

PH.D. THESIS

**EXAMINATION OF INNATE AND ADAPTIVE
IMMUNE RESPONSES INDUCED BY THE
OPPORTUNISTIC HUMAN PATHOGEN *CANDIDA*
*PARAPSILOSIS***

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Introduction

Invasive *Candida* infections pose a serious health problem worldwide, especially among immunocompromised patients. Although the most common cause of invasive candidiasis is *C. albicans*, the prevalence of infections due to non-*albicans* species has been increasing during the last decades. Depending on the geographic region, *C. parapsilosis* is the second or third most commonly isolated species after *C. albicans* causing invasive candidiasis. Although the pathogenesis of invasive candidiasis has been intensively studied during the last years, the majority of studies focuses on the immune response induced by *C. albicans*, while we have little information about the immunological background of *C. parapsilosis* infections.

The purpose of this study was to characterize certain aspects of innate and adaptive immune responses induced by *C. parapsilosis*, as well as their comparison with those induced by *C. albicans*. We have examined the Th polarization in human PBMCs (peripheral blood mononuclear cells) induced by *C. parapsilosis* and *C.*

albicans, as well as the role of pattern recognition receptors and intracellular signaling molecules during the inflammatory response evoked by the two *Candida* species. Furthermore, we have investigated the activation of the inflammasome in THP-1 macrophages following *C. parapsilosis* and *C. albicans* infection.

Methods

Cell isolation and culture

- PBMC isolation
- macrophage differentiation
- culture of mammalian cell lines

In vitro stimulation of PBMCs/macrophages

- *in vitro* stimulation of primary cells and cell lines with different *Candida* strains

Molecular methods

- RNA isolation
- qRT-PCR (quantitative real-time PCR)

Immunological methods

- flow cytometry
- intracellular cytokine staining
- ELISA (enzyme-linked immunosorbent assay)

Other methods

- reactive oxygen species (ROS) measurements
- determination of lysosomal cathepsin B release
- measurement of lactate dehydrogenase (LDH) activity

Results

1. Comparison of immune responses in human PBMCs following stimulation with *C. parapsilosis* and *C. albicans*

1.1. Comparison of T-cell polarization induced by *C. parapsilosis* and *C. albicans*

First, we compared the pro-inflammatory cytokine production in PBMCs following stimulation with heat-killed *C. parapsilosis* and *C. albicans*. We found that PBMCs stimulated with *C. parapsilosis* produced similar quantities of TNF α and IL-6, and approximately 20 % less IL-1 β compared to *C. albicans*-stimulated cells. In case of Th-derived cytokines, we found that *C. parapsilosis* induced significantly lower IFN γ , and higher IL-10 secretion after 48 h compared to *C. albicans*. Furthermore, *C. parapsilosis* stimulated significantly lower IL-17 and IL-22 production after 7 days. Flow cytometric analysis following intracellular cytokine staining confirmed that there was a lower number of IL-

17-producing cells in the CD4⁺ Th population. These results suggest that while *C. albicans* induces a Th1/Th17-dominant Th polarization, *C. parapsilosis* skews the Th balance to the Th2/Treg direction.

1.2. Identification of receptors involved in the immune recognition of *C. parapsilosis*

We next examined the role of Dectin-1, TLR4 and TLR2 in the cytokine production induced by *C. parapsilosis* and *C. albicans*. Following the blocking of Dectin-1, both *C. parapsilosis*- and *C. albicans*-stimulated PBMCs showed significantly lower cytokine (TNF α , IL-1 β , IL-6, IL-10, IFN γ) production, indicating that the receptor plays an important role in the recognition of both species. On the other hand, while inhibition of TLR4 did not affect the cytokine production of PBMCs, our results show that TLR2 is involved in the induction of IL-1 β and IL-6. However, there was no difference in the cytokine production of *C. parapsilosis*- and *C. albicans*-stimulated PBMCs during receptor blocking (although we detected a greater decrease in the levels of TNF α , IL-1 β , IL-6 and

IL-10 following the inhibition of Dectin-1 in *C. parapsilosis*-stimulated cells), indicating that other receptors may be responsible for the different cytokine patterns induced by the two species.

1.3. Examination of intracellular signaling following the recognition of *C. parapsilosis*

The MAPK cascade plays a role in signal transduction following the activation of both TLRs and CLRs, but the role of individual MAP kinases in signaling is less clear. We found that inhibition of the three classical MAP kinases (p38, ERK, JNK) resulted in decreased TNF α , IL-1 β , IL-6, IL-10, IFN γ production in both *C. parapsilosis*- and *C. albicans*-stimulated PBMCs, indicating that all three enzymes are involved in the signal transduction following the recognition of *C. parapsilosis* and *C. albicans*. Furthermore, while the inhibition of p38 and ERK resulted in a greater decrease in the levels of cytokines (TNF α , IL-1 β , IL-6) in *C. parapsilosis*-stimulated cells, blocking the activity of JNK caused a more pronounced decrease in the cytokine

secretion of *C. albicans*-stimulated cells. These results suggest that there is a difference in the relative contribution of p38, ERK and JNK to the resulting cytokine responses in *C. parapsilosis*- and *C. albicans*-stimulated PBMCs.

