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Cytokine modulation, oxidative stress and thymic dysfunctions: Role of age-related changes in the experimental *Trypanosoma cruzi* infection
Age-related thymic dysfunctions and *Trypanosoma cruzi infection*

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ABSTRACT

Aging is linked with a thymic oxidative damage and some infectious diseases such as Chagas' disease may aggravate this process. The aim of this study was to evaluate the production of distinct cytokines as well as the antioxidant/oxidant status of the thymus and thymocytes populations during *Trypanosoma cruzi (T. cruzi)* infection. Young (5 weeks old) and aged (18 weeks old) male Wistar rats were inoculated with blood trypomastigotes forms of the Y strain of *T. cruzi*. On the 16th day after *T. cruzi* infection, increased concentrations of transforming growth factor β (TGF-β), interleukin (IL)-12, IL-17 were detected in aged infected subjects as compared to young infected ones. Interestingly, a reduction in the production of tumor necrose factor (TNF)-α was observed in aged infected rats when compared to young infected subjects. Aged-infected rats presented increased O_2^- levels, compared to young counterparts. Significant raise in the generation of O_2^- in aged infected animals, as compared to uninfected counterparts was observed. Up-regulated expression of Nox2 in the thymus of young and aged infected animals was observed. An increased SOD2 expression was detected in the thymus of young animals infected with *T. cruzi*, when compared to uninfected young rats. Aged animals showed reduced thymus weight and the number of thymocytes. Decreased percentages of $SPCD4^+$ and $SPCD8^+$T cells were detected in aged and control groups when compared to young counterparts. In summary, this is the first data to directly examine the influence of aging on age-related dysfunctions during the acute phase of experimental Chagas disease. Concerning to oxidative stress, it is clear from our analysis that aged infected rats suffer a more intense oxidative damage when compared to young and infected ones. Age and infection triggered a dynamic interplay of cytokines, oxidative stress and thymic dysfunctions which led to impaired response from aged and infected rats. Such findings may have significant functional relevance in therapeutic strategies in order to reestablish the thymic immunological function which occurs in aged and *T. cruzi* infected subjects.

1. Introduction

Chagas' disease is a parasitic infection caused by the flagellate protozoan *Trypanosoma cruzi* that was firstly described in 1909 by the Brazilian physician Carlos Chagas. This disease remains a serious public health problem in Latin America, affecting nearly 8 million people [50], and causing approximately 10,000 deaths every year [57,65]. This disease is now recognized as an emerging worldwide threat to other non-endemic regions, including in the United States, Europe and the Western Pacific Region, as a result of migration of infected people and non-vectorial transmission routes, such as blood transfusion, organ transplantation, and congenitally [67].

The control of the early phase of *T. cruzi* infection requires a complex immune response, which involves cells of both innate and acquired immunity as well as local and systemic production of several pro-inflammatory cytokines, chemokines and other mediators, such as nitric oxide (NO) [22,33], which in turn, lead to effective microbicidal action and important for controlling parasite replication. Although the

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mechanisms underlying the pathogenicity of Chagas' disease are not completely clear, it is well established that thymic impairment and oxidative stress as well as the inefficient scavenging of reactive oxygen species (ROS) may contribute to the dysfunction and pathogenesis during chronic *T. cruzi* infection [31,38].

During *T. cruzi* infection, macrophages are one of the first phagocytes to be invaded and they try to control the progression of the disease by internalizing metacyclic trypomastigotes into the phagosome leading to activation of membrane associated NADPH oxidase [9]. In its active form, NADPH oxidase is responsible for the generation of superoxide anion (O_2^-) against the internalized parasite [47]. Nox2 is the main catalytic subunit of NADPH oxidase that is expressed in phagocytes, where it plays an important role in host defense and in regulating inflammation [56]. In parallel to *T. cruzi* infection, senescence has been widely related to the deleterious effects of ROS, which are important mediators for cellular senescence progression [23]. O_2^- can be enzymatically dismutated by the enzyme superoxide dismutase (SOD), which acts as the first line of antioxidant defense [23].

Several studies have revealed the inefficient functioning of the thymus during Chagas' disease, which induces a strong and transient cell depletion, especially in the cortical zone of this organ [1,29,53], leading to a massive death of cortical $CD4^+CD8^+$ double-positive (DP) thymocytes [29,36]. Furthermore, according to Savino et al. [53] the ability of *T. cruzi* parasites to colonize and multiply in the thymus, during acute infection, is of primary importance, contributing to the host persistent infection.

In the elderly, the features of age-induced impairment of immunological responsiveness also involve regression or involution of the thymus [19,30,60], with an important decrease in tissue mass and thymocyte numbers [48], as well as, reduction in naïve T cell output [2,19]. It has been demonstrated that the transforming growth factor β2 (TGF-β2) may also directly influence T-cell development. It has been suggested that aging exerts a negative influence of TGF-β on thymocyte function, which mainly affect thymic weight and cellularity [24]. Thymus changes throughout T cell development have an important impact in the generation of naïve peripheral T cells, and as a consequence, the thymus has an essential role on the adult T cell pool, influencing the functionality and responsiveness of the T cell repertoire [62].

