PH. D. THESIS

CHARACTERISATION OF *CANDIDA PARAPSILOSIS IN VIVO* INFECTION: THE ROLE OF THE CELL WALL *N*-MANNOSYLATION IN THE VIRULENCE

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Introduction

Candida species are opportunistic fungal pathogens causing severe infections in immunocompromised patients. *C. parapsilosis* is an important opportunistic pathogen, member of the *non-albicans* spp., which is responsible for hospital-acquired infections in neonates and pediatric patients. Although its clinical importance is increasing, our knowledge of the pathogenesis of *C. parapsilosis* is severely restricted.

Therefore, we aimed to develop *in vivo* models to characterize *C. parapsilosis* infections. The cell wall is the immediate contact point between the pathogens and a host, influencing the immune response. Here, we examined the role of cell wall integrity in the immune sensing of *C. parapsilosis in vivo*. The role of *N*-linked mannosylation was investigated using the *C. parapsilosis och1*Δ/Δ (*Cpoch1*Δ/Δ) strain, which displays a severe defect in its *N*-mannan content with elevated β-glucan and chitin levels in the cell wall and showed attenuated virulence in the mouse model of systemic candidiasis.

Methods

In vivo infection models:

- *D. melanogaster* infection with *Candida*
- intravenous infection of newborn mice via external facial vein
- intravenous infection of adult mice via lateral tail vein

Molecular methods

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

Immunological methods

- flow cytometry
- histopathology
- ELISA (enzyme-linked immunosorbent assay)

Other methods

- determination of CFU (colony-forming unit)

Results:

1. The characterization of *C. parapsilosis* infections in *D. melanogaster*

Our results demonstrate, that the Toll pathway in *Drosophila* restrains *C. parapsilosis* proliferation as the Toll pathway mutant, *MyD88*$^{-/-}$ flies succumb to injected *C. parapsilosis.* We found, that however, both the GNBP3 β-glucan receptor and the Persephone protease detection system are required for Toll pathway activation by fungal infections, solely *GNBP3*$^{-/-}$, and not *psh* mutants are susceptible to the *C. parapsilosis* infection. Also, we observed that *MyD88*$^{-/-}$ and *GNBP3*^{hades} flies showed impaired antimicrobial induction following *C. parapsilosis* stimulation. Therefore, we concluded that *C. parapsilosis* cells are detected by GNBP3 which leads to MyD88 signaling, inducing the Toll antifungal humoral defense. Finally, we found that *N*-mannosylation in the cell wall affected the virulence of *C. parapsilosis* in this insect model. The *Cpoch1*Δ/Δ challenged *MyD88*$^{-/-}$ flies showed better survival compared to the reference *C. parapsilosis* strain infected group of flies. Therefore, we

established a *Drosophila* model to analyze the differences in the virulence of the *C. parapsilosis* strains. Furthermore, we also observed that *GNBP3*^{hades} flies displayed similar sensitivity in survival and fungal burdens to *Cpoch1*Δ/Δ as the reference *C. parapsilosis* strain.

2. Investigation of the virulence of *C. parapsilosis* using a new neonatal mouse model to studying systemic candidiasis

In this study, we describe a novel and conveniently applicable intravenous neonatal mouse model to monitor systemic *C. parapsilosis* infection. Using the currently developed model, we aimed to analyze the pathogenic properties of different *C. parapsilosis* strains. We infected 2 days-old BALB/c mouse pups via the external facial vein with different doses of *C. parapsilosis* strains. Homogenous dissemination of yeast cells was found in the spleen, kidney, liver and brain of infected newborn mice. Colonization of harvested organs was also confirmed by histological examinations.

In a comprehensive study with the adult mice infection, we also presented the attenuated virulence of a *C. parapsilosis* cell wall mutant in this model. Significantly less *Cpoch1Δ/Δ* cells were recovered from the spleen, kidney and liver of newborn mice compared to the wild type strain. When investigating the cytokine response of neonatal mice to *C. parapsilosis* infection, we found elevated TNFα, KC, and IL-1β expression in all organs examined when compared to the uninfected control. Furthermore, all three measured cytokines showed a significantly elevated expression when newborn mice were infected with *Cpoch1Δ/Δ* cells compared to the wild type strain. This result further supported the inclusion of cell wall *N*-mannosylation in *C. parapsilosis* pathogenicity.

