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Original article

B cell response during infection with the MAT a and MAT alpha mating types of *Cryptococcus neoformans*

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Abstract

In the present study, we compared the B cell response of BALB/c and C57Bl/6 mice during *Cryptococcus neoformans* infection. This response was investigated using virulent serotype D forms of mating types alpha and a (MAT alpha and MAT a). C57Bl/6 mice showed massive (mainly cerebral) infection by both types, while BALB/c were resistant to infection. Some resistance of C57Bl/6 mice was induced by previous immunization with the capsular polysaccharide from MAT alpha. Passive immunization of C57Bl/6 mice with purified antibody (Ab) obtained from capsular polysaccharide-immunized mice also increased resistance to infection. Both mouse strains showed comparable low IgM response to the capsular polysaccharide from MAT alpha, and only C57Bl/6 mice produced IgM to the polysaccharide of MAT a. Comparable levels of different immunoglobulin (Ig) isotypes against capsular components of MAT alpha and MAT a were detected, and the response of C57Bl/6 mice was higher when compared to that of BALB/c mice. FACS analysis indicated an increase in the percentage of a high-granulosity (side-scatter) splenic subpopulation and in the percentage of splenic Gr-1$^+$ cells in infected C57Bl/6 mice. In addition, the percentage of follicular splenic B cells was decreased after *C. neoformans* infection of C57Bl/6 mice. This response was more pronounced when we investigated infection induced by the MAT a mating type. Taken together, our results indicate that capsular polysaccharide derived from MAT alpha and MAT a types of *C. neoformans* have a stimulatory effect upon B cells but that there is no correlation between resistance of BALB/c mice and Ab production. However, the increase in resistance of C57Bl/6 mice parallels the production of Abs and a major change in splenic cell populations.

Keywords: B lymphocytes; Immunoglobulin; Polysaccharide; *Cryptococcus neoformans*; Mating type; Virulence

1. Introduction

The basidiomicete *Cryptococcus neoformans* is an opportunistic pathogen that causes disease in immunocompromised individuals [1]. This is one of the best models for studying fungal pathogenesis, since there are consistent animal models for investigating its infective cycle [2]. The *C. neoformans* isolates are usually haploid and they present two mating types: MAT alpha and MAT a [3,4]. In clinical and environmental isolates, the ratio between the MAT alpha and MAT a mating types is 40:1, suggesting a selection for MAT alpha [5,6]. The MAT alpha mating type from serotype D presents greater virulence in the murine model, since it induces a higher mortality rate [7]. Studies using serotype D strains have shown that the two mating types show differences in the *STE12* gene, which regulates (among other characteristics) the production of capsule and melanin [8,9]. There is a direct correlation between expression of genes necessary for capsule production and virulence in mice [10,11]. Recently, serotype A congenic MAT a and MAT alpha strains of *C. neoformans* were

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developed and no difference in the levels of virulence between these two forms was observed [12].

The presence of capsule is one of the important virulence factors of *C. neoformans*. Its thickness is increased in vitro by the presence of serum [13], and it is shedded by the fungus in vivo [14]. The glucuronoxylomannan (GXM) is one of the major components of the *C. neoformans* capsule. In the murine model, the administration of anti-GXM monoclonal antibody (Ab) prolongs survival and decreases fungal load [15]. The GXM is weakly immunogenic [16]. Isogenic mouse strains show considerable variability in the capacity to produce Ab after vaccination with *C. neoformans* capsular polysaccharides: BALB/c and C3H/HeJ mice produce a strong Ab response when challenged with purified polysaccharides, while the B10 and A/J strains present a weak response [17].

Previous studies have shown the essential role of T lymphocytes in host defense during the infection induced by *C. neoformans* and the involvement of dendritic cells in the activation of T cells in this model [18–21]. B cells also have an important role in defense. Mouse strains showing a more intense Ab response are more resistant to *C. neoformans*-induced infection [15]. B-cell-deficient mice (like the CBA/N strain) are more susceptible to infection with the fungus [22]. The kinetics of Ab production also has an influence in resistance, since mice that possess a faster Ab response against *C. neoformans* are more susceptible [23].