Since intrinsic defects in aged thymocytes and altered release of immune cell-derived cytokines are classic hallmarks shared by aging and *T. cruzi* infection, we addressed whether the course of the acute *T. cruzi* infection is influenced by age-related changes. The purpose of our study was to investigated the production of IL-2, IL-12, IL-17, TGF-β, TNF-α, the antioxidant/oxidant status of the thymus as well as the determination of thymus weight and thymocytes populations during *T. cruzi* infection. With this purpose, we evaluated some surface markers such as CD25 and CD44 that allow the identification of the stages of T cell development. Other parameters were also evaluated including total thymocytes counting, thymus weight, proportion of T $SPCD4^+$ and $SPCD8^+$ cell subsets by flow cytometry. Regarding oxidative stress, another important consequence of Chagas disease, we analyzed whether senescence would increase O_2^- generation in the thymus of aged infected rats and the ability of SOD in balancing this response.

2. Material and methods

2.1. Animals

All experiments were conducted as per the guidelines of Conselho Nacional de Controle de Experimentação Animal (CONCEA, Brazil) after the necessary approval of the Ethics Committee on Animal Use of the University of São Paulo, Campus of Ribeirão Preto (protocol # 2014.1.928.53.0). Twenty four male Wistar rats (five weeks old), weighing between 180 and 250 g, and 24 male Wistar rats (eighteen months old), (humans between 45 and 50 years) weighing between 500 and 600 g were purchased from the Animal Facility of the University of São Paulo, Campus of Ribeirão Preto. Animals were separated in the following groups: young control (YC), young infected (YI), aged control (AC) and aged infected (AI). Animals were maintained on a 12-h light/12-h dark cycle with propper rodent chow and water ad libitum. To avoid ammonia concentration rat bedding was changed 3 times/week.

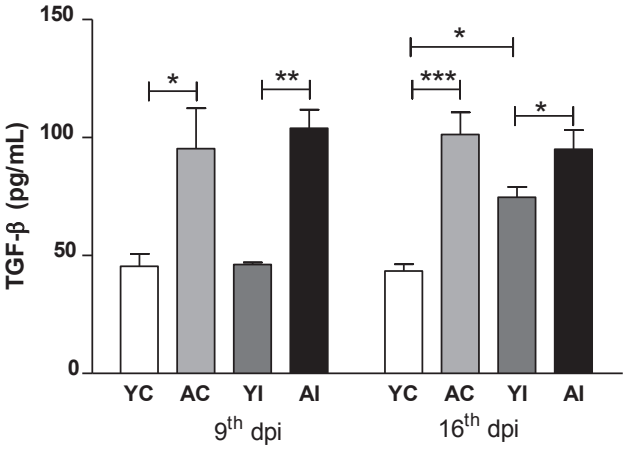

Fig. 1. Serum concentrations of TGF-β in young and aged male Wistar rats infected with 1.0×10^5 blood trypomastigotes of the Y strain of *T. cruzi* during experimental acute infection (9th and 16th day). Analysed groups: young control (YC), young infected (YI), aged control (AC) and aged infected (AI). Results are expressed as the means ± SEM of n = 6 animals. The comparison among groups were performed by One-way ANOVA followed by Bonferroni's multiple comparison test (*$p < 0.05$).

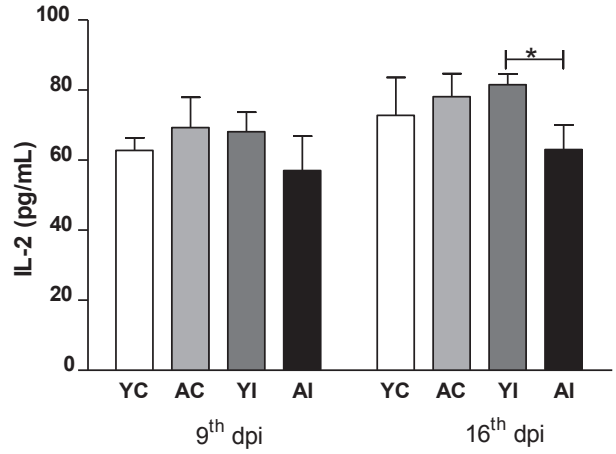

Fig. 2. Serum concentrations of IL-2 in young and aged *T. cruzi* infected male Wistar rats were evaluated in the following groups: young control (YC), young infected (YI), aged control (AC) and aged infected (AI). Results are expressed as the means ± SEM of n = 6 animals. The comparison among groups were performed by One-way ANOVA followed by Bonferroni's multiple comparison test (*$p < 0.05$).

2.2. Reagents and antibodies

RPMI 1640 medium and trypan solution were used (Sigma-Aldrich, St Louis, MO, USA). All anti-rat monoclonal antibodies anti-CD3-Allophycocyanin (APC), anti-CD4- phycoerythrin-Cy7 (PE-Cy7), anti-CD8-peridinin-chlorophyll-protein complex (PerCP), for cell-surface staining were purchased from BD PharMingen (BD Biosciences, San Jose, CA, USA). Fetal bovine serum (FBS) was used (GIBCO-Life Technologies, Baltimore, MD, USA) and immediately before use, solutions were diluted to specific desired concentration with medium.