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Original article

Phagocytic receptors on macrophages distinguish between different *Sporothrix schenckii* morphotypes

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Abstract

Sporothrix schenckii is a human pathogen that causes sporotrichosis, a cutaneous subacute or chronic mycosis. Little is known about the innate immune response and the receptors involved in host recognition and phagocytosis of *S. schenckii*. Here, we demonstrate that optimal phagocytosis of conidia and yeast is dependent on preimmune human serum opsonisation. THP-1 macrophages efficiently ingested opsonised conidia. Competition with D-mannose, methyl α-D-mannopyranoside, D-fucose, and *N*-acetyl glucosamine blocked this process, suggesting the involvement of the mannose receptor in binding and phagocytosis of opsonised conidia. Release of TNF-α was not stimulated by opsonised or non-opsonised conidia, although reactive oxygen species (ROS) were produced, resulting in the killing of conidia by THP-1 macrophages. Heat inactivation of the serum did not affect conidia internalization, which was markedly decreased for yeast cells, suggesting the role of complement components in yeast uptake. Conversely, release of TNF-α and production of ROS were induced by opsonised and non-opsonised yeast. These data demonstrate that THP-1 macrophages respond to opsonised conidia and yeast through different phagocytic receptors, inducing a differential cellular response. Conidia induces a poor pro-inflammatory response and lower rate of ROS-induced cell death, thereby enhancing the pathogen's survival.

Keywords: *Sporothrix schenckii*; Phagocytosis; Opsonins; Glycoproteins; Mannose receptor; TNF-α; Complement receptor; Reactive oxygen species

1. Introduction

Sporothrix schenckii is a pathogenic dimorphic fungus that causes sporotrichosis, an important chronic mycotic infection of the skin or subcutaneous tissues [1]. Epidemiological and experimental evidences suggest that natural infection is initiated by the traumatic introduction of conidia into the skin [2]. In infected tissue, the fungus differentiates into the yeast form and may spread to other tissues [3,4]. Human tissues affected by sporotrichosis show intracellular infection of macrophages (Mϕ's), polymorphonuclear cells, and giant cells, as evidenced by budding yeast observed within these phagocytes [5,6].

Mϕ receptors are central to an effective innate immune response to pathogens. They mediate phagocytosis, signal cascades, intracellular trafficking, inflammatory responses, and antigen presentation [7]. Most receptor–ligand interactions between Mϕ's and microorganisms lead to the destruction of the pathogen, although certain interactions allow for a permissive environment in which the pathogen can survive and proliferate [8]. Some receptors such as the mannose receptor (MR), scavenger receptors, and β-glucan receptors

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may identify specific pathogen-associated motifs, generally carbohydrates, and mediate non-opsonic phagocytosis [9]. Some molecules of the host, like opsonins [10], bind to the surface of microorganisms and promote microbial clearance through activation of phagocytosis. The most well-studied opsonins include the C3bi fragment of the C3 component of the complement system, which binds to complement receptor type 3 (CR3) [11,12], and immunoglobulin, which binds via the Fc domain to the Fc receptor on phagocytes [13,14]. Other humoural components that opsonise foreign agents before they are recognized by a phagocytic receptor include lectins, which bind carbohydrate residues and can mediate interactions between microbial cell wall glycoconjugates and receptors on phagocytic cells [15]. These interactions result in enhancement of internalization, leading to the release of pro-inflammatory cytokines and activation of antimicrobial mechanisms [16–19]. One such receptor is the MR, a pattern-recognition receptor involved in the clearance of endogenous glycoproteins [20]. The MR is also an endocytic receptor that recognizes and efficiently internalizes pathogens through glycosylated molecules with terminal mannose, fucose, or *N*-acetyl glucosamine residues [21]. Although MR is an efficient phagocytic receptor, it appears to require other receptors to trigger an immune response [20].

Several papers have reported the importance of cell-mediated immunity in sporotrichosis [22,23] and the important role of toll-like receptors directing the immune response after first contact with the pathogen [24].

