Immunobiology 217 (2012) 1005–1016

Contents lists available at SciVerse ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

Impaired phagocytic capacity driven by downregulation of major phagocytosis-related cell surface molecules elicits an overall modulatory cytokine profile in neutrophils and monocytes from the indeterminate clinical form of Chagas disease

J.A.S. Gomes[a,b,c,*], A.C. Campi-Azevedo[c], A. Teixeira-Carvalho[c], D. Silveira-Lemos[c,d], D. Vitelli-Avelar[c], R. Sathler-Avelar[c], V. Peruhype-Magalhães[c,e], K.F. Silvestre[a,b], M.A. Batista[c], N.C.C. Schachnik[c], R. Correa-Oliveira[b,f], S. Eloi-Santos[c], O.A. Martins-Filho[c]

[a] Laboratório de Biologia das Interações Celulares, Departamento de Morfologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Brazil
[b] Laboratório de Imunologia Celular e Molecular, Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, Brazil
[c] Laboratório de Biomarcadores de Diagnóstico e Monitoração, Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, Brazil
[d] Laboratório de Imunologia Clínica, NUPEB,UFOP, Ouro Preto, Brazil
[e] Laboratório de Pesquisas Clínicas, Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, Brazil
[f] Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais, INCT-DT, Salvador, Brazil

ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form 11 January 2012
Accepted 15 January 2012

Keywords:
Cell surface molecules
Chagas disease
Cytokines
Monocytes
Neutrophils
Nitric oxide
Phagocytic capacity

ABSTRACT

The distinct ability of phagocytes to present antigens, produce cytokines and provide co-stimulatory signals may contribute to the severity of the outcome of Chagas disease. In this paper, we evaluate the phenotypic features of phagocytes along with the cytokine signature of circulating T-cells from Chagas disease patients with indeterminate (IND) and cardiac (CARD) clinical forms of the disease. Our data demonstrated that neutrophils from IND patients displayed an impaired ability to produce cytokines. A lower *Trypanosoma cruzi* phagocytic index and higher nitric oxide levels were characteristics of monocytes from IND. The impaired phagocytic capacity did not reflect on the levels of anti-*T. cruzi* IgG, but was detectable in the downregulation of Fc-γR, TLR and CR1 molecules. The monocyte-derived cytokine signature demonstrated that a down-regulated synthesis of IL-12 and a modulatory state were evidenced by a positive correlation between IL-12 and IL-10 with a lower synthesis of TNF-α. The down-regulation of MHC-II and CD86 in monocytes supports the occurrence of particularities in the APC-activation-arm in IND, and may be involved in the T-cell pro-inflammatory pattern counterbalanced by a potent IL-10 response. Our findings support the hypothesis that differential phenotypic features of monocytes from IND may be committed to the induction of a distinct immune response related to low morbidity in chronic Chagas disease.

Introduction

Chagas disease, caused by *Trypanosoma cruzi*, is one of the worst public health problems in Latin America, where 15 million people are infected and 90 million are at risk of infection (Dias et al. 2008). The host–parasite relationship determines the onset of the human pathology, which is extremely diverse, varying from a relatively benign and asymptomatic indeterminate form to digestive and/or cardiac clinical forms (Dias et al. 2008; Umezawa et al. 2000). The factors that determine the distinct clinical outcomes, leading to either a mild or a severe form, are not completely understood. However, it is clear that chagasic pathology is associated with host immune response (Bahia-Oliveira et al. 1998; Brener and Gazzinelli 1997; Sathler-Avelar et al. 2009).

Different studies have suggested a possible role for inflammatory cells in host defense due to the ability of monocytes and neutrophils to phagocytize and destroy *T. cruzi* amastigotes *in vitro* (Molina and Kierszenbaum 1987; Villalta and Kierszenbaum 1983, 1984a,b), and by the effector cell activity of these cells in antibody-dependent cellular cytotoxicity against blood and culture forms of

Abbreviations: AUS, autologous plasma; BFA, brefeldin A; BNP, brain natriuretic peptide; CARD, patient with the cardiac clinical form of the disease; IND, patient with the indeterminate clinical form of the disease.

* Corresponding author at: Laboratório de Biomarcadores de Diagnóstico e Monitoração, Centro de Pesquisas René Rachou, FIOCRUZ, Avenida Augusto de Lima, 1.715, Barro Preto, Belo Horizonte, Minas Gerais 30.190-002, Brazil.
Tel.: +55 31 3349 7764; fax: +55 31 3295 3115.
E-mail addresses: jassis@cpqrr.fiocruz.br, estanislau.juliana@gmail.com (J.A.S. Gomes).

0171-2985/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2012.01.014

the parasite (Kierszenbaum 1979; Kierszenbaum and Hayes 1980; Kipnis et al. 1981; Rimoldi et al. 1981; Sanderson et al. 1978). In this context, studies in the past several years have defined the molecular mechanisms of the phagocytic process. Fc-gamma receptors are among the best-studied phagocytic receptors. The key features of Fc-gamma receptor-mediated phagocytosis include the release of proinflammatory mediators such as cytokines and reactive oxygen species (Joshi et al. 2006); however the role of these receptors during *T. cruzi* infection has not yet been established.

