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Chagas disease control and role of zinc supplementation in pregnancy

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Abstract

During normal pregnancy, some changes in cell numbers, phenotype, and activity take place. The impairment of maternal immune function in the course of pregnancy contributes to a decreased resistance against several pathogens. Since scarce data concerning the effects of maternal zinc supplementation during infection are available, the purpose of our current study was to evaluate immune response alterations and zinc levels in pregnant *T. cruzi*-infected Wistar rats under zinc therapy. Pregnant Wistar rats were used in all experiments. $TCD3^+CD4^+$, $TCD3^+CD8^+$, B cells ($CD45RA^+$), splenocyte proliferation, and plasma zinc levels were measured at 14^{th} day after infection (18^{th} day of pregnancy). High proportions of T- and B-cells were found in infected pregnant and zinc-treated animals as compared to pregnant infected and untreated counterparts. Moreover, zinc plasmatic levels from supplemented animals were similar to the non-infected/non-supplemented group. These findings demonstrate that dietary zinc supplementation throughout pregnancy improves immune response during *T. cruzi* infection. These results will base further methods to use zinc in pregnant women affected by *T. cruzi*.

Introduction

From Americas, Chagas disease has reached several continents due to changes in the population migratory pattern. More than 10,000 people die every year with clinical manifestations of Chagas disease. It is estimated that 8 million people are infected by *T. cruzi* and 25 million are at risk of infection [1]. Congenital transmission of *T. cruzi* may occur in acute or chronic phases of infection. For this reason, congenital infection is a serious public health problem, once it extends throughout the woman's fertile life and thus spreads through migrations from endemic to non-endemic areas [2].

The uterine response to pregnancy produces alterations in neuroendocrine and immune functions, requiring induction of a T-cell response/regulation. This T-cell response/regulation is activated since fertilization and plays a key role in the success or failure of pregnancy. This response is also considered a crucial factor in providing a favorable environment within the uterus as fetal compartment expands, differentiates, and mature [3] [4].

The kinetics of *Trypanosoma cruzi* infection in rodents and humans indicates that innate responses are essential to limit parasite replication [5], allowing the host to develop specific adaptive responses which usually enable an infection control. The importance of innate immune cells in protective immunity to *T. cruzi* in early stage of infection is exemplified in mice with genetic deficiency in NK cells, where elevated number of parasites were found during acute phase of *T. cruzi* infection [6]. Studies focusing on host innate immunity against *T. cruzi* have demonstrated the importance of NK cells in protective immunity to parasite by their two major functions, cytotoxicity and cytokine production, especially of IFN-γ. The synthesis of IL-12 by macrophagesA elicits the synthesis of IFN-γ by NK cells [7], which in turn activates phagocytes to control parasite growth, crucial in many aspects of microbial elimination. Strikingly, lethal infection occurs in absence of early IFN-γ production [8] during acute phase of infection. $CD4^+$ and $CD8^+$ T-cell compartments, the main effectors of adaptive cellular immune responses, are also required to control *T. cruzi* replication. Studies have evaluated T-cell subsets, demonstrating the importance of these T-cell-mediated mechanisms for mounting an effective anti-*T. cruzi* immune response, since animals lacking T-cell subsets were extremely susceptible to infection, showing increased parasite burden and shortened survival time

