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DHEA and testosterone therapies in *Trypanosoma cruzi*-infected rats are associated with thymic changes

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ABSTRACT

The ability of the gonadal hormones to influence diverse immunological functions during the course of several infections has been extensively studied in the latest decades. Testosterone has a suppressive effect on immune response of vertebrates and increases susceptibility toward numerous parasitic diseases. Dehydroepiandrosterone is an abundant steroid hormone secreted by the human adrenal cortex and it is considered potent immune-activator. In this paper, it was examined the effects of DHEA and testosterone supplementation in the thymic atrophy in rats infected with *Trypanosoma cruzi*, by comparing blood parasitism, thymocyte proliferation, TNF-alpha and IL-12 levels. Our data point in the direction that DHEA treatment triggered enhanced thymocyte proliferation as compared to its infected counterparts and reduced production of TNF-alpha during the acute phase of infection. Oppositely, the lowest values for cells proliferation and IL-12 concentrations were reached in testosterone-supplied animals. The combined treatment testosterone and DHEA improves the effectiveness of the host's immune response, reducing blood parasites and the immunosuppressive effects of male androgens besides increasing IL-12 concentrations and decreasing TNF-alpha levels.

1. Introduction

Trypanosoma cruzi is the etiologic agent of Chagas' disease, a major cause of morbidity and mortality in Latin America. It is transmitted to humans by the feces blood-sucking triatomine bugs, blood transfusion and through the placenta (Moncayo and Ortiz Yanine, 2006). Treatment and prevention remain unsatisfactory because chemotherapy is nonspecific and generally has toxic side effects; furthermore, no treatment is available for the chronic phase of the disease (Urbina et al., 1996).

During the experimental acute phase, clinical signs are usually mild, despite the high levels of parasitemia in blood, which is characteristic of this stage of infection declining with the onset of immunity. For the control of the parasite load and host survival, the participation of special cells such as NK, T and B lymphocytes are required (Abrahamsohn, 1998; Talerton, 2003; Martin and Tarleton, 2004).

The thymus constitutes the primary lymphoid organ in which bone marrow-derived T cell precursors undergo differentiation. In acute stage of *T. cruzi* infection, this organ is severely affected and becomes atrophic, due to depletion of cortical and immature thymocytes (Wellhausen and Boros, 1982; Wang et al., 1994; Kasempimolporn et al., 2001; Brito et al., 2003).

The depletion of thymocytes during infection can be triggered by diverse stimuli (Pérez et al., 2007). It is well known that a rise in endogenous glucocorticoid levels, caused by activation of the hypothalamus–pituitary–adrenal (HPA) axis can produce a significant thymic cortical depletion (Gruber et al., 1994; Besedovsky and del Rey, 1996).

Additionally, thymocytes apoptosis due to an excessive inflammatory syndrome mainly mediated by tumor necrosis factor-alpha (TNF-alpha) (Roggero et al., 2002, 2004) have been described during parasite infections, including Chagas' disease (Wang et al., 1994; Kato et al., 1997; Savino, 2006). TNF-alpha is a key cytokine produced primarily by monocytes and macrophages which is involved in the host immune response. Low levels of this cytokine confer protection against some infectious agents, but, it also seems to contribute directly to induction of immature T cell death, aggravating the pathogenesis of infections (Hernandez-Caselles and Stutman, 1993).

In contrast to TNF-alpha, IL-12 was originally described as a cytokine with co-mitogenic effect on human T cells (Kobayashi et al., 1989) and improves the resistance to several intracellular pathogens including *T. cruzi* (Trinchieri, 1998; Oliveira et al.,

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2000). IL-12 is released primarily by antigen-presenting cells and acts as a linker between the innate and acquired immunities by inducing the differentiation and proliferation of antigen-specific T cells of Th1 phenotype.

Dehydroepiandrosterone (DHEA) and its sulphate ester (DHEA-S), the most prevalent adrenal steroid hormones in humans, are metabolic intermediates in the production of potent androgens, estrogens and other less well-characterized steroids (Olech and Merrill, 2005);

The endogenous levels of DHEA-S are 250 and 500 times higher than those of DHEA in women and men, respectively (Labrie et al., 1997). DHEA is cleared from the blood at a rate of approximately 2000 mL/day and has a half-life of 1–3 h (Longcope, 1996; Salek et al., 2002). The protein-binding characteristics of the two hormones also influence clearance rates: unlike DHEA, DHEA-S is relatively strongly bound to albumin (Kroboth et al., 1999).

According to Salek et al., 2002 and Frye et al., 2000, the administration of exogenous DHEA increases circulating DHEA and DHEA-S concentrations, but the kinetics is different when compared with endogenous secretion.

