

Trypanosoma cruzi: Plasma corticosterone after repetitive stress during the acute phase of infection

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Abstract

An increased level of plasma corticosterone is one manifestation of severe environmental or physiologic stress. The stress response mediated by the hypothalamic–pituitary–adrenal axis is already known to suppress immunoglobulin production and to impair immune function, but there are few studies relating stress and plasma corticosterone to the outcome of *Trypanosoma cruzi* infection. In this study, male *Wistar* rats were infected with the Y strain of *T. cruzi* and then subjected to repetitive stress by exposure to ether vapor for 1 min twice a day during the acute phase of infection. Stressed animals showed decreased lytic antibody activity and lowered levels of peritoneal macrophages. Despite an increase in the weight of the spleen, histological analyses demonstrated tissue alterations, the presence of amastigote nests, and a complete absence of activated lymphoid follicles. These results suggest that stress-induced increases in plasma corticosterone can suppress the immune response and worsen tissue injury during the acute phase of *T. cruzi* infection.

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Index Descriptors and Abbreviations: *Trypanosoma cruzi*; Macrophages; Stress; Spleen; Lytic antibody; Corticosterone

1. Introduction

Chagas' disease is among the most important parasitic infections in terms of its public health and economic impacts. From a global perspective it represents the third largest tropical disease burden after malaria and schistosomiasis, affecting 20 million people in Latin America (WHO, 2002). *Trypanosoma cruzi* is a potent stimulator of cell-mediated immunity, and induction of macrophage proinflammatory cytokines influences the outcome of infection. The host's resistance during experimental infection by *T. cruzi* is dependent on both innate and acquired immunity, which in turn require the combined activities of macrophages, natural killer (NK) cells, CD4+, and CD8+ T

cells, as well as antibody production by B cells (Brener and Gazzinelli, 1997).

Infection by most pathogenic agents leads to an imbalance of the immune system, creating conditions favorable to the parasite's establishment, and many stressors have been shown to suppress both cellular and humoral immune functions (Khansari et al., 1990; Bohus et al., 1991). The body's principal physiologic response to stress is mediated by the sympathoadrenal system, which is functionally separable into the sympathetic nervous system and adrenal medulla, and the hypothalamic–pituitary–adrenocortical (HPA) axis (Chrousos and Gold, 1992). The early response to acute stress is protective, enhancing immune function, promoting memory to dangerous events, increasing blood pressure and heart rate to meet the physical and behavioral demands of fight or flight, and making fuel available to sustain intensified activity. However, chronic stress

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contributes to the wear and tear that eventually renders the organism more vulnerable to disability and illness (McE-ween, 2000). Cytokines like interleukin-1, interleukin-6, tumor necrosis factor- α (TNF- α) and interferon- γ influence the activity of HPA axis through direct and indirect actions on the central nervous system, pituitary and adrenal glands (Dunn et al., 1999). This functional cascade is generally inhibited or modulated through the immunosuppressive effects of glucocorticoids.

Psychoneuroimmunological factors also represent a source of stress, and their effects have been studied by comparing parasitaemias in males and females kept singly or in groups. The results showed that both genders, when kept alone, developed lower parasitaemia than when they were kept together (Schuster and Schaub, 2001). Indeed, the influences of social environment seem to affect glucocorticoid and testosterone levels in males (Koolhaas et al., 1997).

The adrenocortical hormones like corticosterone and dehydroepiandrosterone acting on the immune system alter the acute phase of *T. cruzi* infection (Santos et al., 2005a,b). Recently we showed that stress in *T. cruzi* infected animals result in elevated parasitemia and loss of normal tissue architecture in the heart and adrenal glands (Santos et al., 2005a). Since corticosterone influences the activity of the immune system, it is important to understand how stress affects its plasma concentration and the course of experimental Chagas' disease. Ether vapor as a stress stimulus causes plasma corticosterone to increase and represents a suitable method to evaluate the influence of the hormone on the development of *T. cruzi* infection. Thus, the aim of the work presented here was to determine whether the increased plasma corticosterone that accompanies repetitive stress can cause alterations in the numbers of peritoneal macrophage cells, lytic antibody percentage and spleen histopathology during the development of experimental Chagas' disease.

2. Material and methods

2.1. Animals and infection

Male *Wistar* rats ($n = 60$) weighing 90–100 g were used for these experiments. The animals were divided in four groups: Stressed Non-Infected, Stressed Infected, Non-Stressed Infected, and Non-Stressed Non-infected. Each group consisted of 15 animals (60 totals) with five from each group being sacrificed on days 7, 14 and 21 post-infection. The rats were intraperitoneally (i.p.) infected with 2×10^5 blood trypomastigotes of the Y strain of *T. cruzi* (Silva and Nussenzweig, 1953) and were kept five to a cage with rodent commercial chow and water available *ad libitum*. The room temperature was $22 \pm 1^\circ\text{C}$, in a 12:12 h light/darkness cycle (lights on from 06:00 to 18:00 h).

2.2. Stress stimulus

Stress was applied to the rats according to the method reported previously (Caldeira and Franci, 2000). Since

stressors are especially considered to become clinically relevant when applied repeatedly, stressed animals were exposed to ether vapor for 1 min twice a day throughout the period of study. The stress exposure was carried out in the morning between 8:00 and 10:00 a.m. and in the afternoon between 2:00 and 4:00 p.m. It was performed in an isolated room separated from all other animals and laboratory personnel and the rats were introduced in a closed container with an ether-soaked rag placed inside. Control animals were kept in their home cage where they could not perceive the reaction of stressed animals after ether exposure.

2.3. Euthanasia

The animals were killed by decapitation to prevent additional stress. The plasma corticosterone is widely used as one indicator of the presence of stressors. However the secretion of this steroid occurs in a pulsatile fashion and blood hormone concentration can change by a factor of 10 or more within minutes (Touma et al., 2004). Thus, we ensured that the relapse time of euthanasia did not exceed 15 min. All procedures were conducted in compliance with the requirements of the Brazilian Committee on Animal Experimentation (COBEA).

2.4. Corticosterone assay

RIA for corticosterone required plasma extraction using ethanol. The antibody and standard used were provided by Sigma (USA) and the ^3H -labeled hormone was from Amer-sham (USA). Assays were performed in duplicate. The lower limit for detection was 2 ng/mL and the intra-assay coefficient of variation was 5%.

2.5. Counting peritoneal macrophages

Macrophages were assessed by the injection of 5 mL of cold RPMI-1640 medium (Cultlab-Campinas Brazil) into the peritoneal cavity. Forty microliters of each harvested peritoneal exudate cell suspension were added to 360 μL of Turkey Solution, and the macrophages were counted in a Neubauer chamber.

2.6. Lytic antibody analysis

The lytic antibody percentage was assessed as described by Levy et al. (1996).

2.7. Histopathology and Karyometry

Spleens were, weighed on an analytical balance and subsequently immersed in a 10% ALFAC solution (alcohol, formaldehyde and acetic acid) and embedded in paraffin. Six-micrometer thick tissue sections were stained with hematoxylin-eosin. Parasite density was estimated in sections separated at 70 μm intervals to avoid recounting

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