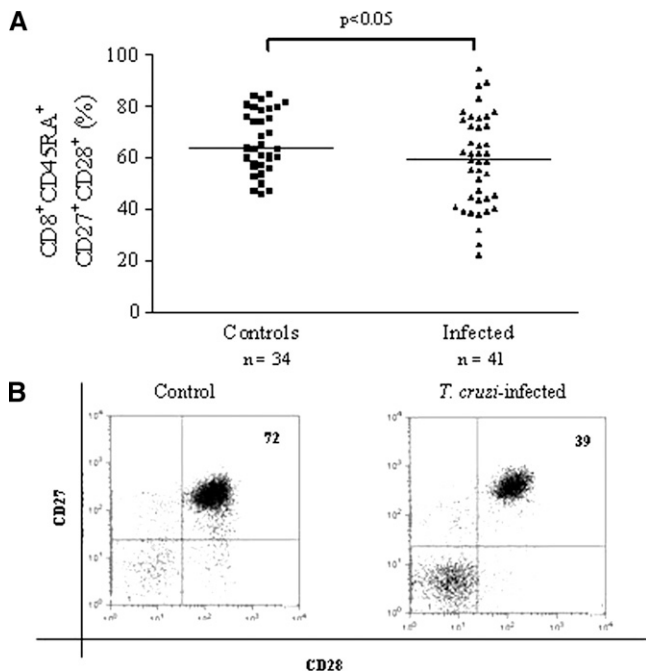


FIGURE 1. (A) Frequency of total naive CD8⁺ T cells in *T. cruzi*-infected children. Each point represents the percentage CD45RA⁺CD27⁺CD28⁺CD8⁺ T cells. Median values are shown by horizontal lines. Comparisons between groups were performed by Mann-Whitney *U* test. (B) Representative flow cytometry profile of CD27 and CD28 expression in the CD45RA⁺CD8⁺ T cell compartment for one uninfected control and one *T. cruzi*-infected child. The numbers in the upper right quadrant show the percentage of total naive CD8⁺ T cells.

and CD8⁺CD45RA⁺ T cells was similar in *T. cruzi*-infected children (mean percentage $\pm$ SD = 27.8 ± 7.7 and 6.7 ± 3.3 , respectively) and uninfected controls (26.5 ± 6.3 and 6.5 ± 3.9 , respectively). Naive (CD27⁺CD28⁺CD45RA⁺) CD8⁺ T cells were decreased in *T. cruzi*-infected children as compared with uninfected controls (Figure 1). Lower percentages of early antigen-experienced (CD45RA⁺CD27⁺CD28⁺) and higher percentages of late differentiated (CD45RA⁺CD27⁺CD28⁺) CD8⁺ T cells were also found in *T. cruzi*-infected children than in the uninfected control group (Figure 2), showing as a whole an activation status of the immune system of *T. cruzi*-infected children.

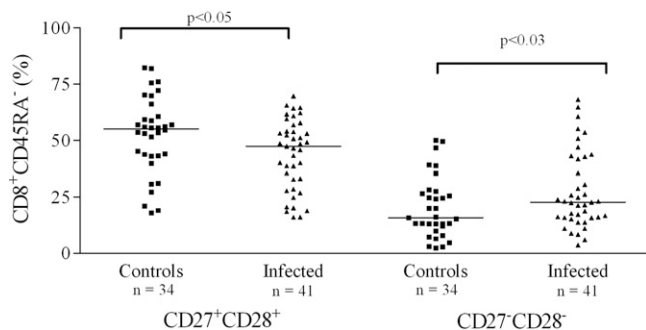


FIGURE 2. Maturation profile of the total memory CD45RA⁺CD8⁺ T cell compartment in *T. cruzi*-infected children. Each point represents the percentage of early (CD27⁺CD28⁺) and late antigen-experienced (CD27⁺CD28⁺) CD45RA⁺CD8⁺ T cells. Median values are shown by horizontal lines. Comparisons between groups were performed by Mann-Whitney *U* test.

We also measured the expression of the IL-7 receptor, involved in the development and maintenance of T cells.¹⁴ Decreased expression of IL-7R on naive (Figure 3A and C) and on antigen-experienced total CD8⁺ T cells with various degrees of differentiation (Figure 3B and D) was observed in *T. cruzi*-infected children compared with uninfected subjects.

Conversely, the analyses of the activation molecules HLA-DR and caspase-3 as well as the marker of replicative senescence, CD57, on naive, memory, and effector total CD8⁺ T cells did not vary in *T. cruzi*-infected children (data not shown).

These findings show that as in chronically *T. cruzi*-infected adult subjects, the naive and antigen-experienced T cell compartments in children in early stages of the disease already reflect the impact of antigen persistence, with naive T cells being likely constantly stimulated and driven into the effector response that may in turn exhaust T cell responses as observed in long-term adult *T. cruzi*-infected individuals.^{10–13} However, these *T. cruzi*-infected children show fewer signs of immune senescence compared with adult subjects who display high levels of CD4⁺ and CD8⁺ T cells expressing CD57 and high levels of apoptosis in the T cell compartment.^{11,13} In pediatric human immunodeficiency virus (HIV) infection a decrease in naive CD8⁺ T cells has also been shown supporting that persistent infections may affect thymic output of naive T cells.¹⁵

Accumulated evidences have shown that the combined action of benznidazole and the host immune system has a role in the efficacy of anti-*T. cruzi* chemotherapy.^{16–19} The less senescent immune status observed in children than in adult *T. cruzi*-infected subjects might support, somehow, previous observations showing that chemotherapy against *T. cruzi* is more effective in children than in adult subjects.^{20–23} Therefore, early treatment could result in higher treatment efficacy.

