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Transcriptome profile of the murine macrophage cell response to *Candida parapsilosis*

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ABSTRACT

Candida parapsilosis is a human fungal pathogen with increasing global significance. Understanding how macrophages respond to *C. parapsilosis* at the molecular level will facilitate the development of novel therapeutic paradigms. The complex response of murine macrophages to infection with *C. parapsilosis* was investigated at the level of gene expression using an Agilent mouse microarray. We identified 155 and 511 differentially regulated genes at 3 and 8 h post-infection, respectively. Most of the upregulated genes encoded molecules involved in immune response and inflammation, transcription, signaling, apoptosis, cell cycle, electron transport and cell adhesion. Typical of the classically activated macrophages, there was significant upregulation of genes coordinating the production of inflammatory cytokines such as TNF, IL-1 and IL-15. Further, we used both primary murine macrophages and macrophages differentiated from human peripheral mononuclear cells to confirm the upregulation of the TNF-receptor family member *TNFRSF9* that is associated with Th1 T-helper cell responses. Additionally, the microarray data indicate significant differences between the response to *C. parapsilosis* infection and that of *C. albicans*.

1. Introduction

Candida parapsilosis has recently emerged as a major human pathogen (Pfaller et al., 2010; Trofa et al., 2008; van Asbeck et al., 2009). Despite the increasing clinical importance, little is known about the host response to *C. parapsilosis* infection. However, defining the interactions between a host cell and *C. parapsilosis* is crucial to understand the molecular details of pathogenesis. In contrast to the massive amount of work that has been described on host defense against *Candida albicans*, there is a paucity of information about the immune response against *C. parapsilosis*. *C. parapsilosis*is typically a commensal of human skin, and its pathogenicity is limited by intact integument. However, the species is known for its capacity to form biofilms on catheters and other implanted devices, for nosocomial spread by hand carriage, and for persistence in the hospital environment (Trofa et al., 2008). In fact, it is now the second or third most commonly isolated *Candida* species from blood cultures worldwide (Cisterna et al., 2010; Horasan et al., 2010; Peman et al., 2005; Tortorano et al., 2011). *C. parapsilosis* is of special concern in critically ill neonates (reviewed by Pammi et al. (2013)), causing more than one-quarter of all invasive fungal infections in low-birth-weight infants in the United Kingdom and up to one-third of neonatal *Candida* bloodstream infections in North America (Benjamin et al., 2003; Clerihew et al., 2007; Neu et al., 2009; Smith et al., 2005). Moreover, it even outranks *C. albicans* infections in some European hospitals (Montagna et al., 2010).

Macrophages are critical cells of the innate immune system, contributing to immediate and robust defense against microbial infections. These cells produce an intricate pattern of cytokines and chemokines that enhance chemotaxis, phagocytosis and microbicidal activity; as well as activate T cells through antigen processing and presentation (Bourgeois et al., 2010; Seider et al., 2010). The pivotal role of macrophages in immune response during candidiasis explains the large number of studies dealing with this interaction, primarily focusing on *C. albicans*. These analyses use different approaches to address the complex host response to *C. albicans*, including human and mouse macrophage-like cell lines (Barker et al., 2005; Heidenreich et al., 1996; Kim et al., 2005; Marcil et al., 2002), as well as primary human monocytes (Cummings and Relman, 2000; Kim et al., 2005; Manger and Relman, 2000). In sharp contrast, little is known about the

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regulation and coordinated expression of genes in macrophages involved in the host response to *C. parapsilosis*. In fact, the significant differences in pathobiology and virulence of *C. parapsilosis* in different patient groups indicate that effective immune responses exists and suggest that they can be different from responses occurring in interactions with *C. albicans* (Trofa et al., 2008; van Asbeck et al., 2009).

In this study, we analyzed the transcriptional responses of murine J774.2 macrophage-like cells to *C. parapsilosis* infection. It is well known that J774.2 cells are able to efficiently phagocytose and kill both *C. albicans* and *C. parapsilosis* (Bertini et al., 2013; Dementhon et al., 2012; Lewis et al., 2012; Tibor Németh et al., 2013). As expected, many genes known to be responsive to *C. albicans* were up-regulated upon *C. parapsilosis* infection. Some of these genes were chosen for quantitative real-time PCR (RT-qPCR) analysis to validate the results obtained by microarray. In addition, further analysis using primary murine macrophages and macrophages derived from human peripheral blood mononuclear cells (PBMCs) was performed on the tumor necrosis factor receptor superfamily member 9 (*TNFRSF9*) gene to confirm that it was highly upregulated, as it had not previously been associated with host responses to *Candida* infections.

2. Material and methods

2.1. Fungal strains and culture conditions

Candida parapsilosis GA1 (Gacser et al., 2007), Candida glabrata CBS 138, Candida albicans ATCC 90028, Candida guilliermondii CBS 566, Candida krusei CBS 573, Candida tropicalis CBS 94, Candida metapsilosis SZMC (Szeged Microbiological Collection) 1548, and Candida orthopsilosis SZMC 1545 were maintained at −80 °C in 35% glycerol. The cells were grown in YPD (1% yeast extract, 2% bactopeptone, 2% glucose). For infection, Candida cells were grown overnight at 30 or 37 °C. Yeast cells in log-phase were washed three times in sterile phosphate-buffered saline (PBS) and counted using a hemacytometer.

