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Invasive fungal disease is a global concern threatening the lives of annually ~1,5 million people. The opportunistic human pathogens within the genus *Candida* are a major cause. These yeasts lead to ~400.000 new invasive infections a year with a mortality of 46–75%. Although *C. albicans* remains the most clinically relevant species the incidence of non-albicans species (e.g. *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C krusei*) are on the rise.

C. parapsilosis is one of the most frequent fungus isolated from candidemia. Due to its biofilm formation patients receiving parenteral nutrition or are treated with other prosthetic devices are at increased risk of dveloping disease. Furthermore, 1/3 of all candidiases affecting neonates are caused by this species.

Invasive fungal infections manifest predominantly in those with impaired immunity. Thus, the study of antifungal immunity is relevant as it is the foundation of immune therapies complementing treatment with antifungal drugs. Much of anti-*Candida* immunity has been explored to date. However, most scientific interest has focused on *C. albicans* while little is known about the interaction of our immune system with non-albicans species. As immune responses triggered by species within even the same genus it is important to identify these processes in a species-specific manner.

Signaling through Syk and CARD9 are crucial is crucial component of immunity against *C. albicans*. This pathway mediates signals from PRRs binding fungal PAMPs and activates effector mechanisms. The involvement of either Syk or CARD9 in the immune recognition of C. *tropicalis* and *C. glabrata* have also been established recently. However, the relevance of these proteins in relation to *C. parapsilosis* remain largely unidentified.

Therefore, the aim of this project was to examine 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 (BMDMs and on multiple occasions PMs) with 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.

Our results are summarized below:

1. **Nuclear translocation of NF-κB p65 upon infection with *C. parapsilosis* is dependent on Syk and CARD9**

Firstly, we implemented immune staining and subsequent imaging flow cytometry to reveal the nuclear translocation of NF-κB 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. Thus NF-κB activation in *C. parapsilosis* infected BMDMs is regulated by the Syk/CARD9 pathway.

2. Syk and CARD9 regulate cytokine production of *C. parapsilosis* challenged murine macrophages

We first 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, TNFα synthesis proved to be lower in $Syk^{-/-}$ and $CARD9^{-/-}$ macrophages than in Wt(Syk) and Wt(CARD9) cells. While chemokine production (KC, MIP-1α 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 case, $Syk^{-/-}$ and $CARD9^{-/-}$ cells failed to produce both TNFα and chemokines as sufficiently as Wt(Syk) and Wt(CARD9) PMs. All in all, 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.

3. Phagocytosis of *C. parapsilosis* and *C. albicans* by murine macrophages is Syk-dependent but CARD9-independent

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 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.

4. Syk but not CARD9 affects the acidification of *C. parapsilosis* or *C. albicans* containing phagosomes in murine macrophages

In this experiment we implemented dual staining of *C. parapsilosis* and *C. albicans* cells with Alexa Fluor® 488/GFP plus pHrodo™ Red, a stain emitting bright fluorescence within acidified phagosomes. Macrophages were coincubated with these yeast cells and then we assessed the proportion of pHrodo™ Red^+ macrophages as percentage of the proportion of $Alexa488^+/GFP^+$ macrophages. This value was lower for *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* or *C. albicans* cells is controlled by Syk but is not affected by CARD9.

5. Killing of *C. parapsilosis* by BMDMs depends on Syk but not CARD9

We then 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, we did not detect defective killing ability of either $Syk^{-/-}$ or $CARD9^{-/-}$ cells.

6. $Syk^{-/-}$ and $CARD9^{-/-}$ bone marrow chimeric mice are susceptible to invasive *C. parapsilosis* and *C. albicans* infections

Having established the *in vitro* participation of Syk/CARD9 signaling in the immunological recognition of *C. parapsilosis*, we sought to determine if it also contributes to resistance to invasive infection caused by this species in a mouse model. Therefore, we retrieved CFUs from organs (spleen, kidneys, liver, brain) and 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. Colonization in organs from $Syk^{-/-}$ and $CARD9^{-/-}$ chimeras was excessively higher than in those of Wt chimeras. Overgrowth of *C. albicans* 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 was pathological. The fungal burden in C. *parapsilosis* infected $Syk^{-/-}$ or $CARD9^{-/-}$ chimeras surpassed that of respective Wt ones. However, the difference reached an extent of multiple orders of magnitude only on day 30 post-infection. At this time point, extensive 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*.

Significance:

The results of the project contributes to our knowledge on the immunological background of *C. parapsilosis* infections. We have demonstrated that Syk and CARD9 take part in the immunological defense against *C. parapsilosis*. We have also confirmed that they are indispensable elements of anti-*C. albicans* immunity. To our knowledge, this was the first example to utilize $Syk^{-/-}$ and $CARD9^{-/-}$ bone marrow chimeras to examine immune responses triggered by *C. albicans* and *C. parapsilosis*. Moreover, some of our results suggest that Syk and CARD9 might regulate responses to these species differently. Combined with literature data, our results point towards the potential of future research on Dectin-2, Dectin-3, Mincle and CR3 in relation to *C. parapsilosis* and on the Syk/CARD9 pathway as a target for anti-*Candida* immune therapy.