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Cellular apoptosis and nitric oxide production in PBMC and spleen from dogs with visceral leishmaniasis

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ABSTRACT

Nitric oxide (NO) is involved in the death of the *Leishmania* parasite and regulation of apoptosis. We quantified the frequency of cells producing NO and its levels in the peripheral blood mononuclear cells (PBMC), leukocytes from spleen in Visceral Leishmaniasis (VL) symptomatic dogs and correlated NO levels with apoptosis and parasite load in the spleen. The percentage of NO+ cells and CD14+/NO+ was higher in PBMC and spleen cells in infected dogs than in controls. The levels of NO+ and CD14+/NO+ cells was higher in PBMC, but lower spleen of dogs infected than compared to control. Late apoptosis rates increased in PBMC and spleen of infected dogs compared to controls, and the NO levels and apoptosis not showed correlation. There was a positive correlation between the percentage of cells producing NO in the spleen and parasite load. The NO participates in the immune response in the canine VL, but it is not apoptosis inducer.

1. Introduction

There is a high worldwide mortality rate caused by VL with approximately 350 million people in 98 countries at risk of infection [1]. Human cases of VL in the Americas, are present in 12 countries; however, 96% of the cases are reported in Brazil. [2]. VL or kala-azar is a zoonotic disease transmitted to mammals by parasites of the genus *Leishmania* [3]. Transmission of *Leishmania* between vertebrate hosts in the New World is by the bite of the blood-sucking sandfly *Lutzomyia* spp [4].

In the urban environment, dogs are considered the main reservoir for the parasite that causes VL and are important in maintaining the epidemiological cycle of the disease because: a) VL is more prevalent in the canine population than in humans, b) infection in humans is usually preceded by canine cases, and c) dogs have a higher amount of parasites in the skin than men, which favors the infection of vectors [5]. The prevalence of leishmaniasis in dogs in endemic areas can reach 20% to 40% of the population [6], and in 2016 this prevalence of canine leishmaniasis was 12.7% of the total population of dogs in this study area [7].

In dogs, VL is a systemic disease and can be fatal if left untreated as the host fails to mount an effective protective response against the parasite. Infection spreads in the body of the host via the lymphatic system or becomes blood-borne reaching organs, such as the spleen, lymph nodes, liver and bone marrow, which is rich in mononuclear phagocytes [3].

Dogs with VL exhibit high T-cell apoptosis rates in the peripheral blood and spleen [8]. In healthy mononuclear cell cultures infected with *Leishmania infantum* a gradual increase in apoptosis was observed in $CD3^+$, $CD4^+$ and $CD8^+$ T cells [9]. In canine VL, the low cellular immune response most likely reflects the depletion of T cells observed in dogs that had high parasitism in the spleen [10]. Immunosuppression associated with chronic infection occurs due to high rates of apoptosis of T cells, and this mechanism could contribute to the disruption of the white pulp of the spleen and decreased levels of T cells in the peripheral blood. [6]

In infected dogs, protective immunity has generally been associated with cellular immune responses, characterized by high proportions of $CD4^+$ T cells and $CD21^+$ B cells [11,12]. High expression levels of IFN-γ, TNF-α and IL-12 [13]. and increased NO were associated with the microbicidal function of which has been demonstrated in vitro [14].

In canine VL, NO is increased in macrophage culture supernatant from asymptomatic dogs in relation to the symptomatic group [15], suggesting that it controls the parasitic load. In addition to functioning

