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Inhalt

Keynote lectures

Keynote lecture I

What are the barriers preventing good clinical outcomes in patients with aspergillosis?

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Historically it was leukaemia and transplant patients who had the highest rates of invasive aspergillosis. The key developments of the fpiinew generation of triazoles depended on studies in these patients, and was critically important, saving millions of lives by now. Now prophylaxis and an appreciation of other risk groups is starting to take centre stage; IA in intensive care (notably VAPA), COPD and to a less extent lung cancer. Much larger numbers of patients live in the community with aspergillosis – chronic pulmonary, ABPA, fungal asthma Aspergillus bronchitis complicating CF or bronchiectasis. Community-based physicians and many respiratory specialists are not well versed in the clinical patterns of and testing for aspergillosis.

The No 1 challenge to improving outcomes is inadequate medical suspicion (until too late) and timely diagnostic testing. No 2 challenge is that culture is insensitive (although improved with higher volume samples). No 3 challenge is that Aspergillus IgG (or IgE) antibody testing is greatly underutilised, and often unavailable. No 4 challenge is that while bronchoscopy and BAL are helpful, it is dangerous for some patients (and possibly for staff if VAPA), and unavailable in many countries. Without it most cases of Aspergillus tracheobronchitis are missed. No 5 relates to the challenges of imaging and its interpretation, especially in non-neutropenic patients. No 6 challenge is the common co-occurrence of other infections and pulmonary conditions, so that aspergillosis is 'disguised'. Common examples include MDR-TB and CPA, NTM infection and CPA, Pseudomonas and Aspergillus bronchitis (ie in CF), multi-antigen sensitization in fungal asthma (house dust mite, cats, dogs etc) – there are numerous other examples. No 7 challenge relates to prescribing and drug interactions. The older azoles (itraconazole and voriconazole) have numerous issues with bioavailability and exposure issues as well as 100's of potential drug interactions. No 8 challenge is the emergence of azole antifungal resistance.

The talk will address these issues and approaches our international community of experts needs to do to improve matters.

Resources

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2. https://en.fungaleducation.org/
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Keynote Lecture II

How does metabolic regulation shape host-fungus interactions?

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In infection, pathogens and hosts experience metabolic stress due to the presence of low nutrients in tissues, and additional restriction and competition for essential nutrients that accompany immune responses and microbial evasion mechanisms. As such, effective metabolic adaptation is essential for survival.

In addition to the production of energy and biomolecular building blocks, metabolic adaptations in immune cells directly regulate antimicrobial programs. This includes the production of cytokines, as well as control over pro-inflammatory cell death pathways that lie downstream of central immune regulators, chiefly inflammasomes. When inflammasomes are activated due to infection-induced immunometabolic stress, the consequent lysis of immune cells releases cytokines and alarmins to amplify the immune response. However, this represents a double-edge sward in the case of fungal pathogens, which do not need immune cells for replication. In this case, the release of fungal pathogens in the process of programmed immune lysis reduces their containment, and could allow for dissemination. Moreover, these are very strong pro-inflammatory responses that, yes, control pathogens but can become a problem and drive poor outcomes if immunopathology is not avoided. This means that understanding how metabolic stress activates inflammation and programmed immune cell death could inform on the ways to restore metabolic health. This would ultimately promote balanced immune responses, as well as organ and tissue function to aid in patient survival.

Towards this goal, we have determined that infection with Candida pathogens disrupts both immune cell metabolism and systemic host metabolism. We are now working towards understanding the consequence of host metabolic stress for immune responses and host survival. We have identified the cell lysis program that responds to Candida-induced glucose starvation of macrophages, discovering the executor of this program and a metabolite that act as its antagonist. Targeting this host factor, potentially with metabolic interventions, has potential to balance immune responses, reduce host damage, increase pathogen containment and ultimately lead to better outcomes of Candida infections.

