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Zinc supplementation increases resistance to experimental infection by *Trypanosoma cruzi*

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Abstract

It is well recognized that zinc is an essential trace element for all organisms, influencing growth and affecting the development and integrity of the immune system. It is also well known that the protective response against *Trypanosoma cruzi* depends on both innate and acquired immunity and for the control of the parasite load and host survival, the participation of special cells such natural killer (NK), T and B lymphocytes and macrophages are required. So the aims of this study were to evaluate the effects of zinc supplementation on the host's immune response infected with *T. cruzi*. Our data point in the direction that zinc supplementation triggered enhanced thymocyte and splenocyte proliferation as compared to unsupplied group of animals. It is also important to emphasize that interleukin-12 (IL-12) participates in the resistance to several intracellular pathogens including *T. cruzi*. Our findings demonstrate an enhanced production of IL-12 during the acute phase of infection in zinc-supplied groups. So we conclude that zinc supplementation leads to an effective host's immune response by up-modulating the host's immune response, thus contributing in the reduction of blood parasites and the harmful pathogenic effects of the experimental Chagas' disease.

Keywords: *Trypanosoma cruzi*; Zinc; Interleukin-12 (IL-12); Thymocyte and splenocyte proliferation

1. Introduction

Chagas' disease, caused by the flagellate protozoan *Trypanosoma cruzi*, is a widespread disease that affects millions of people. According to World Health Organization (2002), 16–18 million people are infected by the parasite in Central and South Americas.

T. cruzi is an obligate intracellular parasite, which invades and replicates within a wide variety of mammalian cells. The infection comprises an acute phase, in which the detection of circulating parasites and intense suppression of the lymphoproliferative responses of spleen cells with parasite antigens are observed (Curotto-de-Lafaille et al., 1990), followed by a chronic phase characterized by the presence of parasites in several tissues such as heart, esophagus, colon and peripheral nervous system (Higuchi et al., 2003).

The ability of hosts to survive the acute phase of infection and to progress to the chronic phase is dependent on both host and parasite genetics. In immunocompetent hosts, the initial infection appears to be readily controlled via elicitation of a variety of immune effector mechanisms, and, as a result, parasite numbers in both blood and tissue drop dramatically to

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nearly undetectable levels in the chronic stage of the infection. The protective response against *T. cruzi* depends on both innate and acquired immunity directed by a type 1 cytokine response. For the control of the parasite load and host survival, the participation of special cells such natural killer (NK), T and B lymphocytes and macrophages are required (Abrahamsohn, 1998; Talerton, 2003; Martin and Tarleton, 2004).

Interleukin-12 (IL-12) participates in the resistance to several intracellular pathogens including *T. cruzi* (Trinchieri, 1998; Oliveira et al., 2000); it is produced during the acute phase and is important in the control of *T. cruzi* parasitism. The neutralization of this cytokine by monoclonal antibodies (mAbs) leads to aggravation of the disease, reinforcing the importance of this cytokine in the *in vivo* resistance to infection (Abrahamsohn and Coffman, 1996; Aliberti et al., 1996; Hunter et al., 1996). IL-12 is an inducer of the type 1 cytokine response, also act as a growth factor, promoting the proliferation of pre-activated T and NK cells, and enhancing the generation of cytotoxic T lymphocytes (Gately et al., 1991; Trinchieri and Scott, 1995). Investigation of mice deprived of IL-12 genes (IL-12 *knockout* mice) has confirmed the important role of IL-12 in controlling parasitism in *T. cruzi* infection. IL-12-deficient mice were highly susceptible to *T. cruzi* infection and succumbed during acute infection, demonstrating the crucial importance of endogenous IL-12 in resistance to experimental Chagas' disease (Müller et al., 2001).

It is well recognized that zinc is an essential trace element for all organisms, influencing growth and affecting the development and integrity of the immune system, but it is also very important in other organ systems (Dardene, 2002). During the last decades the influence of zinc on various cell systems have been investigated. It is clear that this trace element has a broad impact on key immunity mediators, explaining the paramount importance of zinc's status on the regulation of lymphoid cell activation, proliferation and apoptosis.

Based on all these considerations the aims of this work were to evaluate the effects of zinc supplementation during acute phase of the *T. cruzi* experimental infection through the detection of IL-12 concentration and proliferative responses of thymocyte and splenocytes.

2. Material and methods

2.1. Animals

Male Wistar rats (60 animals) 4 weeks old, weighing 90–100 g were used. Rats were obtained from the Facility House of the Universitary Campus of Ribeirão Preto. Animals were divided in groups of $n = 5$ per group/day of experiment: non-infected males-without-zinc supplementation (MWZNI), non-infected males-zinc supplemented (MZNI), infected males-without-zinc supplementation (MWZI), and infected males-zinc supplemented (MZI). Rats were separated in number of five in plastic cages and commercial rodent diet and water were available *ad libitum*. Rat pad was changed 3 times/week to avoid concentration of ammonia from urine. The protocol of this study was approved by the local Ethics Committee protocol number 06.1.427.53.2.

2.2. Parasites and experimental infection

Rats were intraperitoneally (i.p.) inoculated with 1×10^5 blood trypomastigotes of the Y strain of *T. cruzi* (Silva and Nussenzweig, 1953). The experiments were performed in duplicate on 7, 14 and 21 days after infection. Parasitaemia was determined by Brener's method (Brener, 1962). It is important to emphasize that since Wistar rats are normally resistant to most *T. cruzi* strains, we found it necessary to use relatively high inoculums (1×10^5 blood trypomastigotes), which resulted in a more intense pathological response such as enhanced parasitemia and tissues lesions.

2.3. Zinc supplementation

Rats were orally supplied with zinc sulphate (Sigma Chemical Co., MO, USA), at a dose of 20 mg/kg body weight, dissolved in 0.1 mL of distilled water (gavage), once a day over the course of the experiment. Treatment of the infected group started 48 h before infection. Control groups were supplied as prior described.

2.4. Euthanasia

Animals were decapitated with prior anesthesia using thribromoethanol 2.5%.

2.5. Measurement of IL-12 serum levels

The serum levels of IL-12 were quantified using BD Biosciences, San Diego, USA: BD OptEIA^{TM}SET Rat IL-12. This DuoSet ELISA kit contains the basic components required for the development of sandwich ELISA to measure natural and recombinant rat IL-12.