Characterisation of host-pathogen interaction during *Candida* infections

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Candida species are known as members of the normal human flora. However under certain circumstances these commensalist yeasts are able to transform themselves into opportunistic pathogens. *C. parapsilosis* is considered to be the second or third most common *Candida* species causing candidiasis after *C. albicans*. The response of the mammalian immune system given to the *C. albicans* is well-exemined, and based on our pervious work it is clear, that some *Candida* derived lipases play role as virulence factor. On the other hand little is known on the interaction between the immune system and other *Candida* species, like *C. parapsilosis*.

We previously showed that *C. parapsilosis* lipase knockout (LIP-) mutants were significantly deficient in their capacity to produce biofilm, to grow in lipid rich medium, and to survive in macrophages. In an attempt to understand this reduced virulence phenotype, we developed an *in vitro* model system using murine macrophage -like cell line J774.2. We examined the gene expression in J774.2 macrophages infected with wild type (WT) *C. parapsilosis* and LIP- cells. The complex response of murine macrophages to infection with *C. parapsilosis* was investigated at the level of gene expression using Agilent mouse microarray. 155 and 512 genes were identified as being differentially regulated at 3 and 8 hours post infection, respectively. Most of the upregulated genes encoded molecules that were involved in immune response and inflammation, transcription, signalling, apoptosis, cell cycle, electron transport and cell adhesion. Of particular interest were the upregulation of proinflammatory cytokines, typical of the classically activated macrophages such as TNF, IL-1 and IL-15, and also the upregulation of TNF-receptor family members such as *TNFRSF9* 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*.

Flow cytometry analysis proved, that elevated mRNA level of TNFRSF9 correlated to the elevated amount of protein on the surface of J774.2 macrophage cells upon *C. parapsilosis* WT infection. Similar pattern of TNFRSF9 (CD137) regulation could be observed in cells from whole human blood upon *C. parapsilosis* WT infection. To further examine the host pathogen interactions we established a human monocyte (THP-1) cell line infection model. THP-1 cells were infected with eight different *Candida* strains from the parapsilosis *sensu lato* group and subjected for complete transcriptome analysis. In order to profile the transcriptome changes with the best possible resolution, we utilized the robustness and accuracy of the Next-generation Sequencing (NGS) RNA-seq technology.

To further develop our infection models we established an in vitro system using primary human mononuclear blood cells. Monocyte-derived immature and mature dendritic cells (iDCs, mDCs) as well as macrophages (MΦ) co-cultured with live WT or LIP- *C. parapsilosis* strains were studied to determine the host response. We determined that all cell types efficiently phagocytosed and killed *C. parapsilosis*, furthermore our results show that the phagocytic and fungicidal activities of both iDCs and mDCs are more potent for LIP-

compared to WT yeast cells. Notably, MΦ showed eleveted fungal killing activity to LIP- cells but no increased phagocitic capacity was detectable. In addition, the LIP- *C. parapsilosis* cells induce higher gene expression and protein secretion of proinflammatory cytokines and chemokines in all cell types relative to the effect of co-culture with WT yeast cells. Our results show that both DCs and MΦ are activated by exposure to *C. parapsilosis*, as shown by increased phagocytosis, killing and proinflammatory protein secretion. Moreover, these data strongly suggest that *C. parapsilosis* derived lipase has a protective role during yeast:phagocyte interactions, since lipase production in wt yeast cells decreased the phagocytic capacity (in case of DCs) and killing efficiency of host cells and downregulated the expression of host effector molecules.

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