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Keynote lecture III

The Aspergillus exopolysaccharide galactosaminogalactan: More than meets the eye

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The filamentous fungus Aspergillus fumigatus causes invasive, often fatal pulmonary infection in immunocompromised patients. Like many microorganisms, A. fumigatus forms biofilms during pulmonary infection. Biofilms play an important role in the pathogenesis of infection, and help mediate adhesion to host tissues, immune evasion and resistance to antifungals. Using a combined genomics, structural biology and glycobiology approach, our group and others have previously demonstrated that A. fumigatus biofilm formation is dependent on the production of the cell wall and secreted exopolysaccharide galactosaminogalactan (GAG), a heteropolysaccharide composed of α-1,4-linked galactose and partially deacetylated N-acetylgalactosamine. Through studies of the enzymes underlying GAG biosynthesis, we have discovered that GAG plays a role in a wide range of biologic functions during infection including mediating adhesion of hyphae to host cells and abiotic surfaces, and immune evasion. Recent work has discovered that GAG may also function as a toxic molecule that injures host cell membranes, and that GAG is actually comprised of two independently synthesized polymers of different composition and function. This presentation will review key insights into the molecular mechanisms governing GAG biosynthesis, the role of GAG in virulence, and the potential for the development of therapeutic anti-biofilm strategies that target this polysaccharide virulence factor.

Keynote lecture IV

Tracing genomic adaptations in emerging fungal pathogens

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Invasive fungal diseases such as candidiasis, caused by Candida species, are a major public health problem. They are difficult to diagnose and have high mortality rates. Moreover therapeutic options are limited, and resistance to multiple antifungal drugs is increasingly reported, particularly in emerging species such as Candida glabrata, Candida parapsilosis, and Candida auris. Despite their common genus name, Candida pathogens, are evolutionarily diverse and belong to different lineages where the ability to infect humans has emerged independently. Over the last years we have used in vitro evolution, and comparative genomics

approaches to understand how the different pathogenic lineages adapted to humans and how they become resistant to the drugs we use to fight their infections.

Keynote lecture V

Fungal infection at the extreme - the predatory lifestyle of a nematode-trapping fungus

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Many fungi live in association with other organisms. Many mushrooms form a symbiosis with trees, and more than 80 % of the land plants form endo- or ektomycorrhizal associations. In addition, many fungi cause severe problems as pathogens and destroy large amounts of food and feed or infect living plants. A very intriguing interaction is the predatory lifestyle of nematode-trapping fungi. Nematodes are the most numerous animals on earth and live predominantly in soil. Nematode-trapping fungi are also common members of the microbiome and live saprotrophically on organic material. However, when nutrients are limited and nematodes are present, they switch to a predatory lifestyle. They form special trapping devices, catch a nematode, penetrate it and colonise the entire body.

We are interested in the molecular biology of the interaction and discovered in Arthrobotrys flagrans that it recognises Caenorhabditis elegans through nematode pheromones. In other words the fungus "smells" the nematodes. The pheromones trigger a signalling cascade in the fungus which leads to the induction of the formation of adhesive traps. We identified a GPCR responsible for ascaroside “smelling” and found that it signals from the cytoplasmic membrane but also localizes to mitochondria where it stimulates respiration. For the infection A. flagrans applies a battery of small-secreted proteins.

Trap formation requires the formation of ring-like structures, which is quite unusual in biology. We described the cell-to-cell-dialogue which guarantees ring closure.

Another interesting question concerns the overcome of the defence reactions of the nematode. It was thought that the fungus secretes lytic enzymes and digests the nematode. However, we discovered that the fungus secretes in addition different small proteins or peptides, and those peptides are used to penetrate the nematode, paralyse it or reprogram nematode cells. This shows that the interaction is a very sophisticated system which reflects more than 400 Million years of co-evolution.

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Talks

Session 1 - Epidemiologie und Taxonomie

S01-01

Genomic reconstruction of an azole-resistant *Candida parapsilosis* outbreak and the creation of a multilocus sequence typing scheme

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Question

Fluconazole-resistant *Candida parapsilosis* has emerged as a significant healthcare-associated pathogen with a propensity to spread patient-to-patient and cause nosocomial outbreaks, similar to Candida auris. This study investigates a prolonged outbreak of fluconazole-resistant *C. parapsilosis* across multiple years and healthcare centers in Berlin, Germany.

Methods

We used hospital records coupled with whole-genome sequencing to reconstruct the outbreak dynamics and quantify genomic relationships between isolates. Additionally, we used the genomic dataset of 381 global samples to identify loci with high discriminatory power to establish a multi-locus sequence typing (MLST) strategy for *C. parapsilosis*.

Results

A clonal, azole-resistant strain of *C. parapsilosis* was observed causing infections from 2018-2022 in multiple hospitals in Berlin. Temporal and genomic reconstruction of the outbreak indicated that transfer of patients between healthcare facilities was likely responsible for the persistent reimportation of the drug-resistant clone and subsequent person-to-person transmission. Outbreak strains were closely related to strains responsible for an outbreak in Canada and to others isolated in the Middle East and East Asia. Using the novel MLST strategy, isolates were categorized into 62 sequence types, proving the utility of the typing scheme for epidemiology and outbreak investigations as a rapid alternative to whole genome sequencing.

