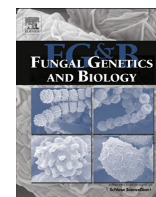
ARTICLE IN PRESS

Fungal Genetics and Biology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi



Binding of the wheat germ lectin to *Cryptococcus neoformans* chitooligomers affects multiple mechanisms required for fungal pathogenesis

Fernanda L. Fonseca [a], Allan J. Guimarães [b], Lívia Kmetzsch [c], Fabianno F. Dutra [a], Fernanda D. Silva [d,1], Carlos P. Taborda [d], Glauber de S. Araujo [e,f], Susana Frases [e,f], Charley C. Staats [c], Marcelo T. Bozza [a], Augusto Schrank [c], Marilene H. Vainstein [c], Leonardo Nimrichter [a], Arturo Casadevall [g,h], Marcio L. Rodrigues [a,i,*]

[a] Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-902, Brazil
[b] Departamento de Microbiologia, Imunologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Brazil
[c] Centro de Biotecnologia and Departamento de Biologia Molecular e Biotecnologia, Universidade Federal do Rio Grande do Sul, Brazil
[d] Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil
[e] Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil
[f] Laboratório de Biologia, Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (INMETRO), Rio de Janeiro, Brazil
[g] Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
[h] Division of Infectious Diseases of the Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
[i] Fundação Oswaldo Cruz (Fiocruz), Centro de Desenvolvimento Tecnológico em Saúde (CDTS), Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Cryptococcus neoformans
Virulence
Lectin binding
Capsule
Chitin oligosaccharides

ABSTRACT

The principal capsular component of *Cryptococcus neoformans*, glucuronoxylomannan (GXM), interacts with surface glycans, including chitin-like oligomers. Although the role of GXM in cryptococcal infection has been well explored, there is no information on how chitooligomers affect fungal pathogenesis. In this study, surface chitooligomers of *C. neoformans* were blocked through the use of the wheat germ lectin (WGA) and the effects on animal pathogenesis, interaction with host cells, fungal growth and capsule formation were analyzed. Treatment of *C. neoformans* cells with WGA followed by infection of mice delayed mortality relative to animals infected with untreated fungal cells. This observation was associated with reduced brain colonization by lectin-treated cryptococci. Blocking chitooligomers also rendered yeast cells less efficient in their ability to associate with phagocytes. WGA did not affect fungal viability, but inhibited GXM release to the extracellular space and capsule formation. In WGA-treated yeast cells, genes that are involved in capsule formation and GXM traffic had their transcription levels decreased in comparison with untreated cells. Our results suggest that cellular pathways required for capsule formation and pathogenic mechanisms are affected by blocking chitin-derived structures at the cell surface of *C. neoformans*. Targeting chitooligomers with specific ligands may reveal new therapeutic alternatives to control cryptococcosis.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Cryptococcosis, the disease caused by the fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*, kills about 650,000 people per year around the world (Park et al., 2009). Treatment of human cryptococcosis is unsatisfactory in many cases (Roy and Chiller, 2011). In this context, efforts to understand how *C. neoformans* and *C. gattii* cause damage to the human host have been actively pursued during the last two decades (Albuquerque and Rodrigues, 2012) and therapeutic alternatives that neutralize cryptococcal virulence factors could be promising alternatives to standard antifungal therapy.

C. neoformans and *C. gattii* are encapsulated eukaryotic pathogens (Zaragoza et al., 2009). The cryptococcal capsule, which is a major determinant of virulence, has been studied in detail in the *C. neoformans* model. The capsular network consists of highly hydrated polysaccharides, including glucuronoxylomannan (GXM), the most abundant component of the capsule, and glucuronoxylomannogalactan (GXMGal), a minor capsular polysaccharide [reviewed in (Zaragoza et al., 2009)]. The complexity of the cell surface architecture of *C. neoformans* is further increased by the presence of other complex glycans, including mannoproteins, glucans and chitooligomers (Rodrigues and Nimrichter, 2012). While

* Corresponding author. Address: Centro de Desenvolvimento Tecnológico em Saúde (CDTS), Fiocruz. Av. Brasil 4.365, Casa Amarela, Manguinhos, 21040-360 Rio de Janeiro, Brazil.
E-mail address: marciolr@cdts.fiocruz.br (M.L. Rodrigues).
[1] Current address: Universidade Federal do ABC, São Paulo, Brazil.