Severe *Sporothrix* infection has been reported among AIDS patients, suggesting that $CD4^+$ T-cell immunity is important for protection against *Sporothrix* [22]. Furthermore, it has been demonstrated that nude mice are highly susceptible to sporotrichosis [22]. Although it is clear that innate immunity is important in limiting the development of the disease, little is known about the receptors mediating phagocytosis of *S. schenckii*. Studies from different laboratories have shown that *S. schenckii* yeast cells are not efficiently internalized by peritoneal Mϕ's unless they are first opsonised with concanavalin A (Con A) or treated with neuraminidase [25]. However, the precise mechanism by which Mϕ's interact with conidia or yeast from *S. schenckii* is unknown.

Phagocytes are important effector cells in the immune response against different fungi, and they must recognize, internalize, and kill pathogens [26]. Since the Mϕ is the primary effector of antimicrobial activity against pathogens, this study aimed to examine the participation of THP-1 Mϕ (THP-1 Mϕ) receptors during the interaction between *S. schenckii* yeast and conidia morphotypes and the Mϕ.

2. Materials and methods

2.1. Reagents

Unless otherwise specified, chemical reagents were obtained from Sigma Chemical Co. (St. Louis, MO); RPMI 1640 tissue culture medium and foetal bovine serum (FBS) were purchased from Gibco Life Technologies (Rockville, MD); and plastic ware was supplied by Fisher Scientific (Pittsburg, PA).

2.2. S. schenckii *culture*

The EH257 strain, which was isolated from a patient with sporotrichosis, was kindly provided by Jorge Mayorga, Instituto Dermatológico, Jalisco, Mexico. Conidial suspensions were prepared by adding sterile phosphate–buffered saline (PBS) to minimal medium slants (6.7 g yeast nitrogen base, 20 g dextrose, and 20 g agar base per litre of distilled water) that were incubated for 7 d at 28 °C. The resulting suspension was filtered with sterile Whatman paper No. 1 to selectively eliminate short hyphae.

2.3. S. schenckii *DNA extraction, amplification, and sequencing*

Genomic DNA was extracted using glass-bead disruption, and DNA was purified with a DNA cleaning and concentrator kit (Zymo Research, CA). Amplification of the nuclear calmodulin (CAL) gene, which is a good identification marker for different *Sporothrix* species, was conducted with the primers CL1 and CL2A [27]; the fragment was sequenced and compared with *Sporothrix*–calmodulin related sequences retrieved from GenBank; and the ClustalW algorithm was used for computer-assisted multiple sequencing. Neighbour-joining analysis was performed using DNAMAN 3.0, and confidence interval was estimated using 1000 rounds of bootstrapping.

2.4. Cell culture

Cells from the human monocytic leukaemia cell line THP-1 [28] were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS and 1% antibiotic–antimycotic (Invitrogen, Gibco BRL, MD) only used during cell culture, 10 mM HEPES buffer (Invitrogen), and 50 μM 2-mercaptoethanol (Invitrogen). THP-1 cells were maintained at 37 °C in a 5% CO_2 atmosphere.

2.5. Differentiation of THP-1 monocytes into Mϕ's

THP-1 cells were cultured on 12 mm round glass coverslips (Bellco, Vineland, NJ) in 24-well plates (Corning, Corning, NY) at approximately 2×10^5 cells per coverslip in RPMI maintenance media containing 10 nM phorbol 12-myristate 13-acetate (PMA). After 24 h, non-attached cells were removed by aspiration, and the adherent cells were washed with PBS prior to the assay. Viability, as determined by trypan blue dye exclusion assay, was always greater than 95%.

2.6. Phagocytosis assays

Differentiated THP-1 Mϕ's were challenged with conidia at a Mϕ:conidia ratio of 1:5 and subsequently incubated at 37 °C in 5% CO_2 for 60 min. At the end of each incubation period, phagocytosis was stopped by washing the Mϕ's with