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Investigation of the role of complement and complement receptors in the modulation of B cell activation by a *Paracoccidioides brasiliensis* cell wall fraction

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Abstract

F1 fraction from *Paracoccidioides brasiliensis* is a potent activator of the complement system. Considering that complement receptors CR1 and CR2 are involved in the regulation of B cell response, we evaluated the in vitro effect of the F1 in the activation of B lymphocytes, as well as the participation of complement receptors in this process. Murine splenocytes were cultured in order to evaluate the expression of CD40, CD45RB and CD69 on B lymphocyte, and IgG and IgM were quantified in the culture supernatant. F1 participated in the activation of B cells, showing a positive modulation effect on all markers analyzed. An increase in the production of IgG was detected in the supernatants when the opsonized F1 fraction was present. Complement receptor blockade with monoclonal antibodies led to a partial reduction in immunoglobulin secretion, suggesting that these receptors, especially CR2, play a role in modulating the function of B lymphocyte stimulated with the opsonized F1 fraction. These results may contribute for a better understanding of the B cell activation and differentiation processes in response to the F1 fraction from *P. brasiliensis*.

Keywords: *Paracoccidioides brasiliensis*; B lymphocyte; Complement system, CR1 (CD35); CR2 (CD21); CD40; CD45RB; CD69

Introduction

Paracoccidioidomycosis is a systemic infection caused by the dimorphic fungus *Paracoccidioides brasiliensis* that primarily involves the lungs and then disseminates to other organs and systems [1,2]. A subclinical infection is established in competent hosts, but, when the host–parasite balance is upset by immunosuppression, the infection progresses and gives rise to a full-blown disease [1,3]. The disease may develop either in the acute (subacute) juvenile or the chronic adult forms [1].

Clinical and experimental aspects of this infection are basically characterized by intense depression in cellular immunity [4], accompanied by polyclonal activation of B cells, causing an increase in gammaglobulin levels [5]. Fungus multiplication results from these alterations.

Polyclonal B cell activation is a well-documented phenomenon in several other infectious diseases [5]. Although the mechanisms that induce this phenomenon in paracoccidioidomycosis have not yet been established, it is known that parasite or parasite-derived antigens can activate B lymphocytes in other diseases, like malaria. However, its regulation can be mediated by distinct immunological mechanisms, depending on the disease [5].

The alkali-insoluble polysaccharide fraction derived from the cell wall of *P. brasiliensis*, F1 fraction or the beta-glucan

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from F1 fraction, has been studied due to its participation in the unspecific polyclonal activation of B cells in paracoccidioidomycosis [6,7]. It was observed that F1 is able to activate rat classical and alternative complement pathways [8] and to stimulate polymorphonuclear cell functions like chemotaxis and respiratory burst of neutrophils [9]. Furthermore, it was demonstrated that F1 fractions, originating from *P. brasiliensis* isolates with different virulence, activate the human complement system and human neutrophils in different manners [10]. The F1 fraction seems to be at least partially responsible for the complement activation that was previously observed with the whole fungus [11].

It is known that complement receptors, CR1 (CD35) and CR2 (CD21), are involved in the regulation of B cell responses. "In vitro" studies support an important function of CR2 in the regulation of B cells response, through the CR2/CD19/CD81 complex. Biochemical studies have demonstrated that cross-linking of the CD21/CD19 complex with the B cell receptor lowers the threshold for B cell activation [12,13]. Thus, it is proposed that complement enhances humoral immunity by lowering the threshold of antigen activation of naive B cells [12].

B lymphocytes activation markers are cell surface glycoproteins expressed as phenotypic markers or identifiers of different stages of maturation. Many of these molecules are involved in the activation or regulation of B lymphocytes. CD45, a critical component in lymphocyte activation, is a transmembrane protein tyrosine phosphatase [14], expressed on all nucleated hematopoietic cells, constituting approximately 5 to 10% of the cell surface [15]. It has been demonstrated to be functionally important by playing a critical role in receptor signaling in both B and T cells, but its function was better established in T cells. These cells express multiple CD45 isoforms, the patterns of which are specific and change depending on the activation and differentiation state of the T cell [16]. B cells predominantly express high molecular weight isoforms of CD45, but few studies have been undertaken to investigate changes in CD45 as a consequence of specific B cell activation signals [16].

CD40 is an integral membrane protein found on B lymphocyte surfaces, dendritic cells, hematopoietic progenitor cells and epithelial cells. It is an approximately 50 kDa glycoprotein, which is a member of the tumor necrosis factor receptor superfamily. It is constitutively expressed on B cells and, its ligand, CD 154 (CD40L), is expressed on activated T helper cells [17,18]. CD40 plays a central role in T-cell-dependent B cell activation and proliferation via its interaction with CD40L. The engagement of CD40 with CD40L on B lymphocytes promotes proliferation, antibody secretion, cytokine production, upregulation of various surface molecules involved in antigen presentation, isotype switching, development of germinal centers and a humoral memory response [17–19].

CD69, an early activation antigen expressed on B and T lymphocytes and NK cells following stimulation by mitogenic agents [20,21], has recently generated interest as a possible marker for use in flow-cytometry-based assays for cellular activation [22]. It is a phosphorylated glycoprotein, as a surface homodimer held together by disulfide bridges. Due to its early activation expression, the CD69 gene has a short and brief expression followed by a fast decrease and degeneration [23].

Considering that the *P. brasiliensis* F1 fraction is a potent inflammation inducer, activates the complement system, and also that complement receptors CR1 and CR2 are involved in the regulation of the B cell response, the goal of this study was to evaluate the in vitro effect of the F1 fraction containing beta-glucan on the activation of B lymphocytes, as well as the participation of complement receptors in this process.

Materials and methods

Animals

BALB/c mice were obtained from the animal house of the University of São Paulo at Ribeirão Preto, S.P. All protocols used are in accordance with the Guidelines for Ethical Care of Experimental Animals of the Institution Animals Care and Use Committee.

Antibodies

Rat anti-mouse CD19-FITC; rat anti-mouse CD40-PE; rat anti-mouse CD45RB-PE; rat anti-mouse CD69-PE; rat IgG2a-PE, isotype control; purified Rat IgG2a, isotype control; purified Rat IgG2b, isotype control (Caltag Laboratories, Inc., Burlingame, CA); rat IgG2a-FITC, isotype control; 8C12-rat IgG2a, κ anti-mouse CR1; 7G6-Rat IgG2b, κ anti-mouse CR2/CR1 (BD-PharMingen, San Diego, CA); purified goat anti-mouse IgG + IgA + IgM (H + L); goat anti-mouse IgM (μ)-HRPO; goat anti-mouse IgG (gamma)-HRPO (Caltag Laboratories, Inc., Burlingame, CA).

Fungus culture and preparation of the alkali-insoluble polysaccharide fraction (F1)

The virulent *P. brasiliensis* Strain 18 was used. Yeast-phase fungal cells were cultured at 37°C for 21 days in Fava Netto's medium [24]. The separated cells were treated with 40% formol and disrupted by ultrasonic vibration [10]. To obtain the alkali-insoluble polysaccharide fraction, the cell suspension was treated with organic solvents and 1 M NaOH, and the final suspension dehydrated at 37°C for 5–6 days, powdered and stored.

Complement

Pools of normal mouse serum (NMS) and of normal human serum (NHS) were employed. Blood samples collected from normal mice and healthy donors were left to clot for 60 min on ice or at room temperature, respectively, before being centrifuged at 500 × *g* for 10 min at 4°C. Sera