PH.D. THESIS

INVESTIGATION OF IMMUNOLOGICAL FUNCTIONS RESULTING FROM THE INTERACTION BETWEEN HUMAN KERATINOCYTES AND *CANDIDA* SPECIES

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Introduction

Invasive fungal diseases are a growing problem today, affecting millions of people worldwide. Opportunistic human pathogenic *Candida* species play a prominent role in the development of these pathologies. Mortality rates from systemic diseases caused by these species can be as high as 75%. *C. albicans* is one of the best known and most widely used species within the genus *Candida*, but other non-*albicans* species also contribute to the pathogenesis of diseases. Species *C. parapsilosis* is known as a common commensal of human skin surfaces and often isolated from the skin. At greatest risk of invasive candidiasis are newborns, the elderly and immunosuppressed individuals.

Most of the studies in recent years have focused on the immune response caused by *C. albicans*. Hence, little is known about non-*albicans* species such as *C. parapsilosis* with respect to the human immune response. The elucidation of the antifungal immune response is an important milestone in the search for effective antifungal therapies. The immune response can show significant

variation even within species of the same genus, and thus there is a need to investigate at the species level.

The human skin is the primary immunological line for our body to fight against many pathogenic microbes, viruses or fungi. The keratinocyte cells found in the epidermis can detect PAMPs through PRRs and induce effector functions through signalling. The literature on effector functions and cell-pathogen interactions is scarce or superficially discussed, especially for non-*albicans* species.

We have investigated in detail the interactions between human skin epithelial cells (keratinocytes) and commensal *C. parapsilosis* and pathogenic *C. albicans* species. We investigated the extent to which keratinocytes are damaged by infection with these two fungal species and their cytokine/chemokine secretion profile. The ability of the fungi to adhere to the surface of epithelial cells. The internalisation capacity of keratinocyte cells. We also used RNA extraction and sequencing to gain more insight into the fungal and cellular changes following infection. Furthermore, the metabolomic changes induced by fungal

infection on the part of the cells are discussed. In addition, all these studies (except RNA sequencing) were performed in the context of pre-incubating keratinocyte cells with the commensal fungus and subsequently infecting them with pathogenic fungi, thus providing information on the primed immunity of the cells.

Methods

Maintenance of keratinocyte cell lines and *Candida* strains for cell-fungus interaction infections: Freezing and maintenance of HaCaT and HPV-KER cells, inoculate of *Candida* strains, infection of keratinocyte cells with *Candida* fungi.

LDH assay: measurement of lactate dehydrogenase release during keratinocyte cell death following *Candida* infection.

Analysis of cytokines/chemokines by immunological method: use of enzyme-linked immunosorbent assay (ELISA).

CFU determination: quantification of fungi after infection of keratinocyte cells with *Candida* species.

Imaging flow cytometry: measurement of the association of GFP-labelled *Candida* with epithelial cells and quantification of pHRodo-labelled *Candida* fungi phagocytosed by keratinocyte cells.

RNA sequencing: RNA sequencing after RNA extraction from keratinocyte cells and *Candida* fungi to determine gene expression changes. Identification of upregulated genes and *ECE1* expression on the fungal side; genes responsible for cytokine/chemokine production, antimicrobial peptide production and additional cellular and biological functions on the cellular side.

Detection of metabolomic alterations in keratinocyte cells: exploration and identification of metabolites in epithelial cells after *Candida* infection using GC-MS technique.

Results

Degree of epithelial cell damage after *Candida* infection and under primed conditions

After infection of keratinocyte cells with *Candida* fungus, the amount of LDH released from epithelial cells was measured, indicating the degree of damage to these cells. Our results show that the damage to keratinocyte cells was significantly increased by *C. albicans* strain SC5314 compared to the control group and the HPV-KER keratinocytes were the most susceptible. This trend was also observed at 24 hours and 12 hours post-infection. At 48 hours post-fungal infection, HaCaT cells were shown to be significantly more sensitive to *C. albicans* strain WO-1 in addition to strain SC5314. Under primed conditions, only HPV-KER cells showed a significant reduction in damage in the case of *C. albicans* strain SC5314 at low infection dose. Different infection doses of the commensal fungus used in priming have an effect on the extent of damage caused by *C. albicans* strain SC5314.

