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Alternative mechanisms of protection of Aspergillus fumigatus conidia against Reactive Oxygen Species.

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Objective: *Aspergillus fumigatus* is the main causative agent of invasive aspergillosis. Upon inhalation conidia pass the mucosal barrier and end up in the lung cavities, the alveoli. Surveilling lung macrophages and type II lung epithelial cells produce amongst others intra-and extracellular reactive oxygen and nitrogen species (ROS/RNS) to prevent fungal growth. ROS consists of superoxides, hydrogen peroxide and hydroxyl radicals. Fungal components involved in protection against these compounds are superoxide dismutase (O_2^-), catalases (H_2O_2), L-DOPA melanine (OH^-) due to the existence of different oxidative states of the quinone residues. *A. fumigatus* produces DHN-melanine present in spores whereas pyomelanine is produced by hyphae. Both types of melanines were suggested to play a role in protection against ROS but the evidence of their protective role is very thin. In addition, how these compounds protect at a chemical level has not been investigated. Our objective is to unravel the protective mechanisms of fungal components against ROS and RNS. We expect that surface exposed and/or secreted fungal component are involved in protection. In this study we have investigated the sensitivity of spores of WT *A. fumigatus* strain and mutants with disruption in genes involved in production of DHN-melanine (PksP), pyomelanine (HppD) or the rodlets (Roda or RodB) for hydrogen peroxide and hydroxyl radicals.

Methods: Spores or fungal cells were incubated with different concentrations of H_2O_2 or hydroxyl radicals for several time intervals and survival was determined after dilution plating on PDA agar media and counting CFU. Spores were plated on minimal medium plates containing different concentrations of H_2O_2 and zones of inhibition were determined. L-tyrosine was added to culture media to determine the role of pyomelanine.

Results: Comparison of sensitivity for hydrogen peroxide or hydroxyl radicals of the WT *A. fumigatus* strain with any of the mutants in different types of assays did not reveal any change in sensitivity for the tested ROS. In contrast, *C. neoformans* grown in the presence of L-DOPA and producing L-DOPA melanine was found to be protected against hydroxyl radicals.

Conclusion: The rodlet and the melanin layer present at the outside of the conidia of *A. fumigatus* does not play a role in the protection against hydrogen peroxide and hydroxyl radicals, this in contrast to the role of L-DOPA-malanine in the cell wall of *C. neoformans*.

The resistance of *fumigatus* spores to hydroxyl radicals is very striking and we propose that spores of *A. fumigatus* have a novel protective mechanism against this component and a possible role of secondary metabolites is discussed.

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Recognition of DHN-melanin by the C-type lectin, MelLec, is required for protective immunity to Aspergillus fumigatus

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Objective: C-type lectin receptors (CLRs) recognize microbial invasion, induce protective immune responses and play a central role in antifungal immunity. Most CLRs recognize conserved carbohydrate structures of the fungal cell wall. Recognition of other fungal virulence factors, including melanin, remain largely undefined. Here, we identify a CLR, Melanin sensing C-type Lectin receptor (MelLec), involved in the recognition of fungal DHN-melanin and describe its role in antifungal immunity to *Aspergillus fumigatus*.

Methods: Soluble protein chimeras consisting of the C-type lectin-like domain of murine or human MelLec fused to the Fc-region of human IgG1 (Fc-MelLec) were generated as probes to screen for recognition of fungi. To characterize the expression of MelLec in mice, we screened various tissues by PCR and generated antibodies against murine MelLec. To explore the role of MelLec *in vivo*, we generated MelLec-deficient mice and performed intravenous infection models with *A. fumigatus* conidia. In humans, associations between susceptibility to the risk of invasive aspergillosis and a common missense single nucleotide polymorphism (SNP) in MelLec were studied in a cohort of stem-cell transplant patients.

Results: Using Fc-MelLec, we were able to show that MelLec recognizes melanin of *A. fumigatus* conidia and other DHN-melanised fungi, but not *A. fumigatus* mutant conidia deficient in DHN-melanin or melanins produced via other pathways. In mice, MelLec RNA was detected in various tissues, with highest expression in the lung. Surprisingly, unlike most other CLRs, presence of MelLec could not be detected on myeloid cell populations by flow cytometry. Instead, ubiquitous expression of MelLec was detected on CD31^{+} endothelial cells in the lung and various other tissues. To understand the role of MelLec in antifungal immunity, we performed *in vivo* infection models. MelLec-deficient mice were significantly more susceptible to systemic infection with *A. fumigatus* conidia compared to wildtype counterparts, and this was associated with significantly increased tissue fungal burdens and inflammatory cytokines. In humans, we could demonstrate that human MelLec also recognizes DHN-melanised *A. fumigatus* conidia. To understand the role of MelLec in human fungal infections, we determined the presence of a SNP in MelLec in stem-cell transplant patients. We found a highly significant association between this SNP and the risk of invasive aspergillosis, with increased risk of disease when the variant was carried by the donor, but not when carried by the recipient. This suggests that in humans, the protective functions of MelLec are primarily mediated by myeloid cells and we could observe that myeloid cells of individuals carrying this SNP produced significantly less pro-inflammatory cytokines following *in vitro* stimulation with *A. fumigatus* conidia.

Conclusion: We have shown that fungal DHN-melanin is sensed through MelLec, a CLR that plays a crucial role in controlling systemic *A. fumigatus* infection in both mice and humans. Our data indicates that identifying genetic variants of MelLec in donors could help reduce the incidence of invasive aspergillosis in stem-cell transplant recipients. Furthermore, our data suggests that MelLec could also play an important role in immunity to other DHN-melanised fungi, such as those that cause phaeohyphomycosis, mycetoma, and chromoblastomycosis.

