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Original article

B-1 cells facilitate *Paracoccidioides brasiliensis* infection in mice via IL-10 secretion

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Abstract

Protective immunity in paracoccidioidomycosis is mainly mediated by cellular immunity. The role of B cells in this disease, in particular B-1 cells, is poorly understood. The aim of this study was to characterize the participation of B-1 cells in resistance or susceptibility of BALB/c and BALB/Xid mice to *P. brasiliensis* (Pb) pulmonary infection. BALB/Xid, which lacks B-1 cells, exhibited higher resistance to infection when compared with BALB/c mice. However, adoptive transfer of B-1 cells to BALB/Xid mice drastically increased the susceptibility of these animals to Pb infection. The fungal burden in BALB/c and B-1-reconstituted BALB/Xid was significantly higher as compared to BALB/Xid strain. Compact, well-organized granulomas were observed in the lungs of BALB/Xid mice, whereas large lesions with necrotic center with a plethora of fungi developed in BALB/c mice. It was also shown that B-1 cells impair phagocytosis of Pb by macrophages *in vitro* via secretion of IL-10, which was increased upon stimulation with a purified Pb antigen, gp43. Finally, *in vivo* blockade of IL-10 led to a better control of infection by the highly susceptible B10.A mouse. These findings suggest that B-1 cells play a major role in resistance/susceptibility to Pb infection in murine models, most likely via production of IL-10.

Keywords: B-1 cells; *Paracoccidioides brasiliensis*; Interleukin-10

1. Introduction

Parasitism is a co-evolutionary and ecological phenomenon. Successful parasites must evolutionarily inherit mechanisms that allow them to invade and exploit the host's economy. Concurrently, parasite antigens trigger the host's cellular and humoral immune responses, which either restrain parasite implantation or fail to get rid of the intruder. The unstable equilibrium between the adaptive mechanisms of parasites and the characteristics of the immune response results in the development of cure, disease or death of the host.

Among the huge number of known etiologic agents of disease, fungi cause a variety of dermatitis and systemic lesions in humans and animals. The importance of systemic mycosis has been amplified by the hazardous role played by these infectious agents in opportunistic infections in HIV-infected subjects [1]. Regardless the clinical and epidemiologic relevance of mycosis, the pathogeny of systemic mycosis in humans and domestic animals is peculiar and poorly understood. Nevertheless, a large volume of experimental data related to the understanding of fungal pathology is available in the literature [1].

Paracoccidioidomycosis, a systemic disease of man whose infective agent is the dimorphic fungus *Paracoccidioides brasiliensis* (Pb), has been extensively investigated. However, the mechanisms determining resistance or susceptibility in man and experimental animals are unclear [2]. Calich et al. [3] have demonstrated that resistance or susceptibility to Pb infection of mice is under strict genetic control. Mice from the

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B10.A strain are highly susceptible to infection with Pb, which causes extensive and disseminated lesions and premature death of the animals. Conversely, mice from the A/Sn lineage are relatively resistant to infection; they survive for a longer time and develop focal lesions with all the characteristics of a granulomatous response. However, the cellular and humoral mechanisms that control resistance or susceptibility to Pb in murine models are not fully understood.

The participation of B lymphocytes in paracoccidioidomycosis, in particular B-1 cells, has not been completely elucidated. B-1 cells are predominantly located in peritoneal and pleural cavities and are characterized by expression of IgM, CD45, CD19 and CD11b [4]. Further, B-1a cells are also identified by expression of CD5 [5,6]. These cells proliferate in cultures of mouse peritoneal adherent cells, differentiate into a mononuclear phagocyte and migrate to inflammatory sites [7]. In addition, B-1 cells are the main source of B-cell-derived interleukin (IL)-10 [8]. In our laboratory, this cell subset was shown to down-regulate macrophage functions *in vitro* via IL-10 secretion [9].

