Lactoferrin, a natural source of peptides that potentiate the antifungal activity of amphotericin B

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S3.4c Oral paper session, September 21, 2022, 4:45 PM - 6:15 PM

Objectives: It is notoriously difficult to prevent and treat fungal infections, however, the natural world has come up with remedies that are non-toxic, effective, and evade resistance. Here we investigate lactoferrin, an iron-binding glycoprotein found in milk, tears, and sweat, for its capacity to inhibit fungi and to synergize with commonly used antifungal drugs, with the aim of determining its mode of action.

Methods: Lactoferrin (LF) was obtained from a commercial supplier and two dairy companies. LF was tested on a set of pathogenic yeast and mold species for inhibition using CLSM microcolony methods. Synergy was determined with antifungal drug amphotericin B (AMB), nystatin (NY), flucytosine (FLC), amphotericin B, itraconazole (ITC), and 5-fluorocytosine (5-FC). The effect of LF on fungal cells was analyzed using scanning electron microscopy (SEM). The active peptides within LF were then predicted from the peptides in all digestions, synthesized, and tested for synergy with amphotericin B (AMB).

Results: LF demonstrated antifungal activity against yeast species Cryptococcus, Candida, and Saccharomyces and was much less effective against molds. Good synergy was achieved with AMB but not azole or echinocandin drugs. While the iron-chelating capacity of LF was important for the antifungal activity, it was not involved in synergy. SEM revealed cell damage suggesting an interaction between AMB, LF, and the fungal membrane or cell wall. A 10-residue peptide from the C-lobe of LF was synthesized and tested for activity and synergy. This peptide, dubbed lactoferrin G (LFG), was inactive alone but was potently synergistic with AMB, indicating a direct role in augmenting AMB activity. Synthetic monomers loaded with amphotericin but not cholesterol were disrupted by AMB + LFG, demonstrating that activity was fungal-specific and was mediated through amphotericin binding.

Conclusions: LF is a complex molecule that causes fungal inhibition via iron binding and when cleaved by papain can produce active peptides. An AMB is a highly toxic treatment, the use of LFG as a synergist could help increase activity while lowering the effective dose, thereby reducing undesirable side effects. The action of AMB + LFG appears dependent on ergosterol, suggesting inhibition will be highly fungal-specific.

S3.4c A pipeline toward the identification of novel antifungal compounds derived from the microbial dark matter

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S3.4c Oral paper session, September 21, 2022, 4:45 PM - 6:15 PM

Background: The current armamentarium of antifungal drugs and the contracted variety in antifungal drug classes combined with the ever-rising threat of resistant fungal pathogens highlighted the urgent need for novel antifungal compounds. Natural
antifungal secondary metabolites have always been the prevalent source for drug development, exemplified by the echinocandins and polyene drug classes. Yet, the golden age discovery platforms were abandoned due to compound rediscovery and its economic return.

Study. In an effort to revert the original success stories, we combined the traditional approach of screening and selecting for antifungal secondary metabolites with modern advances in sequencing, genomics, transcriptomics, metabolomics, and NMR.

Solid bacteria and fungi were isolated through in vitro cultivation via the CIP method. After application of the OSMAC approach, 389 broth were identified with activity against Candida albicans. To prioritize active strains, several criteria were set up; to lower the mammalian host cell toxicity, activity against a broad spectrum of fungal pathogens including wild-type and azole-resistant strains, and established antifungal drug resistance variants and species identification of the producing strain. Continuing, lead hits were purified striking biosafety-based semi-preparative HPLC. The resulting pure fractions were analyzed by tandem LCMS-MS, and proposed structures were later confirmed with NMR in vitro and in vivo validation of the purified compounds will be performed.

Additionally, aside from discovering a novel antifungal compound, another project goal is to gauge if impaired spec.

The research can provide an early indication regarding the mode of action of the present antifungal agent. For this, a PDC study was performed which showed that different antifungal drug classes provide distinct signature responses by which they can be classified. As such, when active strain broth shows unique profile peaks, in comparison with the signature profiles of established antifungal drugs, it suggests that they work through a different mode of action.