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Using Whole-Genome Sequencing to Identify Recent Introductions and Track the Spread of Candida auris in the United States

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Objective: *Candida auris* has emerged as a multidrug-resistant yeast in healthcare settings throughout the world and recently in the United States. The fungus can cause invasive infections associated with high mortality, colonize patients and contacts, and persist on environmental surfaces for months. Whole-genome sequencing (WGS) and phylogenetic analysis of patient isolates from five countries revealed four distinct populations characterized by geography (South Asian, South African, East Asian, and South American clades). We characterized the molecular epidemiology of *C. auris* in the United States to inform understanding of transmission patterns and outbreak response.

Methods: We performed WGS on >300 *C. auris* isolates obtained from clinical and screening cases identified within ten U.S. states: California, Connecticut, Florida, Illinois, Indiana, Maryland, Massachusetts, New Jersey, New York, and Oklahoma. Pairwise SNP comparisons of multiple isolates from the same patient and between patients within a facility with active transmission were examined. We sequenced all isolates using Illumina technology (yielding 50–200X coverage) and used a PacBio *C. auris* reference for comparison. Using the NASP pipeline, we mapped reads using BWA and SAMtools to identify SNPs. We performed principal component analysis using Hamming distance and the adegenet R package.

Results: We found all four known *C. auris* populations represented in the United States. Patient isolates from California, Connecticut, Massachusetts, Maryland, New Jersey, New York, and Oklahoma clustered with isolates from India and Pakistan (South Asian clade). Similar results were found for other states. Patient isolates from Florida, Illinois, and Massachusetts clustered with those from Venezuela and Colombia (South American clade). The patient isolate from Indiana resembled those from South Africa, and two people from New York had isolates that resembled those from Japan and South Korea and clustered to the East Asian clade. Five patients had been hospitalized in a country with known *C. auris* transmission before *C. auris* identification. Isolates from each of these patients were highly related to isolates from the country of previous hospitalization. Little intra-host genetic diversity of *C. auris* was observed within each patient (median SNPs 3, range 0–21). Patient isolates within each facility with active transmission had similarly limited diversity (median SNPs 3, range 0–12).

Conclusion: WGS has helped public health officials define, track, and monitor *C. auris* outbreaks in the United States. These findings suggest multiple recent introductions and support heightened monitoring for *C. auris* in patients recently hospitalized in a country with known transmission. Minimal diversity observed within and between patients in a facility further suggests recent transmission and need for infection control measures.

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Magnetic resonance imaging of infected mouse brains allows non-invasive screening of differences in the virulence of clinical Cryptococcus neoformans strains

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Objective: Variability in the virulence of *Cryptococcus neoformans* strains can influence the outcome of cryptococcal meningoencephalitis. The standard method to assess the *in vivo* virulence of fungal strains uses survival studies in animal models. Unfortunately, these studies only give very limited information and no insights in disease manifestation and progression during the infection. The aim of our study was to validate non-invasive imaging methods for their potential to assess virulence of cryptococcal strains *in vivo*. To this end, we aimed at characterizing potential differences in the course of the disease caused by clinical *C. neoformans* strains in a murine model by use of preclinical magnetic resonance imaging (MRI) and computed tomography (CT).

Methods: 7 clinical *Cryptococcus neoformans* strains (AD2-82a, AD4-47a, AD5-53a, AD2-04a, AD1-83a, AD1-07a, AD4-76a) and a reference strain (H99) were injected intravenously (50 000 cells) in female Balb/C mice (n = 3 per strain). Subsequently, infected animals were scanned every 2–4 days using a 9.4T preclinical MRI system (Bruker Biospin, Ettlingen, Germany). The acquired 3D MRI scans were converted to a 3D model to allow quantification of different disease- and morphology-related readouts. In addition, weekly CT of the mouse lungs was performed (Skyscan 1076, Bruker micro-CT). Animals were followed up during the course of the disease and sacrificed when reaching humane endpoints.

Results: Humane endpoints were reached between 7 (H99, AD2-82a, AD4-47a) and 18 days (AD4-76a) after infection. For all animals, disease was characterized by an increase in the total brain volume and ventricle volume, which could indicate edema of the brain tissue. Furthermore, the phenotypical presentation of disease in infected animals was different between the clinical strains (Fig. 1). Lesions in the brain parenchyma were visible on the MR images as hyperintense spots distributed throughout the brain, whereby the more virulent strains caused larger numbers of lesions. In contrast, the less virulent strains were generally associated with an accumulation of fluid around parts of the meninges. For most strains, hyperintense regions on the outer edges of the brain and meninges could also be observed. Lung CT did not show the development of a pulmonary infection in this model.

Conclusion: In conclusion, MRI can provide additional information on disease manifestation and progression in individual animals, before any symptoms can be detected. We were able to document differences in the disease manifestation between the different clinical *C. neoformans* strains (e.g. time of onset, meningitis, lesion size and numbers). The use of these non-invasive imaging techniques can reduce the ethical burden associated with virulence studies while at the same time providing more insights in disease manifestation.

Picture 1: https://www.eventure-online.com/parthen-uploads/89/8ISH/add_1_421467_c778d2ab-e104-45d6-aae2-e19c67b250d9.jpg

Caption 1: Figure 1: Longitudinal MRI data for animals infected with different clinical Cryptococcus neoformans strains