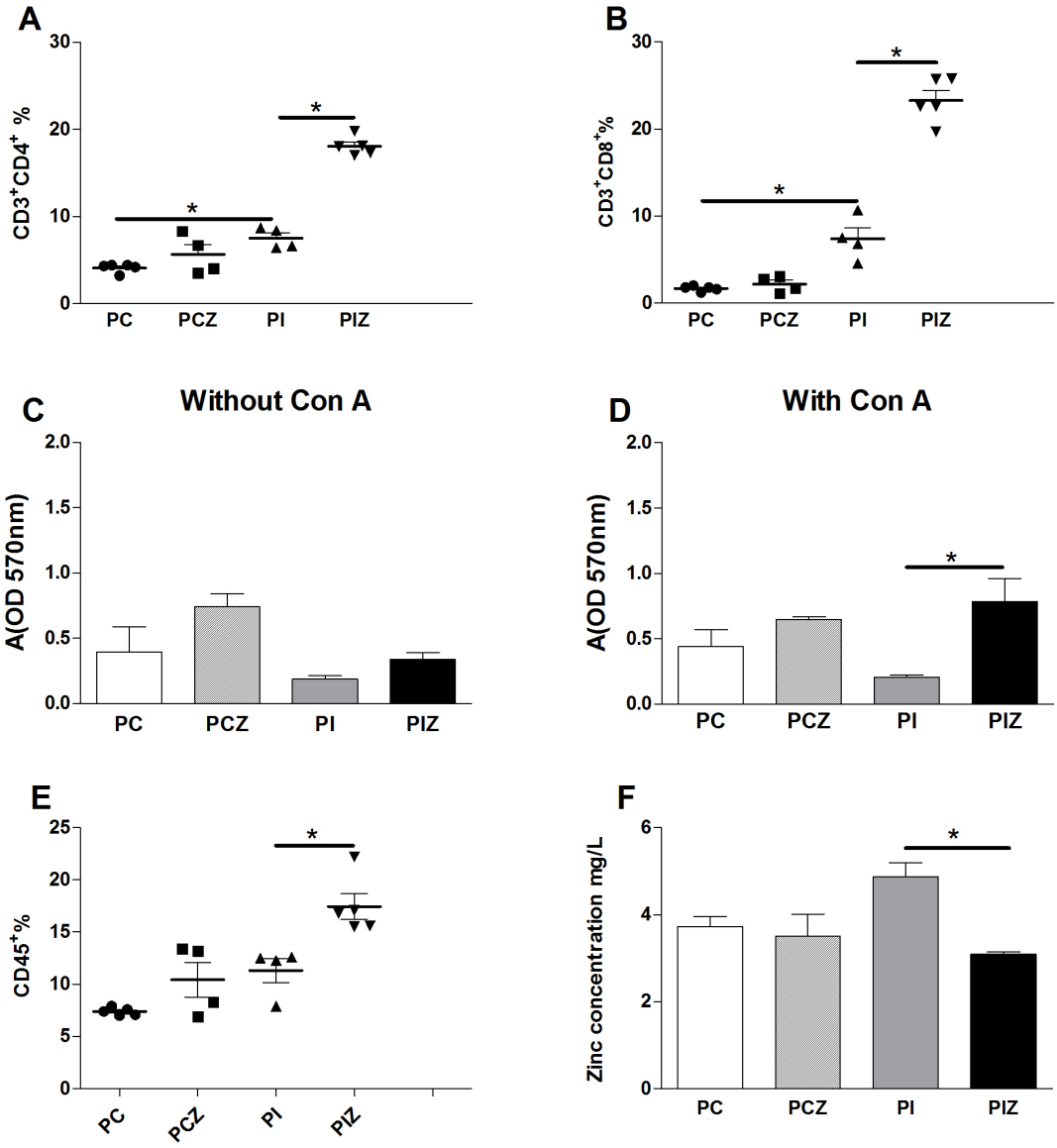
[9].

Some studies confirm a decline of zinc levels with age and have reported beneficial effects of zinc supplementation in aging [10]. Previous reviews have demonstrated that oral zinc supplementation could help reduce the duration and severity of diarrhea or respiratory tract infections [11]. Furthermore, zinc deficiency is usually present during pregnancy and appears to predispose infections [12]. Our previous studies showed several benefits of zinc supplementation during *T. cruzi* infection, represented by significant reduction in parasitemia levels [13]. However, several aspects should be explained about the importance of maternal micronutrient status, covering maternal-fetal relationship in presence of an infectious disease.

To address some of these questions, we have now investigated the course of *T. cruzi* infection using pregnant Wistar rats, evaluating TCD4, TCD8, and B (CD45RA) lymphocyte population by flow cytometry and splenocyte proliferation in pregnant females under effects of zinc therapy.

Objective

The aim of this study was to assess the effects of zinc supplementation on immune response during *T. cruzi* infection in pregnant Wistar rats.



a

Figure Legend

Figure 1. Immune effects of zinc treatment in pregnant rats infected with *T. cruzi*.

Female rats were infected with 1×10^5 blood trypomastigotes of *T. cruzi* Y strain, which induced an acute phase of experimental Chagas disease, using the following groups: pregnant control (PC), pregnant control treated with zinc (PCZ), pregnant infected (PI), pregnant infected treated with zinc (PIZ). Spleen cells and plasma samples were applied in phenotypical analyses and zinc quantification, respectively.