Results. Several species were identified as producing antifungal secondary metabolites that are currently absent in the literature. Either the compound was unknown or literature sources did describe the species as a producer of a known, or variant of a known antifungal compound. Moreover, several species are novel based on BLAST sequencing. Generally producing our current lead hit included: Penicillium, Trichoderma, Parabotrytis, and fungus. At the end of the collection, the Penicillium species appear to produce variants of the antifungal sesquiterpene-precluding proline class.

S3.4d The role of NLRP1 inflammasome in host defense during Talonamyces marneffei infection

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S3.4e Four oral paper session, September 21, 2022, 4:41 PM – 6:15 PM

Talonamyces (Penicillium) marneffei (T. marneffei) is the only thermotolerant pathogen in Talonamyces. The pathogenesis of T. marneffei in mammals is not yet fully understood. Inhalation of T. marneffei conidia without normal clearance may result in conidia dissemination throughout the body and lead to disseminated infection. In TMS patients, study has shown that the abundance of conidia-G18 levels is higher and was correlated associated with the severity of cases and outcomes of post-treatment. That means poor outcome is likely associated with an overly strong immune response. Several studies have identified inflammasome activation as an essential immune response in host defense against fungal pathogens. Among them, NLRP1 inflammasome is the most well characterized. The role of NLRP1 inflammasome in T. marneffei-induced immunopathology remains to be elucidated. Therefore, in the present study, we aimed to address the role played by the NLRP1 inflammasome during Talonamyces marneffei infection in vivo.

We established T. marneffei infected mice pulmonary model with two groups of mice, including the Nlrp1 -/- and wildtype mice. We found that infected mice displayed NLRP1 inflammasome activation and increased production of IL-1β upon pul-

monary T. marneffei infection. Further, we demonstrated that T. marneffei conidia activated the NLRP1 inflammasome both in vivo and in macrophages. And T. marneffei conidia induced IL-1β released by infected macrophages is NLRP1 inflammasome-dependent. In vivo study, we found that NLRP1 contributes to the development of pathology in the early stage of pulmonary T. marneffei infection. However, Nlrp1 -/- mice suffered a similar fungal load to the WT in the middle stage of infection. Yet, the Nlrp1 -/- mice showed a reduced number of fungal colonies in the WT mice at the end stage of infection. Moreover, NLRP1 contributes to pathogenic inflammation in pulmonary T. marneffei infection and contributes to neutrophil recruitment and pulmonary injury.

So, in the present study, we demonstrated that the NLRP1 inflammasome is activated during T. marneffei infection. For NLRP1 inflammasome plays a dual role during pathogenic T. marneffei early inflammatory response inducing a proinflammatory environment, and a subsequent excessive damaging inflammatory response that contributes to pathogenesis and mortality. This study identifies for the first time that activation of the inflammasome in the later stage of TMS detrimentally contributes to pathogenesis and suggests that targeting the inflammasome may be a therapeutic option to treat pathogenic T. marneffei infections.

S3.4e Unraveling the role of DOG genes in a novel alternative pathway of glycerol biosynthesis in Candida albic-

and its influence on virulence

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3S3.4e Four oral paper session, September 21, 2022, 4:41 PM – 6:15 PM

DOG genes, encoding for 2-deoxyribose-6-phosphate phosphatase for low molecular weight phosphates, with an an-

ence biological function. In contrast to bracketed corynabs, which has two DOG homologs, C. albicans only has one DOG gene. We hypothesized that DOG plays an important role under osmotic or toxic stress by biosynthesizing glycerol, which is known to be vital for both cellular formation and virulence of this pathogen, via a novel alternative pathway.