Conclusions

This study underscores the importance of monitoring the epidemiology of *C. parapsilosis* epidemiology, not only in Germany, but worldwide. The emergence of azole-

resistant lineages and an increasing number of nosocomial outbreaks necessitates continuous surveillance and rigorous infection control measures.

S01-02

First transmissions of *Candida auris* in Germany

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Background

Candida auris is known for a rapid development of antifungal drug resistance and resilience to environmental stress. In contrast to most other *Candida* species, *it* can easily be transmitted leading to hospital outbreaks. Though rising, the total number of reported *C. auris* infections is still low in Germany. We collected fungal isolates and patient data from every *C. auris* case reported to the German National Reference Center for Invasive Fungal Infections (NRZMyk).

Objectives

We aimed to characterize isolates from transmission events with a focus on their genome structure and antifungal drug susceptibility.

Methods

Isolates were genetically and phenotypically characterized. We focused on the diversity between closely related isolates from patient-to-patient transmission events. Isolates from a transmission event were further analyzed regarding strain-specific genetic mutations. Antifungal drug susceptibility was tested with broth microdilution according to EUCAST.

Results

In 2023 an almost sixfold increase of *C. auris* primary isolates (n=77) in Germany was observed. 13 cases were associated with infection, 58 isolates derived from colonized patients and in 6 cases no data was available. The majority of *C. auris* primary isolates belonged to clade I (n=66). Almost all primary and follow-up isolates (87/90) showed reduced susceptibility to fluconazole. Echinocandin resistance was still rare (n=1). WGS analysis revealed that 49 primary isolates were assigned to 4 events of transmission in 2023, including one outbreak with 42 affected patients on 4 different hospital units.

Conclusion

We observed a profound rise in German *C. auris* cases. Timely implementation of containment and surveillance strategies are needed to decelerate the spread of *C. auris* in Germany as long as possible. As *C. auris* is a partially notifiable disease in Germany (implemented in 2023; infection only), our data strongly support an inclusion of colonized patients in current reporting obligations.

S01-03

National Cross-Sectional Study of Fungal Infections in Hospitalized Patients in Ecuador: Analysis of WHO Priority Fungal Pathogens (2015-2022)

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Introduction

Fungal infections pose an emerging challenge in global public health, often underestimated. Ecuador's ecological diversity supports pathogen proliferation, but limited data hampers accurate fungal distribution assessment.

Objectives

This study aims to identify the predominant fungal infections in Ecuador between 2015 and 2022 and analyze their geographic distribution based on the WHO fungal priority list(1).

Methods

Fungal infection hospitalizations were analyzed using official national hospital data (INEC (2)) from 2015 to 2022, employing ICD-10 for case identification. RStudio was used for statistics and heatmap visualization.

Results

The hospitalization rate per 100,000 inhabitants in Ecuador was assessed for each province during the study period (Fig. 1). Predominant diseases included candidiasis (7.3) and aspergillosis (1.2) based on cumulative rates. Pneumocystosis and mycetomas had rates of 0.2 and 0.5, respectively. Cryptococcosis and histoplasmosis shared a rate of 1, while coccidioidomycosis, mucormycosis, and paracoccidioidomycosis shared a rate of 0.1 (Fig2. A, B, C).

Conclusion

This study sheds light on the impact of fungal infections in Ecuador, highlighting the urgent need for evidence-based public health interventions. Uncovering these infections' geographical distribution and prevalence lays the groundwork for targeted prevention and treatment initiatives. Moving forward, informed policy decisions and continued research efforts will be essential in effectively combating fungal diseases and safeguarding public health in Ecuador.

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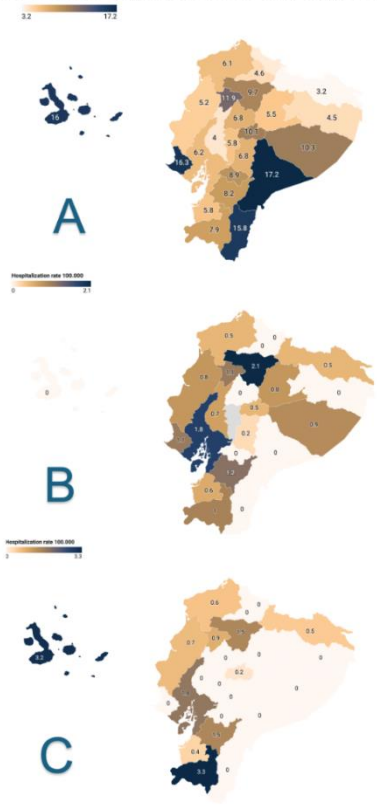
Figure 1

