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Alterations triggered by steroid gonadal hormones in triglycerides and the cellular immune response of *Calomys callosus* infected with the Y strain of *Trypanosoma cruzi*

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Abstract

Calomys callosus is a wild rodent found naturally infected with different *Trypanosoma cruzi* strains. In the work described here, groups of male and female *C. callosus* were subjected to orchiectomy, ovariectomy and sham operation. One month after surgery, animals were inoculated intraperitoneally (i.p.) with 4×10^4 blood trypomastigotes of the "Y" strain of *T. cruzi*. Parasitemia, triglycerides, nitric oxide (NO) and concanavalin A (ConA)-induced proliferation were evaluated. Parasitemia during the course of infection was significantly higher in infected and sham operated animals as compared to infected orchiectomized animals. The opposite was observed in the ovariectomized and infected group. Orchiectomized and infected animals displayed elevated triglyceride levels, as well as a more vigorous immune response, with higher splenocyte proliferation and elevated concentrations of NO. Ovariectomy resulted in an impaired immune response, as observed by a reduction of splenocyte proliferation and NO concentration. The results suggest a pivotal role for gonadal hormones in the modulation of triglyceride levels and the magnitude of the immune response during the acute phase of *T. cruzi* infection.

Keywords: *Calomys callosus*; *Trypanosoma cruzi*; Gonadal hormones; Nitric oxide; Spleen cells proliferation; Triglycerides

1. Introduction

American trypanosomiasis is a protozoan infection caused by *Trypanosoma cruzi* and is an important public health problem in Latin America (WHO, 2002). Infection of the mammalian host with *T. cruzi* results in chronic persistence of the parasite with progressive inflammatory destruction of target tissues.

Calomys callosus (*Rodentia cricetidae*) is a wild rodent that has been found naturally infected with *T. cruzi*, but is more resistant than common laboratory strains of mice, apparently controlling the infection (Borges et al., 1992). *C. callosus* has been used as a model for experimental study of host response to *T. cruzi* infection (Andrade et al., 1994; Magalhães-Santos et al., 2004).

Animals infected with the salivarian trypanosome *Trypanosoma brucei brucei* normally display elevated

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triglyceride levels due to increased production of tumor necrosis factor-α (TNF-α) during the infection's acute phase of the infection, presumably an attempt to control parasite replication. TNF-α suppresses lipoprotein lipase (LPL) synthesis in adipocytes and consequently raises triglyceride levels (Kawakami et al., 1982; Semb et al., 1987). Rabbits experimentally infected with *T. brucei* show suppressed LPL activity, leading to hypertriglyceridemia (Rouzel and Cerami, 1980).

We commonly observe hiperlipemic serum in infected *C. callosus*, even after 12 h of starvation. Since *C. callosus* is resistant to most *T. cruzi* strains, we hypothesized a possible involvement with triglycerides and the immune response to infection. Although *C. callosus* is resistant to the effects of chronic infection by *T. cruzi*, it can be infected with any *T. cruzi* strain. After a normal acute phase, parasites disappear from the circulation and an apparent host–parasite balance is achieved. These features make *C. callosus* an excellent reservoir for Chagas' disease in its normal sylvatic environment. Its ability to control the effects of infection by even the highly virulent Y strain of *T. cruzi* (Mello et al., 1979; Borges et al., 1982, 1992) makes *C. callosus* an excellent experimental model (Magalhães-Santos and Andrade, 2005). Tissue lesions are precocious with intense inflammatory infiltrates and early fibrogenesis, with a spontaneous regression of inflammation and fibrosis (Andrade et al., 1994; Lenzi et al., 1994; Magalhães-Santos et al., 2002, 2004).

It is also known that the protective response against *T. cruzi* depends both on innate and acquired immunity and requires the participation of NK cells, T and B lymphocytes and macrophages (Talerton, 2003), which undergo morphological and biochemical changes consistent with enhanced activity when subjected to appropriate stimuli. Activated macrophages produce and release numerous compounds that regulate the immune response and participate in the destruction of invading pathogens (Turpin and Lopez-Berestein, 1993). NO secretion by activated macrophages is thought to be essential for mediating *T. cruzi* killing during acute murine infection (Vespa et al., 1994; Petray et al., 1995; Rodrigues et al., 2000) especially through gamma-interferon (IFN-γ) and TNF-α activation (Oswald et al., 1994; Silva et al., 1995).

It should also be noted that females are more resistant to *T. cruzi* infection than males, and that orchiectomy enhances the resistance of males (Prado Jr. et al., 1998, 1999). The aims of this work were to evaluate the changes in triglyceride levels during the acute phase of *T. cruzi* infection as well as the influence of ablation of steroid hormone production in orchiectomized and ovariectomized animals on triglyceride profiles and on the magnitude of the immune response.

2. Material and methods

2.1. Animals

All animals were treated according to the international guiding principals for biomedical research involving animal from Cornell for International Organization of Medical Sciences (Cioms, 1985) and the protocol of this study was approved by the local Ethics Committee. Male and female *C. callosus* ($n = 30$, for each sex) 28–30 days old and weighing 20–26 g, were used for the study. The animals were divided in two groups: infected males: infected males (IM), sham infected males (SIM), orchiectomized infected male (ORIM); infected females: infected females (IF), sham infected females (SIF), ovariectomized infected females (OVIF). *C. callosus* were kept in number of five in plastic cages and commercial rodent chow and water available *ad libitum*. Experiments were performed in triplicate.

2.2. Infection

The animals were intraperitoneally (i.p.) inoculated with 4×10^4 blood tripomastigotes of the Y strain of *T. cruzi* (Silva and Nussenzweig, 1953), and experiments were performed on 5, 7, 9 and 14 days after infection. Daily individual parasitemia was determined by Brener's Method (1962).

2.3. Orchiectomy

C. callosus was anesthetized with an i.p. injection of tribromoethanol (0.1 ml of a 2.5% solution/10 g body weight). Animals submitted to orchiectomy underwent an incision in the skin and subcutaneous tissue of the scrotum, ablation of both testes and a suture of the spermatic cord. Those destined for sham operation received only an incision in the scrotum and subsequent suture. After the surgery, animals were kept in sterile cages and pentabiotic was administrated to prevent bacterial infection.

2.4. Ovariectomy

Animals were anesthetized with tribromoethanol (0.1 ml of a 2.5% solution/10 g body weight). After this, one group of animals was submitted to ovariectomy (abdominal cavity opening, ovaries withdrawn and suture) and the other to sham operation (abdominal cavity