Our data are in agreement with those from Laucella and others²⁴ showing high levels of soluble P-selectin and soluble vascular cell adhesion molecule-1 (VCAM-1), which reflects endothelial activation distinctive of an ongoing inflammatory process in *T. cruzi*-infected children. Nevertheless, other authors have reported an overall low immune activation in early indeterminate Chagas disease²⁵ with a mixed pro- and anti-inflammatory cytokine profile.¹⁶

Competent and effective T cell responses are crucial to limit parasite replication and the direct damage that this can do. The results shown herein suggest that the duration of the infection may be implicated in the development of immune senescence in chronic Chagas disease. Additional longitudinal studies will be necessary to ascertain this hypothesis.

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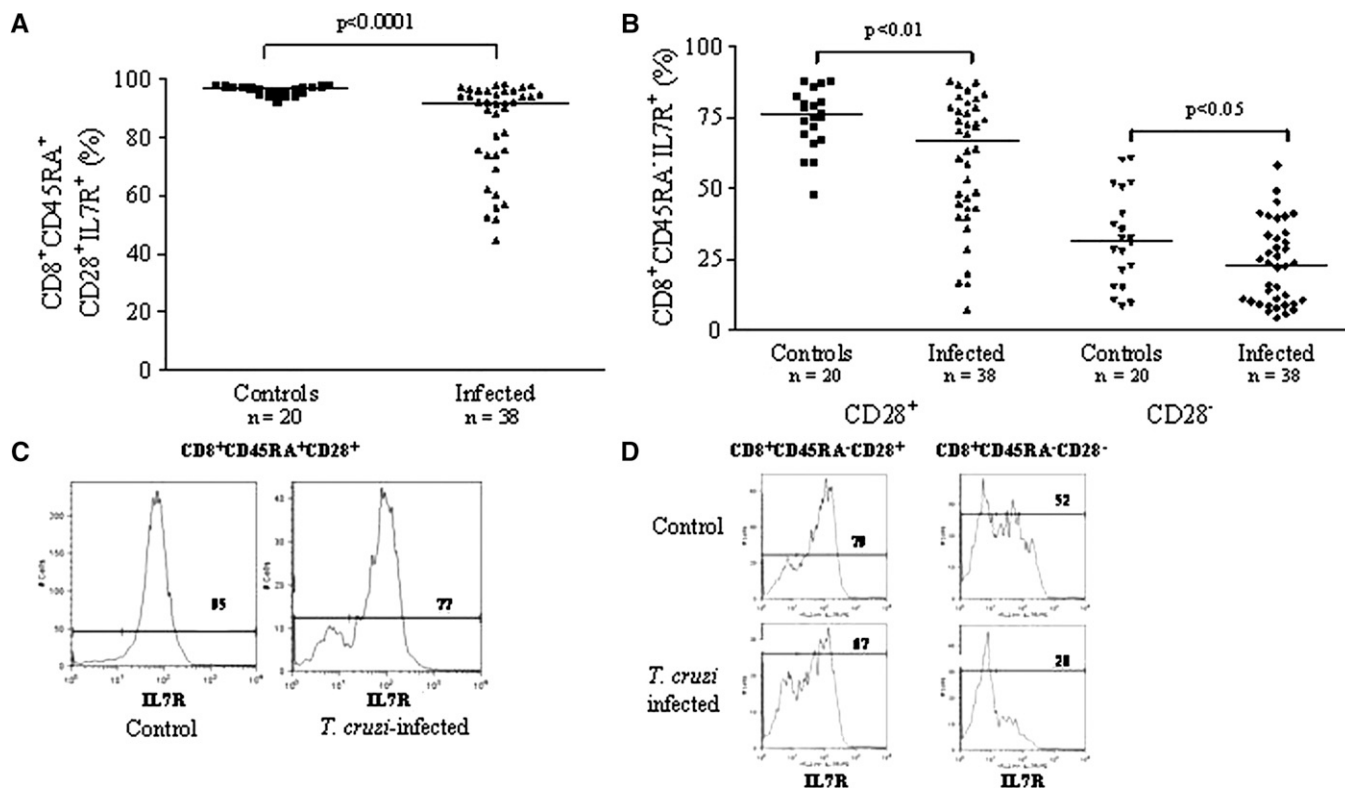


FIGURE 3. Interleukin (IL)-7R expression in naive and antigen-experienced CD8⁺ T cells in *T. cruzi*-infected children. Each point represents the expression of IL-7R on the total (A) CD45RA⁺CD28⁺ and (B) CD45RA⁻CD28⁺ CD8⁺ T cell populations. Median values are shown by horizontal lines. Comparisons between groups were performed by Mann-Whitney *U* test. Representative histogram for the expression of (C) CD8⁺CD45RA⁺CD28⁺IL7R⁺ and (D) CD8⁺CD45RA⁻CD28⁺IL7R⁺ in one *T. cruzi*-infected child and one uninfected control. The numbers show the percentage of naive (C) and memory CD8⁺ T cells that express the IL-7R (D).

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