

607 Developing a chronic model of *Candida albicans* cerebral mycosis through gut colonization



Lynn Bimler¹; ¹Baylor College of Medicine.

RATIONALE: Recent evidence suggests that Alzheimer's Disease (AD) is linked to fungal brain infections. We have previously established an acute model of cerebral mycosis by intravenously injecting the pathogenic yeast *Candida albicans*. The resulting infection induces mild transient memory deficits and fungal induced glial granulomas consisting of microglia and amyloid β deposits surrounding yeast aggregates. This structure essentially duplicates AD's characteristic senile plaque. AD involves numerous senile plaques and tauopathy that presumably accrue over many years potentially from chronic infection. This raises the possibility that *C. albicans* persists in a remote tissue, such as the intestines, from which it periodically mobilizes to chronically re-infect the brain. As both *C. albicans* colonization of the GI tract and low-level candidemia deriving from the GI tract have been documented in humans, we hypothesize that chronic *C. albicans* enteritis leads to low-level transmission of fungal cells into the bloodstream and persistent cerebral mycosis.

METHODS: To test this hypothesis and establish a more translationally relevant chronic model, we administered *C. albicans* to wildtype C57BL/6 mice via oral gavage. After challenge, we confirmed GI colonization past 2 months. Infected mice were tested with a variety of behavioral tests every 2 months. At these time points, mice brains and gut were harvested to confirm infection and examined for histological and proteomic signs of Alzheimer's disease. We also have cultured human brains and examined and sequenced the resultant colonies.

RESULTS: Live yeast are recoverable from the brain as soon as 2 days post gavage and for at least two months. Additionally, these colonies are polymicrobial, consisting of both yeast and bacteria, which is consistent with published analysis and our own cultures of AD brains. Infected mice present with increasingly altered behavior out to 4 months, including increased anxiety and altered fear responses.

CONCLUSIONS: Our study shows mice infected with *C. albicans* develop neuropathology and behavioral changes consistent with Alzheimer's Disease. This research is groundbreaking for the AD field, producing an unprecedented model that could be used for AD therapeutic and mechanistic studies.

608 Proteomic evaluation of spore and hyphae proteomes of *Alternaria alternata*



Michael Strader, PhD¹, Aishwarya Saha², Chantal Fernandes, PhD³, Christian Hadiwinarta¹, Kavita Sharma, PhD¹, Brett Green, PhD⁴, Angela Lemons, MS⁵, Donald Beezhold, PhD⁵, Teresa Goncalves, PhD³; ¹FDA, ²U.S. Food and Drug Administration, ³University of Coimbra, Portugal, ⁴Centers for Disease Control and Prevention, ⁵NIOSH.

RATIONALE: *Alternaria alternata* is associated with allergic diseases, which can be managed with allergen extract-based diagnostics and immunotherapy. The contributions of asexual spores and vegetative hyphae to patient allergen reactivity are uncertain, and commercial extracts are manufactured by unselective extraction without separating these components. Immunotherapy and diagnosis with existing products, while safe and effective, could be improved by better characterization.

METHODS: *A. alternata* spores, germinating spores and hyphae were grown under different conditions and separated by filtration, then extracted and lyophilized for transit. Reconstituted extracts (n=6 for each sample type) were resolved by SDS-PAGE and analyzed by a data independent acquisition strategy using an Orbitrap Fusion Lumos Tribrid Mass Spectrometer. Proteomic differences in allergen profiles and specific ontological groups in each sample type were evaluated using Protalizer software (Vulcan Analytical).

RESULTS: We identified 3638 proteins in spores and hyphae, with 157 proteins upregulated in spores and 55 in hyphae. Comparing spores and germinating spores, 23 proteins were upregulated in spores and 43 in

germinating spores. These observations were reproducible in all replicates. Sialidases predominated in non-germinating spores. IUIS-identified allergens are more abundant in the non-germinating spore proteome, which consists largely of proteins involved in cell wall synthesis, cellular stress responses and maintaining redox balance and homeostasis.

CONCLUSIONS: Spores, germinating spores and hyphae have differentially regulated biological pathways. Known *A. alternata* allergens appear mostly in non-germinating spores. Sialidases might be mechanistically involved in allergen delivery through mucosal surfaces. Information from this study will be important toward understanding allergen reactivity and improving extract potency and specificity.

609 The contribution of yeasts to occupant exposure in an office building with water incursion



Angela Lemons, MS¹, Ju-Hyeong Park, ScD, CIH¹, Tara Croston, PhD¹, Jean Cox-Ganser, PhD¹, Brett Green, PhD²; ¹National Institute for Occupational Safety and Health, ²National Institute for Occupational Safety and Health.

RATIONALE: Occupants of a water-damaged office building experienced post occupancy-onset asthma that was associated with exposure to fungi in floor dust. In this study, measures of fungal burden and diversity were employed to determine the fungi present in the environment that could contribute to work-related illness.

METHODS: Floor dust was collected from workstations in an office building that had known water incursion prior to (n=27) and one year following (n=27) building remediation. Viable culture, ergosterol measurements, and internal transcribed spacer (ITS) region sequencing were utilized to determine fungal load and diversity within office floor dust.

RESULTS: Viable culture and ergosterol concentrations demonstrated slight decreases in fungal load following remediation that were not significant. Interestingly, 60% of culturable fungi in pre-remediation samples were unicellular yeasts. ITS sequencing revealed many of these to be Ascomycota yeasts belonging to the order Saccharomycetales as well as Basidiomycota yeasts in the orders Tremellales and Cystofilobasidiales. Following remediation, yeasts continued to dominate the culturable fungi (78%) and many of the same species remained most abundant. Fungi in the orders Pleosporales and Capnodiales were also highly abundant in pre-remediation and to a greater extent in post-remediation samples.

CONCLUSIONS: These data suggest that unicellular yeasts may have significant contributions to personal exposure in water-damaged indoor environments. Remediation did not significantly alter yeast burden or diversity. Other fungal species, specifically those in the order Pleosporales, were abundant in pre- and post-remediation environments, suggesting that while remediation may decrease fungal burden, it does not necessarily alter the diversity of fungi present in indoor environments.