2. Comparison of inflammasome activation induced by *C. parapsilosis* and *C. albicans*

We next examined the production of cytokines in PBMCs stimulated with live *C. parapsilosis* and *C. albicans*. We found that while both species induced similar TNF α and IL-6 production, PBMCs infected with *C. parapsilosis* produced significantly less IL-1 β compared to *C. albicans*-stimulated cells. Next we examined the potential mechanisms underlying this difference. The production of mature IL-1 β in monocytes and macrophages is dependent on caspase-1 and the activation of the inflammasome; however, while caspase-1 is constitutively active in monocytes, in macrophages it is activated by the cleavage of pro-caspase-1 during inflammasome activation. During our study, we

examined the activation of the inflammasome in PMA-treated THP-1 macrophages. We found that *C. parapsilosis* induced the secretion of IL-1 β only after a relatively long incubation and when added in a high dose, while *C. albicans* induced high levels of IL-1 β already after a few hours. Although, hyphae formation has been shown to play an important role in inflammasome activation by *C. albicans*, our results show that secretion of IL-1 β is independent of the presence of pseudohyphae following stimulation with *C. parapsilosis*. Furthermore, we found that the level of IL-1 β mRNA and pro-IL-1 β in THP-1 cells was similar following stimulation with *C. albicans* or *C. parapsilosis*, indicating that the difference in secreted IL-1 β levels originates from the differential processing of IL-1 β protein. Using different chemical inhibitors, we showed that mature IL-1 β is produced by a similar mechanism in *C. parapsilosis*- and *C. albicans*-stimulated THP-1 cells, and the process is dependent on caspase-1, caspase-8, Syk and TLR4. Using NLRP3- and ASC-deficient THP-1 macrophages, we confirmed that IL-1 β secretion in response to *C. parapsilosis* and *C. albicans* is NLRP3 inflammasome-dependent. The three

most important mechanisms involved in the activation of the NLRP3 inflammasome in macrophages is the production of ROS, release of lysosomal cathepsin B and the decrease of intracellular K^+ concentration. Our results show that IL-1 β secretion following *C. parapsilosis* and *C. albicans* stimulation is K^+ -efflux-dependent. However, inhibition of cathepsin B did not affect the production of IL-1 β , although *C. albicans* induced higher cathepsin B release in THP-1 cells compared to *C. parapsilosis*. Furthermore, inhibition of NADPH-oxidase significantly decreased the levels of both intracellular pro-IL-1 β and secreted IL-1 β in THP-1 cells, indicating the possible role of ROS in inflammasome activation. Furthermore, *C. albicans* induced significant ROS production in THP-1 cells, while *C. parapsilosis* did not induce the generation of ROS during the first four hours of infection. We also showed that the secretion of IL-1 β is dependent on phagocytosis, and that *C. albicans* cells are phagocytosed more rapidly by THP-1 macrophages than *C. parapsilosis* cells. Taken together, our results suggest that multiple mechanisms play a role in the relatively low IL-1 β production during *C. parapsilosis* infection.

Summary

We have shown that:

1. *C. parapsilosis* induces similar TNF α and IL-6, but significantly lower IL-1 β production in PBMCs compared to *C. albicans*
2. While *C. albicans* induces Th1/Th17-dominant polarization in PBMCs, *C. parapsilosis* infection is characterized by a Th2/Treg-biased response
3. Dectin-1 plays an important role in the recognition of both *C. parapsilosis* and *C. albicans*
4. p38, ERK and JNK MAP kinases play an important role in cytokine induction following the recognition of *C. parapsilosis* and *C. albicans*, but their relative contribution to the resulting cytokine response differs for the two species
5. *C. parapsilosis* induces significantly less IL-1 β secretion in THP-1 macrophages than *C. albicans*, that originates from the low level of pro-IL-1 β processing

6. The production of IL-1 β induced by *C. parapsilosis* in THP-1 macrophages is dependent on caspase-1, caspase-8, Syk, TLR4, ASC and NLRP3
7. IL-1 β production induced by *C. parapsilosis* is independent of the presence of pseudohyphae
8. *C. albicans* induces significantly more ROS production and cathepsin B release in THP-1 macrophages compared to *C. parapsilosis*
9. The production of IL-1 β induced by *C. parapsilosis* and *C. albicans* is dependent on K⁺ efflux and NADPH-oxidase, but independent of cathepsin B activity
10. *C. albicans* is phagocytosed more rapidly by THP-1 macrophages compared to *C. parapsilosis*

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