1087-1845/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.fgb.2013.04.005

Please cite this article in press as: Fonseca, F.L., et al. Binding of the wheat germ lectin to *Cryptococcus neoformans* chitooligomers affects multiple mechanisms required for fungal pathogenesis. Fungal Genet. Biol. (2013), http://dx.doi.org/10.1016/j.fgb.2013.04.005

the functions of GXM have been extensively explored during the last two decades (Zaragoza et al., 2009), the knowledge on how GXMGal and mannoproteins impact cryptococcal pathogenesis has advanced more significantly only during last years. On the other hand, the roles of glucans and chitooligomers in the interaction of *C. neoformans* with host cells remain virtually unknown.

Chitooligomers or chitooligosaccharides are chitin-derived structures composed of three to twenty residues of β1,4 linked *N*-acetylglucosamine. In *C. neoformans*, they are embedded within the capsular network (Fonseca et al., 2009; Rodrigues et al., 2008) and interact with GXM to form complex glycans (Rodrigues et al., 2008). Chitin-derived oligomers were also shown to regulate capsular architecture in *C. neoformans* cells from both *in vitro* cultures and infected tissues (Fonseca et al., 2009), implying an indirect role in the cryptococcal pathogenesis. Chitooligomers have been detected at the capsular surface (Rodrigues et al., 2008), suggesting their potential for recognition by host receptors possibly affecting cryptococcal pathogenesis.

Fungal chitooligomers are recognized with high affinity by the wheat germ lectin (WGA), a reagent that is commonly used to study cellular distribution and functions of chitin and chitin-like molecules (Orgad et al., 1984). The addition of WGA to *Fusarium* sp cultures resulted in morphological alterations of the germ tubes, including vacuolation of the cellular content and lysis of cell walls, culminating in the prevention of spreading of the fungus within the host (Ciopraga et al., 1999). Indeed, chitin-derived oligomers have been implicated with cell division in different models (Chen and Contreras, 2004).

Considering the virtually unknown role of chitin-derived oligomers in the pathogenesis of fungal infections and their recently described functions in the physiology of *C. neoformans* (Fonseca et al., 2009; Rodrigues et al., 2008), we aimed at determining how these molecules affect pathogenic mechanisms of this fungus. Based on a model where chitooligomer exposure at the cell surface was blocked with WGA, we demonstrated that these glycans are involved in brain colonization of mice and interaction with phagocytes. Unexpectedly, blocking of chitooligomers with the lectin resulted in defective capsule formation and down regulation of genes required for synthesis, cellular traffic and signaling pathways controlling capsular components. These results reveal novel functions for surface glycans in *C. neoformans*.

2. Methods

2.1. Microorganism and growth conditions

The *C. neoformans* strain used in all experiments in this study was the standard serotype A isolate H99 (Loftus et al., 2005). The only exception was the use of the *C. gattii* standard serotype B strain R265 (Gillece et al., 2011) as a control in scanning electron microscopy procedures utilizing the lectin WGA as an inhibitor of capsule formation. Yeast cells were inoculated into 100 ml Erlenmeyer flasks containing 50 ml of minimal medium composed of glucose (15 mM), $MgSO_4$ (10 mM), KH_2PO_4 (29.4 mM), glycine (13 mM), and thiamine-HCl (3 μM) (pH 5.5). Fungal cells were cultivated for 2 days at 30 °C, with shaking. Yeast cells were obtained by centrifugation, washed in phosphate-buffered saline (PBS) and counted in Neubauer chamber. All media were prepared with apyrogenic water, and glassware was rendered sterile and pyrogen free by heating to 190 °C for 4 h.