(A) Phenotypical analysis of T $CD_3^+CD_4^+$ lymphocyte population.

(B) Phenotypical analysis of T $CD_3^+CD8^+$ lymphocyte population.

(C, D) Effects of zinc treatment on splenocyte proliferation.

(E) Phenotypical analysis of $CD_{45}RA^+$ (B cells).

(F) HPLC quantification of zinc in plasma samples.

The results were presented as mean and standard error of the mean, n = 5/group/day of experiment. * represents groups significantly different compared to PC and PI (p <0.05).

Parasites

Rats were intraperitoneally inoculated with 1×10^5 blood trypomastigotes of the Y strain of *T. cruzi* on 3rd day after pregnancy [13]. The assays were performed on 18 days after pregnancy (14 days after infection). It is important to emphasize that because Wistar rats are normally resistant to most *T. cruzi* strains, we found it necessary to use relatively high inoculum (1×10^5 blood trypomastigotes).

Pregnancy

Female Wistar rats weighing 180–200 g were used. Animals were randomly distributed into groups: pregnant control (PC), pregnant control treated with zinc (PCZ), pregnant infected (PI), and pregnant infected treated with zinc (PIZ). A total of 5 animals were used per group (2 animals per cage). 1 male Wistar rat was introduced into each cage and was allowed to mate with 2 females. The vaginal plug appearance was designated as being at day 1 of gestation. Rodent diet and water were available ad libitum.

Treatment scheme

Rats were treated by oral route through gavage with 20 mg/kg/day zinc sulfate (Sigma Chemical Co. MO) (da Costa et al. 2013). The treatment was started 24 h after parasite infection and was maintained until 18 days after pregnancy.

Euthanasia

Animals were decapitated on 18th day of pregnancy with prior anesthesia using 0.25% tribromoethanol (10 mL/kg), administrated intraperitoneally.

Flow cytometry assay

Cells were dispersed from spleen by extrusion through a 70 µm nylon cell strainer, macerated in RPMI 1640 medium to produce a single cell suspension. 2×10^6 cells from the suspension of each organ from each experimental group were placed in 96 well round bottomed plates for flow cytometry analysis. Following Fc receptor blocking, cells were incubated with combinations of monoclonal antibodies anti-CD3-fluoresceinisotiocianate (FITC), anti-CD4-allophycocyanin (APC), anti-CD8-peridin clorofil protein (PERCP), anti-CD45RA-phycoerythrin (PE), as well as immunoglobulin isotype-matched controls. Stained cells were stored for analysis in PBS containing 0.01 mL sodium azide and 1% paraformaldehyde, in sealed tubes held in the dark. All steps were performed at 4°C. Analysis of these cells was performed using a Becton Dickinson FACScan flow cytometer with DIVA-BD software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Proliferation assay

Spleens were aseptically removed. To prepare a single cell suspension, cells were teased out into serum-free RPMI 1640 medium. After centrifugation at 300 *g* for 10 min at 4°C, the pelleted cells were resuspended in RPMI 1640 containing 5% FBS (2×10^6 cells/mL) and added to 96 well flat bottomed plates (0.1 mL/well). The cells were subsequently stimulated with 4 µg/mL Concanavalin A (Sigma) and incubated at 37°C in a humidified 5% CO_2 atmosphere for 72 h. The cells were incubated with MTT reagent (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) for 4 h, followed by lysis using acidic isopropanol and measurement at 570 nm in an ELISA reader (Sunrise Tecan).

Zinc determination in plasma samples (ICP-MS)

For zinc determination in plasma samples, a method described previously [26] was adopted with few modifications. Briefly, samples were diluted 1:20 into a 15 mL polypropylene Falcon® tube (Becton Dickinson) with a solution containing 0.01% (v/v) Triton® X-100, 0.5% (v/v) nitric acid, and 10 µg L-1 of each one of Rh (the internal standard). Analyses were carried out with an inductively coupled plasma mass spectrometer equipped with a reaction cell (ICP-MS ELAN DRCII, PerkinElmer, SCIEX, Norwalk, CT) operating with high-purity argon (99.999; Praxaair, Brazil). Sample introduction system was composed by quartz cyclonic spray chamber and a Meinhard® nebulizer connected by Tygon® tubes to the ICP-MS's peristaltic pump (set at 20 rpm). The ICP-MS was operated with Pt sampler and skimmer cones purchased from Perkin Elmer.

