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Purinergic enzymatic activities in lymphocytes and cardiomyocytes of mice acutely infected by *Trypanosoma cruzi* modulating the inflammatory responses

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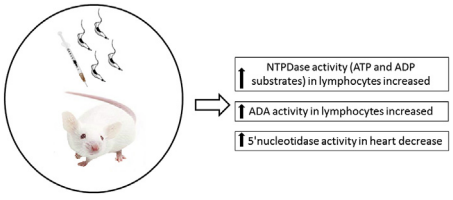
HIGHLIGHTS

- Chagas disease is a zoonosis caused by *Trypanosoma cruzi*.
- Activation of NTPDase can reduce ATP levels, an important inflammatory mediator.
- The ADA also had increased activity; however, by hydrolyzing adenosine, an anti-inflammatory molecule.
- These alterations may implicate in the pathophysiology of Chagas disease related of inflammatory response.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to evaluate the activity of purinergic enzymes in lymphocytes and cardiac tissue of mice experimentally infected by *Trypanosoma cruzi*. Twelve female mice were used, divided into two groups (n = 6): uninfected and infected. On day 12 post-infection (PI), the animals were anesthetized and after euthanized, and samples were collected for analyses. Infected mice showed reduction in erythrocyte counts, hematocrit and hemoglobin concentration, as well as reduced number of total leukocytes in consequence of neutrophilia ($P < 0.01$). The number of monocytes increased in infected mice ($P < 0.001$), however the number of lymphocytes and eosinophils did not differ between groups ($P > 0.05$). The E-NTPDase (ATP and ADP substrate) and E-ADA activities in lymphocytes increased significantly in mice infected by *T. cruzi* ($P < 0.01$). In the heart, multiple pseudocysts containing amastigotes within cardiomyocytes were observed, as well as focally extensive severe necrosis associated with diffuse moderate to severe inflammatory infiltrate of lymphocytes. Although, the NTPDase activity (ATP and ADP substrate) in the cardiac homogenate did not differ between groups, a reduction on 5'-nucleotidase activity ($P < 0.001$) and an increase in the ADA activity in infected animals ($P < 0.05$) were observed. Thus, animals infected by *T. cruzi* experienced the disease, i.e., showed anemia, leucopenia, and

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heart lesions. Associated with this, purinergic enzymes showed altered activities, which might be related to the modulation of the inflammatory response.

1. Introduction

Chagas disease is a zoonosis caused by *Trypanosoma cruzi* (Chagas, 1909), present mainly in Latin America and remains a serious health problem affecting about 6–7 million people in the American continent. Also, more than 25 million people are at risk of contracting Chagas disease in the world (WHO, 2015). This disease is featured for two phases: acute and chronic. The acute phase, which appears after one week of *T. cruzi* infection, causes low (<10%) mortality. After 1–2 months of infection, the immune system partially controls infection that persists for all life. Approximately 60–70% infected untreated people never develop clinical manifestations (indeterminate phase). However, the remaining 30–40% develop symptomatic chronic phase of the disease 10–30 years after infection, characterized by cardiomyopathy, megacolon, and megaesophagus (Chatelain, 2015; Steverding, 2014; Teixeira et al., 2011). These pathological changes might be due to different mechanisms and may affect other systems, such as the purinergic system that is involved in many physiopathological events in several acute diseases (Castilhos et al., 2015; Oliveira et al., 2012; Tonin et al., 2013), as investigated in this study.