2.2. Macrophages

Bone marrow-derived macrophages (BMDM) were obtained from wild type (WT) or *Tlr2*−/− mice (C57BL/6, gender matched, 6–10 week old) (Marim et al., 2010). The animals were kept at 25 °C with free access to chow and water in a room with a 12-h light/dark cycle. The Animal Ethics Committee at the Federal University of Rio de Janeiro approved the animal protocols. Bone marrow was harvested from the tibias and femurs from mice and differentiated into BMDM as described previously (Marim et al., 2010). The cells were suspended at 5×10^6/10 ml in RPMI 1640 medium supplemented with 20% of FCS and 30% of supernatant from L929 cells cultures. After 3 days, fresh supplemented medium was added to the cell cultures. Macrophages were collected at day 6, and 5×10^5/well were plated in 24-well plates in RPMI 1640 medium with 10% of FCS. Cells were cultivated at least 12 h before further experimental procedures.

2.3. Interaction of C. neoformans with mammalian cells

For interaction with host cells, control fungi or WGA-treated cryptococci were used. Treatment with WGA consisted of incubation of yeast cells (5×10^6 cells/ml) for 30 min at 37 °C with the lectin at 10 μg/ml in PBS. The cells were washed in PBS by centrifugation and then stained with 0.5 mg/ml fluorescein isothiocyanate (FITC; Sigma) in PBS (25 °C) for 10 min (Barbosa et al., 2006). Staining with FITC was monitored by flow cytometry and fluorescence microscopy, which revealed that both control and WGA-treated fungi had similar levels of fluorescence (data not shown). Fungal suspensions were prepared in DMEM at a ratio of 10 yeasts per host cell. Interactions between fungal and host cells occurred at 37 °C with 5% CO_2 for 12 h. Incubation time was based on previous phagocytosis studies developed in our laboratory showing that 60–70% of the phagocytes become infected and efficiently internalize *C. neoformans* after overnight incubations in the absence of opsonins (Kmetzsch et al., 2011a,b,c, 2010). In some systems, the medium of interaction was supplemented with the trisaccharide $(GlcNAc)_3$ at 10 μg/ml (Sigma; Richmond, VA). Cells were washed three times with PBS to remove non-adherent yeasts. After removal from the plastic surface with a cell scrapper, the cells were analyzed by flow cytometry as described previously (Barbosa et al., 2006). Control preparations were developed as described above by using uninfected cells and non-stained yeast (data not shown).

2.3.1. Animal infection

Before infection, fungal suspensions were treated for 1 h at 37 °C with PBS (control) or with the same buffer supplemented with WGA (10 μg/ml, final concentration). The cells were then washed with PBS to remove unbound WGA, collected by centrifugation and adjusted to the density of 10^6 yeast cells per 50 μl of PBS. Female BALB/c mice (6–8 weeks old, $n = 10$) were first submitted to anesthesia through intraperitoneal administration of ketamine (10 mg/kg) and xylazine (4 mg/kg). The fungal suspension was then inoculated intratracheally. Alternatively, mice were infected with *C. neoformans* as described above, but with no previous treatment with WGA. In these systems, mice were given WGA during infection in five doses with 48 h intervals. The first dose was administered 24 h after infection. Each dose corresponded to 50 μl PBS containing 10 μg of WGA, to mimic the lectin-cryptococci ratio (10 μg/10^6 cells) used in the in *vitro* macrophage assays. Control systems included infected mice given similar volumes of PBS. To evaluate whether WGA alone could induce acute toxicity, the lectin was also administered to uninfected mice in a similar fashion, with all animals remaining alive for at least 30 days (data not shown). Animals were monitored daily for mortality or, alternatively, sacrificed 5 days post-infection for counting colony forming units (CFUs). For this analysis, brains and lungs were excided, macerated in PBS and plated onto Sabouraud dextrose agar, as previously described (Rodrigues et al., 2007b). Histopathologic analy-

Please cite this article in press as: Fonseca, F.L., et al. Binding of the wheat germ lectin to *Cryptococcus neoformans* chitooligomers affects multiple mechanisms required for fungal pathogenesis. Fungal Genet. Biol. (2013), http://dx.doi.org/10.1016/j.fgb.2013.04.005

Download English Version:

https://daneshyari.com/en/article/8470959

Download Persian Version:

https://daneshyari.com/article/8470959

Daneshyari.com