Moreover, recent evidence suggests that neutrophils can contribute significantly to the immune response by modulating both cellular and humoral immunity, especially via the synthesis and release of immunoregulatory cytokines (Mantovani et al. 2011). It has been hypothesized that neutrophils may be involved in immunomodulation in experimental Chagas disease. Chen et al. (2001) observed that neutrophils play opposite roles in infected BALB/c and C57BL/6 with respect to protection or exacerbation of *T. cruzi* infection, possibly through the modulation of Th1/Th2 in different directions. Muniz-Junqueira et al. (2004) showed that patients with the cardiac clinical form of Chagas without congestive cardiac failure presented reduced phagocytic capacity of monocytes and neutrophils. On the other hand, patients with heart failure showed phagocytic function of these cells similar to healthy subjects, suggesting that the depressed immune function of monocytes and neutrophils was reversed in the presence of heart failure. A possible hypothesis to explain the results is that it was due to an increased production of cytokines, such as TNF-α, capable of influencing phagocyte functions when heart failure is present in patients with Chagas disease. Therefore, heart failure and phagocyte function show that the immune system may be differently activated in distinct clinical conditions according to the heart functional status, which suggests that the disturbances characteristic to each condition *per se* appear to affect one another.

Sathler-Avelar et al. (2003) have demonstrated that early acute human Chagas disease is not associated with monocyte activation. However, patients with early indeterminate Chagas disease show increased expression of HLA-DR by pro-inflammatory monocytes. Vitelli-Avelar et al. (2006) further suggested that the activation of the innate immune response during the early stages of human Chagas disease, through recruitment and activation of monocytes, could be an important bridge between innate and adaptive immunological events in early *T. cruzi* infection.

The toll-like receptor family (TLRs) is a major class of receptors for microbial pathogen-associated molecular patterns (PAMPs) and endogenous ligands (Takeda et al. 2003). Some reports have shown that TLR-2, TLR-4 and the related signaling pathway play an important role in the initial recognition of *T. cruzi* and may regulate the initial pro-inflammatory response during infection with the parasite but may also contribute to the severity of the disease (Almeida and Gazzinelli 2001; Gazzinelli et al. 2004; Junqueira et al. 2010).

In addition, the cytokine profile is fundamental in the regulation of immune response. IFN-γ plays a crucial role in the activation of macrophages, which mediate the killing of intracellular pathogens including protozoan parasites. The killing of pathogens is mediated by nitric oxide (NO), which is produced by activated macrophages and is cytotoxic for intracellular microorganisms (Vespa et al. 1994). The presence of inflammatory and anti-inflammatory cytokines is found in peripheral blood cells of patients with indeterminate and cardiac clinical forms. Various studies have shown that IFN-γ, TNF-α and IL-12 are important in the control of this infection by ensuring the induction of an efficient adaptive host response (Abrahamsohn 1998). Sathler-Avelar et al. (2006) demonstrated that despite a slight increase in IL-12$^+$ monocytes, equivalent numbers of TNF-α$^+$ and IL-10$^+$ monocytes during early-indeterminate Chagas disease resembled that observed in healthy uninfected children. Souza et al. (2004) demonstrated that monocytes from indeterminate disease patients display higher levels of IL-10. On the other hand, cardiac disease patients present a higher ability to produce TNF-α, suggesting a role for pro-inflammatory monocytes in the development of cardiac disease (Vitelli-Avelar et al. 2008). Moreover, Souza et al. (2004) demonstrated that monocytes from cardiac disease patients display higher levels of TNF-α both *ex vivo* and after *in vitro* infection with live parasites. In this context, the characteristics of monocytes from indeterminate disease patients are consistent with the establishment of a modulatory response, whereas monocytes from cardiac disease patients may elicit T cell activation in the presence of high levels of TNF-α, leading to a decrease or increase in the phagocytic capacity of monocytes and an exacerbated inflammation depending on the concentration of this cytokine.

Thus, we aimed to investigate how *in vitro T. cruzi* infection leads to distinct changes in the functional properties of circulating neutrophils and monocytes from IND and CARD, and to examine the possibility of establishing biomarkers that allow forecasting of the therapeutic outcome.

Patients, materials and methods

Study population

The patients who agreed to participate in this study were identified and selected from the Outpatient Referral Center for Chagas Disease of the Hospital das Clínicas at the Federal University of Minas Gerais (UFMG), Brazil. Serology for Chagas disease was determined by two or more tests (indirect immunofluorescence, ELISA or indirect hemagglutination) and patients were considered infected when at least two different tests were positive. In this study, we used 11 samples from chagasic patients with chronic disease. According to their clinical records, the chronic chagasic patients were divided into two categories, namely indeterminate (IND) and cardiac (CARD) clinical forms. Patients presenting asymptomatic *T. cruzi* infection, classified as IND ($n = 5$; 4 males and 1 female; mean age = 42.5 ± 5.5 years, ranging from 35 to 52 years), had no clinical manifestations of the disease other than their positive serology. Patients with cardiac dysfunction, CARD ($n = 6$; 1 male and 5 females; mean age = 44.0 ± 8.7 years, ranging from 33 to 56 years), presented dilated cardiomyopathy and were diagnosed by a detailed clinical examination, including eletrocardiography, 24-h Holter examination and chest X-ray. Seronegative adults were included in this study as non-infected individuals ($n = 7$, 4 males and 3 females, mean age = 29.0 ± 9.7 years, ranging from 18 to 47 years).

Informed written consent was obtained from all participants. This work complied with resolution number 196/1996 from the National Health Council for research involving humans and was approved by the Ethical Committee at Centro de Pesquisas René Rachou (CPqRR/FIOCRUZ protocol 11/2004), Belo Horizonte, Minas Gerais, Brazil.

Blood samples

Five milliliters of whole peripheral blood were collected in EDTA (Becton Dickinson, CA, USA) anticoagulant for hemogram analysis. Forty milliliters of heparinized (Becton Dickinson, CA, USA) peripheral blood were collected from each participant and used for *in vitro* phagocytic capacity assay as well as intracellular nitric oxide assessment. Five milliliters of peripheral blood were collected without anticoagulant to obtain the autologous plasma (AUS) samples. These samples were inactivated by heating for 30 min at 56 °C, and then maintained at −20 °C prior to use in the short-term cultures of whole blood samples and the