DHEA displays a number of remarkable actions, especially on immune system, where it counteracts immunosuppressive effects of glucocorticoids (Hampl and Vondra, 2006). DHEA-S circulates freely and becomes active when its sulphate group is cleaved at a particular site, such as a lymph node (Daynes et al., 1990a; Morfin and Courchay, 1994). There, DHEA may exhibit microenvironment-specific, activity-enhancing T lymphocyte functions (Daynes et al., 1990b). This hormone acts as powerful down-regulators of the pro-inflammatory cytokines, such as TNF-alpha (Degelau et al., 1997; Knoferl et al., 2003) and its administration in mice and rats is likely to be useful in the protection against a variety of lethal infections with bacteria (Ben-Nathan et al., 1999), virus (Loria et al., 1988) and parasites, including *T. cruzi* (Santos et al., 2005).

Several studies link sex differences in immune function with circulating steroid hormones. It is widely accepted that immunity is sexually dimorphic and the gonadal steroids directly influence the cells and tissues of immune system and thus susceptibility to disease. Concerning to parasitic infections, for *T. cruzi*, males are relatively more susceptible than females and orchiectomy leads animals to enhanced resistance against the parasite (Prado et al., 1998, 1999; Filipin et al., 2008).

Gonadal hormones, especially testosterone, have long been known to decrease the structure and function of the thymus as well as lymphopoiesis in this and other tissues. Removal of androgens by castration results in thymic enlargement correlated with increased thymocyte proliferation, even in aged animals, whereas androgen replacement reverses these effects (Olsen and Kovacs, 1996, 2001).

Thus, the aims of this current study were to evaluate the possible influences of DHEA and testosterone administration, both in combination and individually, over the thymocyte depletion during *T.cruzi* infection in *Wistar* rats and how these hormones may affect the T cells proliferation during the infection, by comparing thymocytes proliferation, TNF-alpha, IL-12 levels and blood parasitism.

2. Materials and methods

2.1. Animals

Male Wistar rats, four weeks old, weighing 90–100 g were used. Rats were obtained from the Facility House of the University Campus of Ribeirão Preto. Animals were divided into the following groups: control group: non-infected and untreated rats (C); infected and untreated group (I); infected and testosterone-supplied group (IT); Infected and DHEA-treated group (ID); Infected and testosterone/DHEA treated (ITD). A number of five animals were used per group/per day of experiment. All experiments were performed twice in order to verify the reliability of data. Rats were kept in number of five per cage and commercial rodent diet and water were available *ad libitum*. Rat pad was changed three times/week to avoid accumulation of ammonia from urine. The protocol of this study was approved by the local Ethics Committee.

2.2. Parasites and experimental infection

Rats were intraperitoneally (i.p.) infected with 1×10^5 blood trypomastigotes of the Y strain of *T. cruzi* (Silva and Nussenzweig, 1953). This strain has been maintained in the laboratory by serial blood passages in mice. Experiments were performed on 7 and 14 days post infection. Parasitemias were evaluated by counting (optical microscope, 40× objectives) the number of trypomastigotes in 5 μL samples of heparinized blood (Michelin et al., 2005), as described by Brener (1969).

2.3. Testosterone (T) supplementation

Animals from testosterone-supplied groups received intramuscular injections (0.1 mL) of testosterone cypionate, diluted in mineral oil, at a dose of 5 mg/day/kg body weight, once a day at the same time and during the course of the experiment (Baltaci et al., 2006), while the control group received only mineral oil injections. Treatment schedule started at the same day of infection.

2.4. DHEA treatment

Animals from DHEA-treated groups received, subcutaneously (Wittert et al., 1991; Kroboth et al., 1999), 0.1 mL of DHEA (Sigma Chemical Co.), at a dose of 40 mg/day/kg body weight, dissolved in absolute ethanol (0.05 mL) and an equal volume of distilled water (0.05 mL), once a day at the same time and during the course of the experiment (Santos et al., 2005, 2007, 2008; Filipin et al., 2008; Brazão et al., 2009; Kuehn et al., 2009; Caetano et al., 2009), while the control group received only phosphate-buffered saline (PBS) injections.

Treatment schedule started at the same day of infection and subcutaneous administration was chosen because prior work showed it to be more efficient than the oral or percutaneous routes (Labrie et al., 1996).

2.5. Euthanasia

Animals were decapitated with prior anesthesia using tribromoethanol 2.5%, subcutaneously.

2.6. Thymocytes proliferation assay

Thymi were aseptically removed. To prepare a single-cell suspension, the cells were teased out in serum-free RPMI-1640 medium (Cultlab-Campinas Brazil). After centrifugation for 10 min at 300*g* at 4 °C, pelleted cells were resuspended in RPMI-1640 containing 5% FCS (Sigma) and added to 96-well microplates (0.1 mL/well) at a cell density of 5×10^6/mL. The cells were subsequently stimulated with Concanavalin A (Con-A 4 μg/ml; Sigma) and incubated at 37 °C in humidified 5% CO_2 atmosphere for 72 h. The experiments were performed in triplicate with a final volume of 0.2 mL/well. Cellular proliferation was determinated by MTT assay. After incubation of the cells with the MTT reagent for approximately 4 h, acidified isopropanol was added to lyse the cells and solubilize the purple formazan salt crystals. The sam-