

PH.D. THESIS
**FUNCTION OF SYK/CARD9 SIGNALING IN THE
IMMUNE RECOGNITION OF *CANDIDA*
*PARAPSILOSIS***

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Introduction

Invasive fungal disease is a global problem threatening the lives of annually ~1.5 million people. The opportunistic human pathogens in the genus *Candida* are a major cause. These yeasts lead to ~700,000 new invasive infections annually with a mortality of 46–75%. Although *C. albicans* is the most clinically relevant species, the incidence of non-albicans species is rising. *C. parapsilosis* is one of the commonest fungi isolated from candidemia. Those receiving parenteral nutrition or are treated with other prosthetic devices as well as neonates are at increased risk of developing disease caused by this yeast.

Invasive fungal infections mostly develop when immunity is impaired. This makes the study of antifungal immunity relevant as it gives foundation to antifungal immune therapies. Much of anti-*Candida* immunity has been explored to date. Nevertheless, most research has focused on *C. albicans* while little is known about the interaction of the mammalian immune system with non-albicans species. As immune responses triggered by

species within even the same genus may differ, it is important to identify these processes in a species-specific manner.

Signaling through Syk and CARD9 is a crucial component of immunity against *C. albicans*. This pathway mediates signals from PRRs binding fungal PAMPs and activates effector mechanisms. However, the relevance of these proteins in relation to *C. parapsilosis* remains largely unidentified.

In this thesis, we examined the role of Syk and CARD9 in the context of *C. parapsilosis* infections. First, we compared the responses of *C. parapsilosis* challenged Syk^{-/-} or CARD9^{-/-} murine macrophages with those of control Wt(Syk) and Wt(CARD9) cells. Then we investigated the susceptibility of Syk^{-/-} or CARD9^{-/-} bone marrow chimeric mice to *C. parapsilosis* invasive infection. Most experiments were also performed using a *C. albicans* reference strain.

Methods

Fungal and host cell cultures and *in vitro* coinubation (infection): bone marrow derived macrophages (BMDMs) and peritoneal macrophages (PMs), infection of macrophages with *Candida* cells.

In vivo candidiasis model: intravenous infection of bone marrow chimeric mice with *Candida* cells.

Microscopy: bright field microscopy for the study of cell cultures and histological preparations.

Imaging flow cytometry: for monitoring the nuclear translocation of NF- κ B p65, phagocytosis of *Candida* cells by macrophages and subsequent phagosome acidification.

Immunological methods for detecting cytokines:

-Proteome Profiler

-ELISA

Determination of fungal colony forming units: for the quantitative determination of live fungal elements after

coincubation with macrophages or isolation from infected organs.

Results

Examination of nuclear translocation of NF- κ B p65 in BMDMs stimulated with *C. parapsilosis*

We implemented immune staining and subsequent imaging flow cytometry to reveal the nuclear translocation of NF- κ B p65 in BMDMs stimulated with *C. parapsilosis* strains. We detected decreased translocation in Syk^{-/-} and CARD9^{-/-} macrophages compared to Wt(Syk) and Wt(CARD9) cells. However, the absence of Syk or CARD9 did not hinder the translocation in BMDMs treated with LPS as positive control. Therefore, we concluded that NF- κ B activation in *C. parapsilosis* infected BMDMs was regulated by the Syk/CARD9 pathway.

Investigation of cytokine production of murine macrophages challenged with *C. parapsilosis* and *C. albicans*

We investigated the cytokine expression of *C. parapsilosis* stimulated BMDMs by the Proteome Profiler Mouse Cytokine Array Panel A and then by ELISA. For comparison, we also included the *C. albicans* strain in this experiment. Regardless of *Candida* strains or species, TNF α synthesis was lower in Syk^{-/-} and CARD9^{-/-} macrophages than in Wt(Syk) and Wt(CARD9) cells. While the chemokine production (KC, MIP-1 α and MIP-2) of *C. parapsilosis* treated Syk^{-/-} BMDMs was intact, *C. albicans* infected cells of this genotype yielded less chemokines than Wt(Syk) BMDMs. However, CARD9^{-/-} BMDMs were characterized by compromised chemokine production irrespective of *Candida* species. We then studied the cytokine production of PMs. In this set-up, Syk^{-/-} and CARD9^{-/-} cells failed to produce both TNF α and chemokines as effectively as Wt(Syk) and Wt(CARD9) PMs. Overall, these results suggest that the cytokine response of murine macrophages to *C. parapsilosis* is dependent on Syk and CARD9. We also confirmed the

importance of this signaling pathway in the cytokine production triggered by *C. albicans*. Additionally, we observed some species-specific differences.

Characterization of phagocytosis of *C. parapsilosis* and *C. albicans* by murine macrophages

Using imaging flow cytometry, we monitored the phagocytosis of Alexa Fluor[®] 488/GFP labelled *C. parapsilosis* and *C. albicans* cells by macrophages. Syk^{-/-} BMDMs and PMs internalized the yeast cells of both species less effectively than their Wt(Syk) counterparts. On the other hand, CARD9^{-/-} macrophages ingested both species normally. The phagocytic capacity of murine macrophages was therefore Syk-dependent but CARD9-independent.