Cytokine/chemokine production by keratinocytes in response to *Candida* infection and in primed immunity cases

The production of cytokine/chemokine by HaCaT and HPV-KER cells following *Candida* infection was determined by ELISA. We found that IL-6 cytokine and IL-8 chemokine production were significantly increased in both cell lines upon *C. albicans* infection compared to the control group. In the primed condition, no elevated production of either IL-6 or IL-8 was systematically detected, but in the vast majority of cases, pre-incubation with live *C. parapsilosis* CLIB214 strain resulted in significantly elevated IL-6 and IL-8 secretion upon *C. albicans* infection in predominantly HPV-KER cells.

Candida cell adhesion to keratinocytes under non-primed and primed conditions

The extent of adhesion of *Candida* fungal cells to keratinocytes was determined by CFU counting. We spread the fungi, gained from the supernatant and associated with cells, on YPD medium after infection. It was concluded that the adhesion ability of *C. albicans*

strains is significantly higher compared to *C. parapsilosis* strains. This ability can be attributed to the true hyphal formation property of *C. albicans* strains and the higher expression of their adhesins. *C. parapsilosis* strains, in the absence of the aforementioned properties, do not show a significantly higher adhesion ability after a longer infection period than was observed for the shorter incubation. Under primed conditions, no detectable change in the ability to associate was observed compared to the non-primed condition.

Phagocytic ability of keratinocyte cells against *Candida* fungus in non-primed and primed state

The ability of epithelial cells to phagocytose *Candida* fungal cells was assessed by flow cytometry after staining the fungus with pHRodo stain. This dye emitted an elevated signal upon internalisation into the cells under the prevailing acidic conditions. As keratinocytes are not typically phagocytic cells, phagocytosis was only a few percent of the total population measured, but *C. albicans* strains were more able to be phagocytosed by epithelial cells compared to *C. parapsilosis* strains. This may be

attributed to the fact that *C. albicans* strains have a more pronounced association with cells, which is an important condition for the presence of phagocytosis. In the primed case, after infection with *C. albicans* strain SC5314 following pre-incubation, the internalization efficiency of cells was significantly reduced compared to the non-primed state, with no change for *C. albicans* strain WO-1. Hence, the primed condition did not show more efficient phagocytosis on the keratinocyte cell side against *C. albicans* strain SC5314.

Gene expression changes following cell-fungal interaction and antimicrobial peptide response of keratinocytes

RNA was extracted from *Candida* fungal cells and keratinocyte cells and RNA sequencing was used to obtain more detailed information on the changes caused by cell-fungal interaction. The *ECE1* gene is a key virulence factor in *C. albicans* strains and we explored the expression of this gene. We conclude that the interaction of the fungus with keratinocyte cells does not increase significantly the expression of this gene, but shows significantly high expression in its basal state, especially

in the case of *C. albicans* strain SC5314. In our experiments, we could not detect antimicrobial peptide production in either HaCaT or HPV-KER cell lines upon *Candida* fungal infection. Furthermore, during an infection, the fungal side showed significantly increased expression of genes that help to increase fungal adhesion, reduce free radical stress and increase regulation of fungal metabolism and transporter activity. On the other hand, keratinocyte cells showed significant upregulation of processes such as signalling, cytokine/chemokine production and reduction of ROS levels.