The known classical pathway of glycerol production begins when the glycerol-3-phosphate dehydrogenase (GPD) is converted into glycerol-1-phosphate (G-P) by a pair of glycerol-3-phosphate dehydrogenase (GPD1 and GPD2). This enzyme is additionally involved in the production of DHAP from DGDH, thereby allowing the synthesis of glycerol in the absence of the classical pathway. Overexpression of the DOG genes restored the osmotic-tolerance of the gpd1Δ gpd2Δ double deletion strain (Fig. 1A, B). Furthermore, we found that DOG1 (2.5 g/disk) can form a clear inhibition zone, but not DOG2 (2.5 g/disk) (Fig. 1A, B). In addition, we observed C. albicans cells from the broth microdilution assay on YPD solid medium and found that cells treated with DOG1 (∼27 μM) could not survive on YPD solid medium, while cells cultured with GPD could (∼4 l) (Fig. 1C). It is worth noting that DOG inhibitor effects on filamentation and hyphal formation is at 12.5 μM (Fig. 2a, b), which are key virulence factors of pathogenic fungi. Besides, DOG (∼50 μg/ml) significantly prolonged the median survival time from 5 days (control group) to 15 days and improved the survival rates from 0% (control group) to 62.5% (Fig. 2a, b). Furthermore, we found that DOG exhibited reduced low toxicity to mammalian cells with EC50 28.57 ± 2.49 (Fig. 2b, d), which was much higher than MIC against pathogenic fungi.

Conclusion. Our main highlight that DOG has a broad-spectrum antifungal activity with low MIC50 values and high safety and potency antifungal efficacy in vivo and will be beneficial to the treatment of devastating invasive fungal infections.

S3.5a Contamination in sand and water of sea, lakes, and river beaches. Risk factor for fungal human disease

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S3.5 Environmental exposure - risk for human fungal diseases, February 21, 2022, 4:45 PM – 6:15 PM

Marine and freshwater bodies, such as rivers and lakes are known as possible niches harbouring microbes that may affect human health. These ecosystems have been extensively researched, and even regulated, for the presence of bacterial contamination. Fungal contamination has been researched and lacks regulation, nevertheless the recent finding that the antifungal resistant yeast Candida auris has been isolated from a marine ecosystem.

The present lecture will focus on a recent collaborative study, involving 13 countries, spanning from the Atlantic to the Eastern Mediterranean coast, and including the Italian lakes and the Atlantic, Baltic, and Black Sea. The study explored fungal contamination of sand and water of the sea, lakes, and river beaches in 91 sampling sites, and water of 67 of these.

This study considered several fungal parameters, all fungi, several species of the genera Aspergillus and Candida plus other yeasts, antifungal fungi, and dematiaceous fungi and dermatophytes. The study took in account four variables that might influence the results of the analytical parameters, such as: (i) coast or inland location, (ii) urban and nonurban sites, (iii) period of the year, (iv) geographical proximity and type of substrate.

A sub-millenial median was found to be 80 Colony-Forming Units (CFU) of fungi per grams of sand in coastal and inland freshwaters, with variability between 0 and 6480 CFU. For freshwater sites, that number was 201.7 CFU (0, 6440 CFU) (P = 0.01) and for coastal sites was 74.6 CFU (0, 3487 CFU). For coastal waters and all others, the median was 0 CFU but, for freshwaters 0.7 (0, 310.5 CFU) (P = 0.001). The fungal load in freshwater was far better correlated in all environmental samples (Fig. 1).

Some of these will be presented herein. The lecture will also include, as an example, the data collected at the Israeli Mediterranean coast. This study included a series of sand and water of six urban beaches, from north to south of the Israeli Mediterranean Coast. Sand samples were extracted by water, and the water was cultured and quantitated. Water samples were quantitated as well. The fungi were identified phenotypically, by MALDEP®-300M system and ITS sequencing. The results revealed that about 90% of the isolates were molds and about 20% yeasts. The mold species included opportunistic pathogens and potential allergens. Aspergillus fumigatus, Nocardia, Penicillium, and Mucorales species. Yeast isolates included Candida, Cryptococcus and Rhodotorula species. The most abundant fungi were C. albicans and C. tropicalis, with yeast and mold isolation.

The results suggest that beaches should be monitored for fungi for safer use and better management, and for the benefit of public health.