Previous work on B-1 cells–Pb interaction has shown that gp43 secreted by Pb stimulates the production of TNF-α and IL-10 *in vitro* when B-1 cells and macrophages are present, though IL-10 compromises granuloma formation [9,10]. Further, CBA/NXid mice, deficient in B-1 cells, exhibit a higher DTH response after Pb antigen inoculation in comparison to CBA/J or CBA/NXid reconstituted with B-1 cells [11]. These results suggest that B-1 cells might contribute to susceptibility in experimental paracoccidioidomycosis. Moreover, B-1 cells are shown to be detrimental in other mouse models of microbial infection, such as experimental Chagas' disease, leishmaniasis and *Staphylococcus aureus*-induced arthritis [8,12–14].

Herein we demonstrate the involvement of B-1 cells in the progression of experimental infection by Pb. Results show that B-1 cells lead to increased mortality of Pb-infected BALB/c mice. We also unearth evidence that animals fail to control infection due to impairment of macrophage function under the effect of B-1-secreted IL-10.

2. Material and methods

2.1. Animals

Eight- to ten-week-old male BALB/c, BALB/Xid and B10.A mice were provided by the animal facility of the Departamento de Imunologia, Instituto de Ciencias Biomedicas, Universidade de São Paulo and CEDEME (Universidade Federal de São Paulo). Animal handling and housing were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals.

2.2. Yeast culture and infection

Pb18, a high-virulence isolate of *P. brasiliensis* [15,16], was maintained in Sabouraud–dextrose–agar semi-solid medium (Difco, Surrey, UK) at 37 °C, with weekly passages. For infections, 3-day culture yeasts were washed in PBS, counted and tested for viability with Janus green staining [15]. Cells were resuspended in sterile PBS at 1×10^6 viable cells/40 μl. Mice were anesthetized with 2% tiazine chlorhydrate (Bayer, Sao Paulo, Brazil) and 5.7% ketamine chlorhydrate (Parke-Davis, Sao Paulo, Brazil) according to the manufacturer's instructions and injected intratracheally (i.t.) with a tuberculin syringe.

2.3. Antigen purification

Gp43 was purified by affinity chromatography using the monoclonal antibody 17C [16] coupled to Sepharose CNBr-4 (Pharmacia, Uppsala, Sweden) from *P. brasiliensis* suspension cultures. After dialysis in PBS, antigen purity was monitored by SDS–PAGE and the concentration measured by the Bradford assay [17].

2.4. Pulmonary fungal burden

Presence and viability of Pb in lungs of infected animals was assessed by counting colony forming units (CFU), as described elsewhere [18]. Tissue samples were fixed in 10% formaldehyde and embedded in paraffin. Five-micrometer sections were stained with hematoxylin–eosin for further histological analysis by light microscopy.

2.5. B-1 cell cultures for in vitro assays and in vivo reconstitution

B-1 cells were obtained from the peritoneal cavity of BALB/c mice as described elsewhere [7]. Briefly, peritoneal cells were collected from the abdominal cavity of mice by washing with RPMI-1640 medium. Cells (2×10^5 cells/ml) were dispensed onto glass dishes (Corning Costar, Tokyo, Japan) and incubated at 37 °C in 5% CO_2 for 1 h. Non-adherent cells were discarded and the adherent monolayers were rinsed with medium. Subsequently, RPMI-1640 containing 10% FCS (R10) was added to the cultures, followed by incubation at 37 °C in 5% CO_2 for 5 days with no medium renewal. After this procedure, B-1b cells are the main cell type in the non-adherent cell population, according to Almeida et al [7]. The high percentage (>90%) of B-1 cells in these cultures was routinely analyzed by FACS. Reconstitution of Xid mice with these cells was made by intraperitoneal (i.p.) route with RPMI-1640 medium as the vehicle.

2.6. Analysis of cell phenotype

B-1b cells obtained as described before were stained with monoclonal antibodies and analyzed with the FACSCalibur System (BD Biosciences, USA). The following primary antibodies were used: phycoerythrin (PE)-conjugated rat anti-mouse CD19, fluorescein-isothiocyanate (FITC)-conjugated rat anti-mouse CD5 (Pharmingen), allophycocyanin (APC)-conjugated rat anti-mouse CD11b, CyChrome-conjugated rat anti-mouse CD23 (BD Pharmingen, USA). Cell labeling was carried out according the manufacturer's protocol.