Metabolomic changes in keratinocytes after non-primed and primed *Candida* infection

Following infection of HaCaT and HPV-KER epithelial cells with *Candida* fungi, the cells were disrupted and their contents analysed by GC-MS to reveal their metabolomic changes in response to infection. In general, infection with *C. albicans* strain SC5314 induced significant metabolic changes, often elevated levels and less frequently lower production, in both non-primed and primed conditions compared to the uninfected control

group. Although in many cases changes were also observed in *C. albicans* WO-1 strain, the extent was less pronounced compared to *C. albicans* SC5314 strain. The production of metabolites responsible for enhancing epithelial barrier functions, promoting cell differentiation, collagen synthesis, inflammatory processes and antioxidant properties showed a significant change compared to the control group.

Summary

1. Damage to keratinocyte cells after 12 and 24 h of *Candida* infection is significantly increased in *C. albicans* strains and greater cell death is observed in HPV-KER cells. After 48 h of infection, HaCaT cells become more sensitive. In the primed state, significantly lower damage is measured in HPV-KER cells to low infection dose of *C. albicans* SC5314 strain.
2. Keratinocytes show significantly higher production of IL-6 cytokine and IL-8 chemokine when infected with *C. albicans* strains (mainly SC5314 strain). In the

primed condition, significantly elevated cytokine/chemokine production is observed under certain pre-incubation conditions, especially in HPV-KER cells compared to non-primed conditions.

3. *C. albicans* strains are able to adhere to keratinocytes to a significantly greater extent than *C. parapsilosis* strains. Under primed conditions, the efficiency of adhesion is unchanged compared to non-primed conditions.
4. The phagocytic ability of epithelial cells is low, but they are more efficient at internalising *C. albicans* strains. During primed infection with *C. albicans* strain SC5314, the ability of epithelial cells to internalise was significantly reduced compared to that measured in the non-primed condition.
5. The *ECE1* gene expression of *C. albicans* strains does not change when interacting with keratinocyte cells, showing high levels at baseline and higher expression in SC5314 strain compared to WO-1 strain. HaCaT and HPV-KER cells did not show antimicrobial peptide production following *Candida* infection. During cell-fungal interaction, fungal side processes

were significantly increased: adhesion, reduction of free radical stress, increased metabolism and transporter activity; cell side processes: signalling, cytokine/chemokine production and ROS reduction.

6. Epithelial cell metabolism shows greater changes to infection with *C. albicans* strains, under both primed and non-primed conditions. Significantly elevated metabolite-related functions compared to the control group: cell differentiation, collagen synthesis, inflammatory processes, antioxidant property, barrier role.

List of publications

Publications related to the topic of the dissertation:

Ádám Novák, Erik Zajta, Máté Csikós, Emese Halmos, Márton Horváth, Orsolya Tildy, András Szekeres, Gergő Svorenj, Nikolett Gémes, Gábor Szebeni, Renáta Tóth, Attila Gácser* (2025): Comprehensive analysis of human keratinocyte interactions with *Candida albicans* and *Candida parapsilosis*. - *Virulence*. doi: https://doi.org/10.1080/21505594.2025.2532815. IF: 5.40

Other publications related to the topic of the dissertation:

Erik Zajta, Katalin Csonka, Adél Tóth, László Tiszlavicz, Tamás Németh, Anita Orosz, **Ádám Novák**, Máté Csikós, Csaba Vágvölgyi, Attila Mócsai, Attila Gácser (2021): Signaling through Syk or CARD9 Mediates Species-Specific Anti-*Candida* Protection in Bone Marrow Chimeric Mice. - *mBio* 12: 4 Paper: e01608-21, 18 p. doi: https://doi.org/10.1128/mbio.01608-21. IF: 5.85

Other publications:

M.J. Hernández-Chávez, D.M. Clavijo-Giraldo, **Á. Novák**, N.E. Lozoya-Pérez, J.A. Martínez-Álvarez, R. Salinas-Marín, N.V. Hernández, I. Martínez-Duncker, A. Gácser, H.M. Mora-Montes (2019): Role of Protein Mannosylation in the *Candida tropicalis*-Host Interaction. - *Frontiers in microbiology* 10 Paper: 2743, 18 p. doi: https://doi.org/10.3389/fmicb.2019.02743. IF: 5.08

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