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Original article

Impact of *Trypanosoma cruzi* infection on nitric oxide synthase and arginase expression and activity in young and elderly mice

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ABSTRACT

Elderly organisms are more susceptible to infectious diseases. However, the impact of aging on antiparasitic mechanisms, especially the nitric oxide pathway, is poorly understood. Using an integrated *in vivo* and *in vitro* model, we compared the severity of *Trypanosoma cruzi* infection in young and elderly (8 or 72 weeks old) mice. Forty C57BL/6 mice were randomized into four groups: Y-inf, young infected; Yn-inf, young uninfected; A-inf, aged infected; An-inf, aged uninfected. Parasitemia was measured daily, and animals were euthanized after 15 days of infection. *Trypanosoma cruzi*-induced inflammatory processes were analyzed in blood and heart samples, as well as in bone marrow-derived macrophages (BMDMs) co-cultured with splenocytes isolated from young or elderly mice. Our results indicated upregulated IgG2b and IL-17 production in elderly animals, which was not sufficient to reduce parasitemia, parasitic load and myocarditis to levels observed in young animals. The higher susceptibility of elderly mice to *T. cruzi* infection was accompanied by reduced cardiac inducible nitric oxide synthase (iNOS) gene expression, nitric oxide (NO) and IFN-γ levels, as well as an antagonistic upregulation of arginase-1 expression and arginase activity. The same responses were observed when BMDMs co-cultured with splenocytes from elderly mice were stimulated with *T. cruzi* antigens. Our findings indicate that elderly mice were more susceptible to *T. cruzi* infection, which was potentially related to an attenuated response to antigenic stimulation, inhibition of iNOS gene expression and NO production, and antagonistic upregulation of arginase gene expression and activity, which created favorable conditions for heart parasitism and myocarditis development.

1. Introduction

Chagas disease is a neglected parasitic disease caused by the protozoan *Trypanosoma cruzi*, which is closely associated with poverty [1]. Estimates indicate that approximately 7 million people are infected by *T. cruzi* worldwide, and at least 41,000 new cases are reported each year [2,3]. The disease is endemic in Latin American countries, in which around 13% of the population is at risk of infection, and more than 10,000 deaths attributed to *T. cruzi* are registered per year [3,4]. Due to the immigration of infected people, vertical transmission and the donation of infected tissues and organs, the disease has spread to non-endemic areas such as Europe, Japan, North America and Australia [5,6].

Chronic cardiomyopathy is the most serious manifestation of Chagas disease, occurring in 30–40% of infected individuals [7,8]. This condition is related to a higher risk of death due to heart failure, which is invariably associated with severe reactive myocardial fibrosis, cardiomegaly, microvascular and electromechanical dysfunction [9,10]. Although cardiomyocyte parasitism plays an important role in the direct heart damage associated with acute infections [11], the organ deterioration observed in chronic infections is mainly due to immunomediated lesions associated with *T. cruzi*-induced upregulation of the T helper 1 (Th1) phenotype [12,13].

The immunological response is directly related to parasitic control, and plays a central role in the host's susceptibility and/or resistance to *T. cruzi* infection [14]. Aligned with the response typically observed in

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