Acidification of phagosomes containing *C. parapsilosis* in murine macrophages

We implemented dual staining of *C. parapsilosis* and *C. albicans* cells with Alexa Fluor[®] 488/GFP plus pHrodo[™] Red, a dye emitting bright fluorescence within acidified phagosomes. Macrophages were coincubated

with these yeast cells and the proportion of pHrodo™ Red⁺ macrophages as percentage of the proportion of Alexa488⁺/GFP⁺ macrophages was assessed. This value was lower in the case of *C. parapsilosis* or *C. albicans* infected Syk^{-/-} BMDMs and PMs than for Wt(Syk) cells. However, the absence of CARD9 had no effect on this feature. Therefore, we concluded that the acidification of phagosomes containing *C. parapsilosis* cells is controlled by Syk but is not affected by CARD9. Furthermore, we have confirmed that this process is also Syk-dependent in the case of *C. albicans*.

Study of elimination of *C. parapsilosis* by murine macrophages

We studied the elimination of *C. parapsilosis* by macrophages by CFU determination after coincubation. While Syk^{-/-} BMDMs killed *C. parapsilosis* less efficiently than Wt(Syk) cells, CARD9 did not affect this process. In the case of PMs, however, the killing ability was not defective in either Syk^{-/-} or CARD9^{-/-} cells.

Susceptibility of bone marrow chimeric mice to systemic candidiasis

We retrieved CFUs from selected organs (spleen, kidneys, liver, brain) and the blood of animals intravenously injected with *C. parapsilosis* on days 2, 5, 7 and 30 post-infection. As *C. albicans* infected Syk^{-/-} and CARD9^{-/-} chimeras did not survive until the later time points, these and their Wt controls were assessed for fungal burden on day 2 post-infection only. Colonization by *C. albicans* in organs of Syk^{-/-} and CARD9^{-/-} chimeras was at least 1 order of magnitude higher (brains, kidneys) than in those of Wt chimeras. Excessive growth of this yeast in the kidneys of Syk^{-/-} and CARD9^{-/-} chimeras was confirmed by histological observations and we also detected necrotic areas and signs of inflammation. The gross morphology of these kidneys appeared pathological. The fungal burden in *C. parapsilosis* infected Syk^{-/-} or CARD9^{-/-} chimeras surpassed that of respective Wt ones. The extent of this difference was multiple orders of magnitude on day 30 post-infection. At this time point, expansive growth of *C. parapsilosis* could be visualized in the kidneys of the mutant mice. To conclude, we

confirmed the profound role of Syk and CARD9 in the resistance against *C. albicans* and proved that they are also involved in the systemic resistance against *C. parapsilosis*.

Summary

1. NF- κ B activation triggered by *C. parapsilosis* is Syk- and CARD9-dependent in BMDMs.
2. The cytokine production of *C. parapsilosis* infected BMDMs and PMs is (partially) Syk- and CARD9-dependent.
3. The phagocytosis of *C. parapsilosis* and *C. albicans* cells by BMDMs and PMs is Syk-dependent but CARD9-independent.
4. Acidification of phagosomes containing *C. parapsilosis* is Syk-dependent but CARD9-independent in BMDMs and PMs.
5. Elimination of *C. parapsilosis* by BMDMs is Syk-dependent but CARD9-independent.

6. Resistance to systemic infection by *C. parapsilosis* is dependent on Syk and CARD9 expression in cells of hematopoietic origin in the mouse model.

List of publications

Publications forming the basis of the doctoral procedure:

Tóth A*, **Zajta E***, Csonka K, Vágvölgyi Cs, Netea MG és Gácsér A (2017): Specific pathways mediating inflammasome activation by *Candida parapsilosis*. – Scientific Reports 7: 43129. doi: <http://doi.org/10.1038/srep43129>, IF: 4,122

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Csonka K, Vadovics M, Marton A, Vágvölgyi Cs, **Zajta E**, Tóth A, Tóth R, Vizler Cs, Tiszlavicz L, Mora-Montes HM, Gácsér A (2017): Investigation of OCH1 in the virulence of *Candida parapsilosis* using a new neonatal mouse model. – Frontiers in Microbiology 8: 1197. doi: <https://doi.org/10.3389/fmicb.2017.01197>, IF: 4,019

Other publications related to the topic of the dissertation:

Csepregi JZ, Orosz A, **Zajta E**, Kása K, Németh T, Simon E, Fodor Sz, Csonka K, Barátki BL, Kövesdi D, He Y-W, Gácsér A és Mócsai A (2018): Myeloid-specific deletion of Mcl-1 yields severely neutropenic mice that survive and breed in homozygous form. – *The Journal of Immunology* 201 (12): 3793-3803. doi: <https://doi.org/10.4049/jimmunol.1701803>,
IF: 4,718

Other publications:

Zajta E, Varga T, Kovács GM és Dima B (2019): New insights on *Hygrophorus penarioides* and *H. penarius* (Hygrophoraceae, Agaricales) from Hungary. – *Phytotaxa* 392 (2):127-139. doi: <http://dx.doi.org/10.11646/phytotaxa.392.2.2>,
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Knapp DG, Kovács GM, **Zajta E**, Groenewald JZ és Crous PW (2015): Dark septate endophytic pleosporalean genera from semiarid areas. – *Persoonia* 35: 87–100. doi: <http://dx.doi.org/10.3767/003158515X687669>, *IF*: 5,725

Zajta E (2012): Distribution of *Hygrophorus* species in Hungary. – *Mikológiai Közlemények, Clusiana* 51 (2): 223-240.

<https://www.researchgate.net/publication/314086246>,

IF: 0

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