Statistical analysis

The results are expressed as mean ±SEM. A value of p <0.05 was considered statistically significant. Differences among control and infected groups were determined by one-way ANOVA with Bonferroni's posttest. Statistical analyses were performed using Graph Pad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA). **Results &**

Discussion

Zinc deficiency usually results in impaired immune function, increasing the susceptibility to infectious illnesses [14] and is related to adverse pregnancy disorders [15]. Zinc levels are also correlated with growth, brain development, cognitive performance, and infertility [16]. Pregnancy is a complex immunological condition that requires an adequate fetomaternal immune adjustment in order to avoid fetus rejection. Several changes occur during this period, shifting the balance between Th2 (humoral immunity) and Th1 (cell-mediated immunity), in favor of Th2 [17]. Although the immunological changes may be beneficial to maintaining fetus during pregnancy, this condition may result in increased susceptibility to infection establishment.

T. cruzi infection slightly improved $CD3^+CD4^+$ and $CD3^+CD8^+$ cells, which was significantly enhanced when infected animals were zinc-supplemented (Fig. 1A and 1B, respectively). T-cells have been strongly linked to inflammation modulation in *T. cruzi* infection, leading to an antigen-specific cellular immunity. Components of cell-mediated immunity demonstrate a crucial importance on the control of intracellular forms of the parasite, once genetically modified mice lacking T-cell subsets were extremely susceptible to *T. cruzi* infection [9]. Cytokines produced by T lymphocytes trigger important mechanisms, acting as growth factor in $CD8^+$ lymphocyte effector functions. Interestingly, it was shown that passive transfer of lymphoid cells restores the resistance of knockout animals against *T. cruzi* infection [18] [19]. Among immune cells affected during zinc deprivation, especially T-cell subsets are noted to have the highest susceptibility, with reduced T-cell numbers. There is also an imbalance between Th1 and Th2 responses, with major production and release of Th2 cytokines as well as compromised immune defense mediated by T-cell [20].

Splenic cell proliferation was responsive to zinc supplementation, whereas *T. cruzi* infection decreased cell proliferation (Fig. 1C and 1D). The protective role of zinc on lymphoid cell proliferation may be explained by its involvement in nucleic acid synthesis and anti-apoptotic properties [21]. Zinc supplementation also altered $CD45RA^+$ (B cells) profile in infected/zinc-treated animals (Fig. 1E). Inflammatory T-cell behavior in animals infected with *T. cruzi* is also tightly regulated by a network of B cells, once the clearance of an established infection requires a successful humoral response [22]. Zinc deficiency is associated with several immune adverse effects, compromising cellular and humoral response [23].

Zinc levels are highly dependent on tissue stocks, once serum Zn is only a small percentage (0.1%) of whole-body-mass Zn content [24]. Higher concentrations of zinc found in plasma of infected animals (Fig. 1F) probably are a result of metal release from tissue stocks, which are useful to enhance an efficient cellular immune response in host. When animals were zinc-supplemented, serum metal levels were maintained as observed in controls (Fig. 1F), probably avoiding zinc depletion from stocks. Zinc depletion is also important, once the metal deficiency is related to susceptibility to childhood diarrhea, acute respiratory infections, malaria, as well as diseases associated with impaired cellular immunity such as tuberculosis and leishmaniosis [25]. Further experiments will be performed to understand the immunological events during pregnancy, demonstrating mechanisms by which the course of *T. cruzi* infection is influenced by zinc.

Conclusions

Zinc supplementation has a beneficial effect on pregnant rats infected with *T. cruzi*. As observed in several diseases, zinc supplementation elevates the number of cells related to specific immune response. This work will guide the use of zinc on pregnant women affected by Chagas disease.

Additional Information

Methods

Parasites

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Supplementary Material

Please see https://sciencematters.io/articles/201612000008.

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Ethics Statement

The protocol of this study was approved by the local animal ethics committee (CEUA, protocol no. 01.193.53.4).

Citations

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