Several studies suggest that the production of Ab is important in protection against *C. neoformans* [24,25]. The protecting effect of Ab depends on both direct action on the fungus and indirect action, decreasing the magnitude of the inflammatory reaction [26]. The production of Ab does not have only protecting effects: it could even exacerbate the infection caused by *C. neoformans* [27,28]. The protecting efficiency of monoclonal Ab against *C. neoformans* depends on the amount, specificity and immunoglobulin (Ig) isotype [24,27–31]. Recent studies have shown that the Ig isotype produced can determine the pattern of binding of an Ab to the *C. neoformans* polysaccharides [32].

The immune response (and resistance) to *C. neoformans* varies among different mouse strains. When infected with a highly virulent strain of *C. neoformans*, the CBA/J, C.B-17 and BALB/c strains are more resistant and control the infection, while C57Bl/6 mice show a chronic pulmonary infection [33–36]. C.B-17 mounts a Th1 response that correlates with resistance to the organism, whereas the absence of this response in C57Bl/6 mice induces susceptibility [36]. In C56Bl/6 mice, IL-5 production is detected in the lung, and the pulmonary cell infiltrate is rich in eosinophils [37]. Also, the inflammatory response against *C. neoformans* determines susceptibility. Highly virulent strains of *C. neoformans* induce a down-modulation of the granuloma induction [38]. The inflammatory response and cytokine production by susceptible and resistant mouse strains have been widely investigated. However, no studies have investigated the response of B cells induced by different *C. neoformans* strains. In the present study, we compare the B cell response of both BALB/c and C57Bl/6 mice during *C. neoformans* infection. This response was investigated using virulent serotype D yeast forms of MAT alpha and MAT a.

2. Material and methods

2.1. C. neoformans strains and polysaccharide purification

The strains of *C. neoformans* used in this study were B-3501 (mating type alpha) and B-3502 (mating type a) [3]. Both isolates are encapsulated and are of serotype D. These strains were kindly provided by Dr. Tamara Doering (Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA). Yeast forms were obtained during exponential growth in synthetic medium [39] at 37 °C in a shaker at 100 rpm. The cells were collected by centrifugation and exhaustively washed with buffered saline before infection. The capsular polysaccharide was isolated from concentrated dialyzed culture supernatant by repetitive ethanol precipitation and purified by anion-exchange chromatography on a Mono Q HR 16/10 (Pharmacia) column equilibrated and eluted for 15 min with 0.01 M sodium phosphate buffer, pH 7.0, and then with a linear gradient of NaCl (0.1 M) in the same buffer over 120 min at a flow rate of 3 ml/min. The capsular polysaccharide-containing fractions were detected by phenol/sulfuric acid assay and dialyzed and lyophilized.

2.2. Animals, infection and immunization

BALB/c and C57Bl/6 mice (males and females) with age ranging from 6 to 8 weeks were obtained at the Central Animal Facility from Instituto de Microbiologia Professor Paulo de Góes. The animals were used according to institutional policies for care and maintenance of experimental animals. Infection was performed by injection of 10^5 yeast forms by the intravenous route. This parasite dose was in the range used in previous intravenous infection studies [23] and did not induce mortality during our 3-week study (data not shown).

Some animals received intraperitoneal injections of a saline solution of capsular polysaccharides. The immunized animals were bled by the tail vein at different time points. Sera were obtained and kept at –20 °C until use.

For passive immunization experiments, sera were obtained from C57Bl/6 mice 15 days after immunization with capsular polysaccharide from B-3501 MAT alpha. Antibodies from pooled serum were purified by precipitation with 50% ammonium sulfate. One milligram of purified Ab was injected intraperitonealy 1 h prior to infection and 8 days after infection. Fungal load was determined 14 days after infection, as detailed below.

2.3. Fungal load determination

Spleen, lung and brain were obtained from infected animals after different periods of infection and were macerated