The purinergic signaling system plays an important role in modulating the inflammatory and immune responses by extracellular purines such as ATP and adenosine (Yegutkin, 2008). High ATP levels can interact with P2 receptors, developing a pro-inflammatory profile in immune cells (Bours et al., 2006). Moreover, ATP and ADP induce cardiovascular effects, such as vasodilation, increased heart rate, and platelets aggregation (Agteresch et al., 1999; De Vente et al., 1984). However, the nucleoside adenosine (Ado) acts like an anti-inflammatory compound in cells, and can protect the heart from some diseases (Bours et al., 2006; Cronstein, 1994). The levels of these extracellular nucleotides are controlled by nucleotidases that are soluble enzymes throughout interstitial tissues or anchored in the plasma membrane of cells. The enzyme NTPDase (EC 3.6.1.5) or E-NTPDase (CD39), when anchored in lymphocytes, are responsible for the hydrolysis of ATP and ADP, into AMP. The nucleotide AMP is hydrolyzed by 5′-nucleotidase (EC 3.1.3.5) into adenosine, and adenosine undergoes deamination into inosine by adenosine deaminase (ADA; EC 3.5.4.4) or E-ADA when anchored in lymphocytes (Colgan et al., 2006; Zimmermann, 2001). Several studies are revealing the physiological importance of the nucleotidases in the heart during parasitic diseases (Robson et al., 2006). Considering that, *T. cruzi* infection induces systemic inflammation and cardiac pathophysiological changes, the aim of this study is to investigate the activities of the nucleotidases and E-ADA in lymphocytes and cardiac tissue of mice experimentally infected by *T. cruzi*.

2. Materials and methods

2.1. Chemicals

The substrates ATP, ADP, AMP and adenosine, as well as Trizma base, Coomassie Brilliant Blue G and bovine serum albumin were obtained from Sigma Chemical Co (St. Louis, MO, USA) and K_2HPO_4 from Reagen. All chemicals used in this experiment were of high purity.

2.2. Strain Y

This study used blood trypomastigotes of *T. cruzi* known as strain Y (Silva and Nussenzweig, 1953) cryopreserved in liquid nitrogen. This strain causes acute disease in laboratory animals.

2.3. Animal model and experimental design

Twelve female (Swiss) mice (45 days of age, 20–30 g of body weight) from the Central Animal House of the Universidade Federal de Santa Maria (UFSM) were used in this experiment. They were maintained in a room with constant temperature (23 ± 1 °C) on a 12 h light/dark cycle with free access to feed and water. They were divided into two groups: uninfected (the control group), and infected group with six animals each. Animals of the infected group were intraperitoneally inoculated with blood containing 10^4 trypomastigotes of *T. cruzi* (strain Y) from a mice previously infected.

2.4. Blood parasitemia evaluation

The infection was monitored by counting the number of motile parasites in 5 μL of fresh blood sample drawn from the lateral tail vein, as recommended by a standard protocol (Brener, 1962). The number of blood trypomastigotes was recorded in a two-day interval from 2 to 12 days post-infection (PI), and the number of parasites was expressed as parasites/mL of blood.

2.5. Sample collection

Twelve days PI, the animals were anesthetized with isofluorane and humanely euthanized by decapitation. Blood samples were stored in tubes containing 7.2 mg of dipotassium EDTA anticoagulant for hematological analyses, and tubes containing citrate as anticoagulant for isolation of lymphocytes. Thereafter, their heart were removed and a portion weighed and homogenized with Tris–HCl 50 mM and 4 mM EDTA (to exclude possible interference of endogenous divalent cations). Each homogenate was centrifuged at 2200 g for 10 min with the supernatant collected and frozen at −20 °C until analyses.

2.6. Hematological analyses

Hematological parameters were assessed in whole blood collected in tubes containing EDTA (Vacutainer®) using an automatic counter COULTER T890® (Coulter Electronics, Inc, Hialeach, FL, USA). Total leukocytes (WBC), total erythrocytes (RBC), hematocrit (Ht), and hemoglobin concentration (Hb) were measured. Blood smears were fixed in methanol and stained with Instant-Prov (NewProv®) stain for the determination of differential WBC counts where at least 200 WBCs counts were performed.

2.7. Isolation of lymphocytes

Lymphocytes-rich mononuclear cells were isolated from peripheral blood collected with 129 mM sodium citrate as anticoagulant and separated on Ficoll-Histopaque density gradient, as described by Böyum (1968). Protein in lymphocytes was measured