3. Detailed characterization and comparison of wild type and cell wall mutant *C. parapsilosis* infection in a mouse model

The *Cpoch1Δ/Δ* showed significantly reduced fungal loads in the kidney, liver and spleen of mice compared to those infected with the wild type *C.*

parapsilosis strain. Based on the flow cytometric data we found that the wild type *C. parapsilosis* induced the recruitment of neutrophil granulocytes, macrophages and dendritic cells to the infected organs. In comparison to the wild type *C. parapsilosis,* a significantly lower macrophage and dendritic cell count was detected in the kidney of *Cpoch1*Δ/Δ-infected mice. During the determination of the cytokine concentration in the kidney, we found that at the early stages of the infection wild type *C. parapsilosis* induced the TNFα, IL-1β, IL-6, GM-CSF and IFNγ production, which contributes to the effective elimination of the fungal cells from the host. With the decrease in *C. parapsilosis* fungal burdens, we found the secretion of the anti-inflammatory cytokines such as IL-4, IL-13, IL-27 and IL-9, indicating the development of immune tolerance to *C. parapsilosis*. Cytokine measurement from this organ showed the induction of IL-27, IL-9 and IL-23 cytokines induced by *Cpoch1Δ/Δ*.

4. Investigating the role of the Dectin-1 receptor in an *in vivo* mouse model during systemic *C. parapsilosis* infection

Intravenous infection with wild type *C. parapsilosis* cells resulted in similar fungal burdens in the kidney, liver and spleen of both wild type and Dectin-1$^{-/-}$ mice. No differences were observed in the composition of immune cells in the kidney of wild type and Dectin-1$^{-/-}$ mice after the wild-type *C. parapsilosis* stimuli. Our purpose was to evaluate whether the presence of Dectin-1 contributes to the reduced virulence of *Cpoch1*Δ/Δ *in vivo*. According to our data, absence of Dectin-1 did not influence fungal clearance in mice after infection with the less virulent *Cpoch1*Δ/Δ strain. Interestingly, in Dectin-1$^{-/-}$ mice we detected less effective neutrophil, but normal macrophage and dendritic cell infiltration in the kidney upon infection with the *Cpoch1*Δ/Δ strain. However, this phenomenon did not influence the elimination of the *N*-mannan mutant strain.

Summary

We have shown that:

1. *C. parapsilosis* cells are detected by GNBP3 which leads to MyD88 signaling, inducing the Toll antifungal humoral defense.

2. The *N*-mannosylation in the cell wall affected the virulence of *C. parapsilosis* in this model. The *Cpoch1*Δ/Δ challenged *MyD88*$^{-/-}$ flies showed better survival compared to the reference *C. parapsilosis* strain infected group of flies. Therefore, we established a *Drosophila* model to analyze the differences in the virulence of the *C. parapsilosis* strains.

3. We describe a novel and conveniently applicable intravenous neonatal mouse model to monitor systemic *C. parapsilosis* infection.

4. Using a comparative analysis, we demonstrated that the newborn mice model can be used to observe the virulence of different *C. parapsilosis* strains.

5. Compared with adult mice, newborn mice are more susceptible to infection with wild-type and cell wall mutant *C. parapsilosis* strains.

6. The wild-type *C. parapsilosis* induces the recruitment of neutrophil granulocytes, macrophages and dendritic cells and trigger the production of TNFα, IL-1β, IL-6, GM-CSF and IFNγ at the early phase of the infections, which can promote the effective elimination of fungal cells.

7. The absence of the cell wall N-mannan side chains in the *C. parapsilosis* cell wall resulted in decreased virulence and lower induction of immune cell infiltration and triggers the production of anti-inflammatory cytokines.

8. The Dectin-1 receptor is not involved in the immune sensing of wild-type *C. parapsilosis* and has no effect on the virulence properties of the *N*-mannosylation mutant strain. Therefore, our study suggests that the immune response induced by *C. parapsilosis* is independent of Dectin-1 recognition during a systemic infection.

Publications:

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