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Changes in the pro-inflammatory cytokine production and peritoneal macrophage function in rats with chronic heart failure

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Abstract

Chronic heart failure (CHF) is a state of immune activation, and pro-inflammatory cytokines play an important role in its development and progression. Macrophages (Mϕs), when activated, are the main source of pro-inflammatory cytokines. We measured interleukin-6 (IL-6), interleukin (IL-1β) and tumor necrosis factor - α (TNF-α) production after lipopolysaccharide (LPS)-stimulation, as well as peritoneal Mϕs migration, phagocytic capacity, chemotaxis index, and hydrogen peroxide production, in an attempt to clarify the role of this cell in an animal model of CHF. Ligature of the left coronary artery or sham operation was performed in adult Wistar rats. After 12 weeks, resident and total cell number, phagocytic capacity, chemotaxis index, and hydrogen peroxide production in Mϕs were significantly higher in CHF than in control rats. The production of IL-6 and TNF- α was similarly significantly enhanced in CHF as compared with controls. Mϕs obtained from CHF rats were more responsive to LPS, suggesting the existence, *in vivo*, of possible factor(s) modulating the production of pro-inflammatory cytokines. The results demonstrated that there is modification of peritoneal Mϕs function along CHF development, possibly contributing to the pathophysiological process in the establishment of CHF.

Keywords: Chronic heart failure; Peritoneal macrophages; Chemotaxis; Hydrogen peroxide; Pro-inflammatory cytokines

1. Introduction

Phagocytic cells constitute the first line of immune defense against external agents. The main phagocytes are polymorphonuclear cells in the blood, and macrophages (Mϕs), in the tissues. Both types of cells carry out non-specific functions through phagocytosis. This process is divided into several stages, from the moment the pathogenic agent is reached, until its destruction: mobility directed by a chemical gradient or chemotaxis, ingestion, and digestion of the foreign agent [1,2].

Mϕs are widely recognized as cells that play a central role in the regulation of immune and inflammatory processes, as well as in tissue remodeling. Peritoneal macrophages represent a macrophage population with specific properties [1]. In response to Lipopolysaccharide (LPS)[1] endotoxin, macrophages secrete pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-12 [3]. Pro-inflammatory cytokine production can affect other functions of Mϕs, namely adherence to tissue, chemotaxis, and digestion of foreign agents [4].

The development of chronic heart failure (CHF) includes phenotypic changes in a variety of homeostatic systems so that, as the disease advances, CHF may be seen as a multi-system disorder with its origins in the heart, but

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[1] *Abbreviations used:* CHF, chronic heart failure; IL-1β, interleukin-1beta; IL-6, interleukin-6; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor–alpha; Mϕs, macrophages.

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embracing many extra-cardiac manifestations [5,7]. Immunological abnormalities are recognized in this context, in particular, changes in the expression of mediators of the response of the immune system [8]. High levels of the pro-inflammatory cytokine tumor necrosis factor α (TNFα) are found in the circulation and in the myocardium of patients with CHF. This cytokine has been implicated in a number of pathophysiological processes that are thought to be of relevance for the progression of CHF [9,10]. Immune activation with CHF may be secondary to endotoxin (lipopolyssaccharide) exposure, due to altered gut permeability with bacterial translocation and endotoxaemia [5,6,11].

The exact role of Mϕs in the progression of CHF has not been fully elucidated and these cells may serve as an important source of cytokines and growth factors [12,13].

The aim of the present work was to study pro-inflammatory cytokine production and other functions of peritoneal Mϕs in rats with chronic heart failure as induced by myocardial infarction.

2. Materials and methods

2.1. Animals

A total of 33 male Wistar rats ranging in age from 6 weeks (weighing ~250 g), obtained from the Animal Breeding Unit, Institute of Biomedical Sciences, University of São Paulo, were used. They were housed, five per cage, receiving food and water *ad libitum,* in an animal room under 12 h light–dark cycle, at 22 ± 1 °C and 60 ± 5% humidity. The experiments were carried out after acclimation for a week.

The investigation conformed to the Guide for the care and use of laboratory animals published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Committee of Ethics in Animal Experimentation of the Institute of Biomedical Sciences, University of São Paulo.

2.2. Surgical preparation

Rats were initially anesthetized with 3% halothane, tracheotomy was performed, and the animals were placed under a rodent respirator apparatus (Harvard model 680), and maintained under a 2% halothane oxygen mixture. The heart was exposed through left thoracotomy between the fifth and sixth ribs (1.5 cm in diameter) and the pericardium was opened. In animals in which a myocardial infarction (MI) was produced, a 9–0 Ethylon suture was placed under the left main coronary artery at a point 1–2 mm distal to the edge of the left atrium and a ligature was performed at the artery [14]. Sham-operated animals underwent the same procedure except that the suture under the coronary artery was left untied. Muscle and skin incisions were closed with separate purse-string silk sutures (size 0) and the lungs were fully expanded. The heart was then returned to its normal position and the thorax immediately closed. Each animal was allowed a minimum of 4 weeks of recovery.

2.3. Experimental design

After the myocardial infarction (MI) preparation, animals were allowed a minimum of 4 weeks of recovery (this being the necessary time to achieve the development to chronic heart failure state) [15], and then, maintained for a further 8 weeks for observation. The animals were killed by decapitation without anesthesia.

2.4. Echocardiography

After 12 weeks, all rats underwent echocardiographic evaluation. Briefly, under mild sedation the chests were shaved and they were placed on prone decubitus position. Echocardiograms were performed with a HP Sonos 2500 sector scanner equipped with a 12 MHz phase-array transducer. M-mode tracings were recorded for measurement of anterior/posterior wall thickness and left ventricular internal dimensions. All the values corresponded to the average of three measurements of three consecutives cardiac cycles.

2.5. Determination of left ventricular infarct size

After fixation in formalin for a minimum of 24 h, the left ventricle was cut into four transverse sections from base to apex in parallel with the atrio–ventricular groove. The four sections of the left ventricle were then dehydrated in alcohol, bathed in xylol, and embedded into paraffin. Transverse sections (10 μm thick) were obtained, mounted, and stained with Masson's trichrome stain from which hematoxylin was omitted, as to provide maximum discrimination between the fibrous area of infarct and the muscle. These sections were then measured with a planimeter Digital Image Analyzer (Carl Zeiss), according to the technique described by [14].

2.6. Collection of peritoneal suspensions

The abdomen was cleansed with 70% ethanol, the abdominal skin was carefully dissected without opening the peritoneum, and 6 ml of Phosphate–buffered saline (PBS) adjusted to pH 7.4 were injected intraperitoneally. The abdomen was massaged and 90–95% of the injected volume was recovered. The peritoneal resident Mϕs, identified by morphology and non-specific esterase staining, were counted and then adjusted in the same medium to 5×10^6 Mϕs per ml. Cell viability was confirmed by the Trypan-blue exclusion test (>95%). At least 92% of the peritoneal exudate cells were Mϕs.

2.7. Preparation of zymozan (*Saccharomyces cerevisiae*)

Thirty-five milligrams of zymozan in 100 ml PBS (Sigma, St. Louis, MO, USA) was boiled for 30 min and