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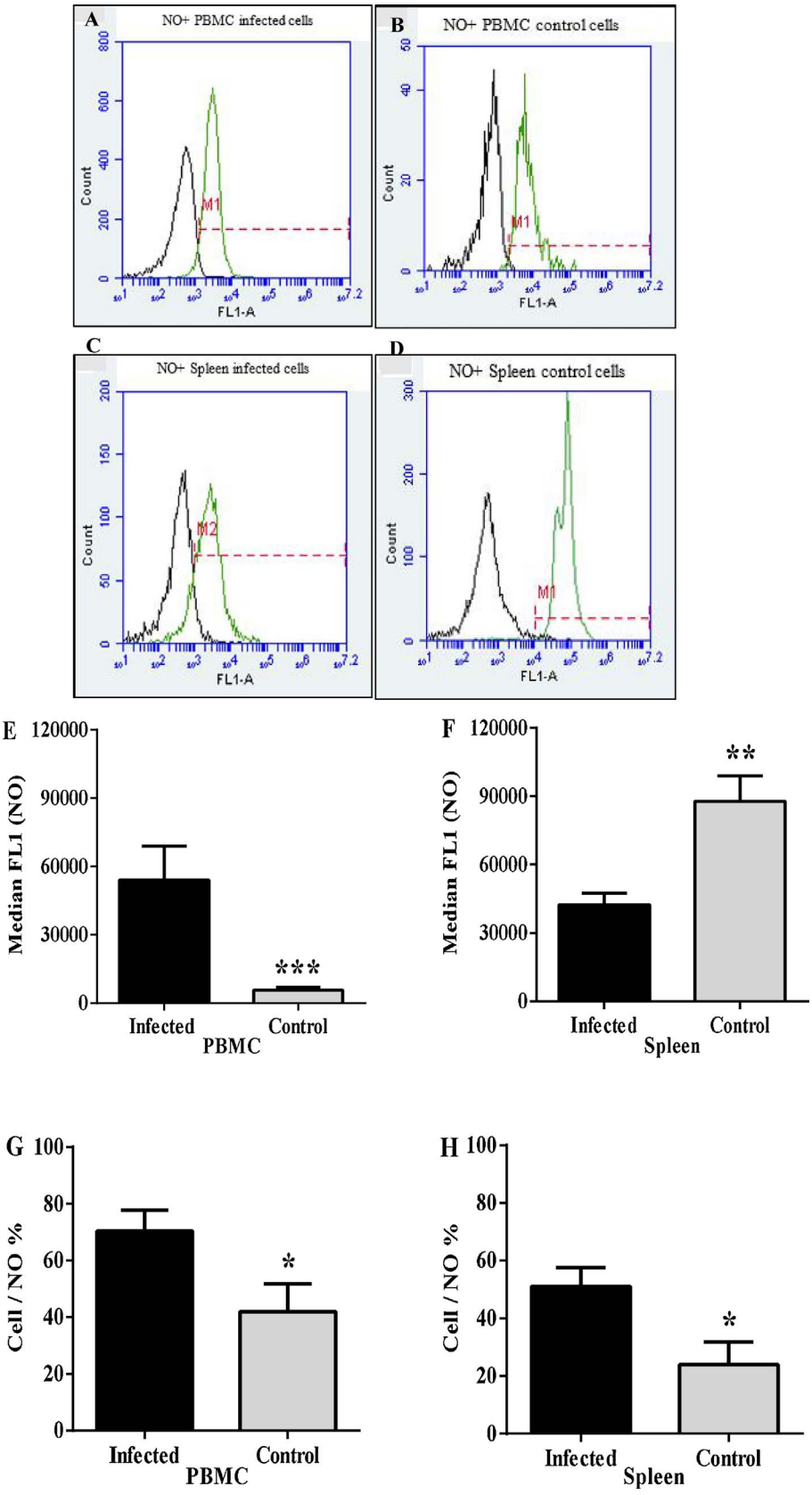

Fig. 1. Overlay histogram shows a representative example of the non-stained cells (dark), NO+ labeled (green) from PBMC (A, B), and splenic (C, D) of canine infected and control. Median of NO+ in PBMC (E) and spleen (F) cell. The mean and standard error of mean (SEM) are shown (*). Percentage of NO+ producing cells in PBMC (G) and the spleen (H) of dogs with VL and the control group. Amount of NO+ produced by PBMC and the leukocytes from splenic cells of dogs positive for VL and the control group. Data were analyzed using the Mann-Whitey test (*$P < 0.05$). (Infected – black bars), (Control – gray bars). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

as a microbicide, the NO may be involved in the immunopathology of the disease because in different parasitic infections its overproduction is associated with the absence of a lymphocyte response and apoptosis in different cell types [16]. NO can induce apoptosis in response to inflammatory reactions by different pathways inhibiting respiration of mitochondria to release cytochrome c that activates caspases, or by activating protein kinases, p38, MAPK, Janus kinase e ERK 1 and 2, leading to pro apoptotic signals, with accumulation of p53 and