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Receptor expression and epithelial damage response to pathogenic Mucorales species characterized in an alveolar bilayer model

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Background: *Mucorales* are an important cause of life-threatening systemic infections in immunocompromised patients, but their interplay with host immunity is scarcely assessed. The predominant site of infection in haematological patients is the lung. This study sought to employ an alveolar trans-well bilayer model to characterize invasiveness and host response in the pulmonary context.

Material/methods: 5 x 10^5 human pulmonary artery endothelial cells and 5 x 10^5 epithelial cells (A549) were seeded on the lower and upper side of a trans-well membrane with 3 µm pores, respectively. The upper compartment was inoculated with 2.5 x 10^5 vital resting conidia of *R. arrhizus*, *R. pusillus*, or *C. bertholletiae*. Simultaneously, 2.5 x 10^5 monocyte-derived dendritic cells (moDCs) or plain EGM-2 medium were added. 24 h after infection, 2.5 x 10^5 neutrophils or plain medium were

added to the lower compartment. 6 h later, RNA samples (A549 ± moDCs) and culture supernatants were harvested. Transcriptional levels of pattern recognition receptors, cytokine genes, and damage response genes were studied by RT-qPCR. Culture supernatants from the upper compartment were analysed using a magnetic bead multiplex cytokine assay. *Mucorales* DNA was quantified by an 18S ribosomal DNA qPCR assay.

Results: A constant increase of *Mucorales* DNA was detected in the lower compartment over time indicating fungal penetration of the epithelial barrier. Strongly elevated IL1β (161-335-fold), IL12-p70 (68-289-fold), IL8 (13-23-fold), TNFα (54-89-fold), IL6 (13-24-fold), and G-CSF (10-21-fold) secretion from moDCs was observed in comparison to uninfected samples, with the strongest elevations being caused by *R. pusillus*. MIP1α and GM-CSF release was slightly induced upon *Mucorales* infection (2.9-13-fold). While CC5 (RANTES) and MCP1 secretion from moDCs was stimulated by the fungi, the release of these cytokines from A549 cells was lowered in presence of *Mucorales*, whereas transcriptional levels in A549 cells were unaltered. Assessing receptor expression, slight transcriptional induction of ICAM-1 on A549 cells (2.1-2.9-fold) and strong upregulation on moDCs (5.9-8.4-fold) was noted. Compared to uninfected controls, the transcription of TLR4 (relative expression: 0.16-0.29) and CLEC7A/Dectin-1 (relative expression: 0.02-0.06) was significantly lowered in *Mucorales*-infected samples. Similarly, reduced transcription of CLEC6A/Dectin-2 was observed in response to the fungi (relative expression: 0.04-0.23).

Conclusions: In this study, a previously described alveolar bilayer model was adapted for assessment of *Mucorales*-host interaction in the pulmonary context. While a robust pyrogenic response of moDCs was induced by the studied *Mucorales* species, reduced pro-inflammatory epithelial cytokine secretion appears to be attributable to significant epithelial damage caused by the fungi. As C-type lectin receptors are frequently downregulated upon ligand binding, the markedly lowered CLEC6A and CLEC7A expression provides further evidence for a role of Dectin-1 in the recognition of *Mucorales*, but may also be indicative of Dectin-2 involvement in host response to *Mucorales*.