2.2. J774.2 cell cultivation

The murine macrophage cell line J774.2 (BALB/c) was maintained in DMEM medium (Lonza) supplemented with 10% heat-inactivated fetal bovine serum (Lonza) and 1% 100x Penicillin–Streptomycin solution (Sigma). Macrophages were incubated under the following conditions: 37 °C, 5 v/v% CO_2 and 100% relative humidity. J774.2 cells were plated at 5×10^6 cells per well in six-well plates for RNA isolation, or 5×10^5 cells on tissue culture coverslips placed into a 24-well plate for scanning electron microscopy (SEM). The J774.2 cells were co-incubated at an effector-to-target ratio of 1:5 with *C. parapsilosis* cells.

2.3. Isolation of murine peritoneal macrophages

Peritoneal macrophages were harvested from euthanized BALB/c mice by peritoneal lavage by using 10 ml of sterile ice cold PBS. The cells were collected (120 g, 10 min), suspended in ACK lysis buffer and incubated for 10 min on ice to eliminate red blood cells. The suspension was centrifuged again (120 g, 10 min) and the pellet was suspended in 37 °C DMEM medium (Lonza) supplemented with 10% heat-inactivated FBS and 1% Penicillin–Streptomycin solution. Cells were counted by using a hemocytometer and plated out into 12-well plates. They were allowed to adhere for one hour, then washed with pre-warmed PBS and cultured in DMEM medium. Cells were incubated as described above and co-incubated at an effector-to-target ratio of 1:5 with *C. parapsilosis* cells.

2.4. Human PBMC isolation and macrophage differentiation

Peripherial Blood Mononuclear Cells (PBMCs) were isolated from buffy coat blood sample deriving from a healthy donors by using Ficoll-Paque Plus (GE Healthcare) density gradient centrifugation. Experiments were performed according to the institutional regulation of the independent ethics committee of the University of Szeged. Cells were suspended in RPMI medium (Lonza) containing 10% heat-inactivated human serum (Lonza) and 1% Penicillin–Strepromycin solution (Sigma). Monocytes were allowed to adhere to the surface of the plate for two hours then carefully washed with pre-warmed PBS. They were incubated for five days in the same medium under the conditions mentioned above and allowed to differentiate into macrophages. Differentiated cells were co-incubated at an effector-to-target ratio of 1:5 with *C. parapsilosis* cells.

2.5. Phagocytosis assay (quantitative imaging)

For the analysis of phagocytosis by quantitative imaging, yeast cells were labeled with the fluorescent dye Alexa Fluor 647 carboxylic acid, succinimidyl ester (Invitrogen) as described (Tibor Németh et al., 2013). J774.2 macrophages were co-cultured with the labeled *Candida* cells in 12-well plastic cell culture plates at an effector:target ratio of 1:5 for 15, 30, 60 or 120 min to allow phagocytosis. Macrophages were washed extensively with PBS after the incubation period in order to eliminate non-phagocytosed *Candida* cells. Afterwards, macrophages were gently suspended to a single cell suspension by pipetting, harvested by centrifugation, suspended in 50 μL FACS buffer (0.5% FBS in PBS) and measured on a FlowSight instrument (Amnis). Data were analyzed using *IDEAS* Software (Amnis).

2.6. Scanning electron microscopy

J774.2 and *C. parapsilosis* co-incubation was performed on coverslips (Sarstedt) in a 24 well-plate at 37 °C and 5% CO_2 for 1 h. Cells were washed twice with PBS, fixed and dried as described by Van de Velde et al. (2010) with the following modifications. Samples were fixed with 2.5% glutaraldehyde in Sorenson-buffer (pH = 7.5) instead of cacodylate-buffer overnight at 4 °C. The samples were serially dehydrated in 50% ethanol (2 × 15 min on ice), 70% ethanol (2 × 15 min on ice), 80% ethanol (2 × 15 min on ice), 90% ethanol (2 × 15 min on ice), 95% ethanol (2 × 15 min on ice), and then absolute ethanol (2 × 15 min on ice). Samples were then held in tert-butyl alcohol:absolute ethanol 1:2, 1:1, 2:1 one after the other for 1 h each at room temperature. Then 100% tert-butyl alcohol was applied for 1 h at room temperature. Finally, the coverslips were frozen in 100% tert-butyl alcohol at 4 °C, freeze dryed overnight, and fixed on aluminum stubs with double adhesive carbon tapes. Samples were coated with gold in a Quorum Technologies SC 7620 'Mini' sputter coater, and observed by using a Hitachi S-4700 scanning electron microscope.

2.7. Acridine orange/crystal violet staining

The staining was performed based on the protocol of Miliotis (Miliotis, 1991) with slight modifications. J774.2 and *C. parapsilosis* co-incubation was performed on coverslips (Sarstedt) in a 24 well-plate. Samples were stained for 30 s in 0.01% acridine orange solution recovered in PBS, and then washed three times in PBS for 30 s. To quench the extracellular fluorescence 0.15 M crystal violet dissolved in PBS was applied for 30 s, then the dye was removed and the samples were washed three times in PBS. Coverslips were mounted on slides in PBS by using colorless nail polish. The staining protocol was performed at room temperature in dark. Samples were observed by using Olympus DP-72 fluorescent microscope.