Figure 1.- Adjusted Hospitalization Rate per 100,000 Inhabitants for Fungal Pathogens in Ecuador by Province (2015-2022) Based on WHO Priority List

Province	Aspergillosis	Candidiasis	Coccidioidomycosis	Cryptococcosis	Histoplasmosis	Mucormycosis	Paracoccidioidomycosis	Mycetomas
Azuay	1,2	8,2	0,0	1,5	0,1	0,2	0,4	0,5
Bolívar	0,0	5,8	0,0	0,0	1,0	0,0	0,0	0,0
Cañar	0,0	8,9	0,0	0,0	0,4	0,0	0,0	0,0
Carchi	0,0	3,8	0,0	0,0	0,0	0,0	0,0	0,0
Chimborazo	0,2	6,8	0,0	0,0	0,6	0,0	0,2	0,0
Cotopaxi	0,0	6,8	0,0	0,0	0,0	0,0	0,0	0,4
El Oro	0,6	5,8	0,0	0,4	1,0	0,0	0,0	0,1
Esmeraldas	0,5	6,1	0,0	0,6	0,6	0,2	0,0	0,3
Galápagos	0,0	16,0	0,0	3,2	3,2	0,0	0,0	0,0
Guayas	1,8	6,2	0,1	1,8	1,7	0,2	0,0	0,6
Imbabura	0,0	4,6	0,0	0,0	0,0	0,0	0,0	0,4
Loja	1,0	7,9	0,0	3,3	0,2	0,4	0,4	3,9
Los Ríos	0,7	4,0	0,0	0,0	0,7	0,0	0,0	0,1
Manabí	0,8	5,2	0,0	0,7	0,5	0,1	0,1	0,3
Morona Santiago	0,0	17,2	0,0	0,0	0,0	0,0	0,0	1,6
Napo	0,8	5,5	0,0	0,0	0,0	0,0	0,0	0,8
Orellana	0,0	4,5	0,0	0,0	0,6	0,0	0,0	0,0
Pastaza	0,9	10,3	0,0	0,0	1,9	0,0	0,0	0,9
Pichincha	2,1	9,7	0,3	1,5	1,8	0,1	0,1	0,4
Santa Elena	1,1	16,3	0,0	0,0	0,0	0,0	0,0	0,0
Santo Domingo de los Tsáchilas	1,1	11,9	0,0	0,9	0,9	0,5	0,0	0,5
Sucumbíos	0,5	3,2	0,0	0,5	0,5	0,0	0,0	0,0
Tungurahua	0,5	10,1	0,0	0,2	0,0	0,2	0,0	0,5
Zamora Chinchipe	0,0	15,8	0,0	0,0	0,9	0,0	0,0	0,0
Total	1,2	7,3	0,1	1,0	1,0	0,1	0,1	0,5

Figure 2

Figure 2.- Geographic Heat Maps of Hospitalization Rates in Ecuador (2015-2022) Illustrating Fungal Diseases with Highest Burden



A.- Aspergillosis. B.- Candidiasis. C.- Cryptococcosis

S01-04

Revisions in the taxonomy of Mucorales and their benefit for the medical mycology

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Taxonomy of many fungal groups is currently in a state of flux because a large part of their diversity has not been discovered and/or described yet. Molecular phylogenetic analyses usually result in an increasing number of species and changes of taxonomic concepts that often found to be annoying, especially when they involve changes in species names. In my presentation, I will use the example of the Mucorales to show that medical mycology benefits from taxonomic revisions in a long term. First of all, a reliable diagnosis of the causative agents of mucormycosis depends on a revised taxonomy. For example, new species concepts of the *Mucor circinelloides* complex allow now the identification of clinical isolates of the genus *Mucor* to the species level. As a consequence, we receive a clear picture of the epidemiology and medical importance of the particular species. Based on these results experimental research groups changed their model organism from *M. lusitanicus* (formerly *M. circinelloides* f. *lusitanicus*) to *M. circinelloides*, the most important pathogen in the genus *Mucor*. Correct species identification due to revised species concepts also result in more precise antifungal susceptibility profiles and the detection of taxon specific differences. Furthermore, taxonomy also provides valuable information of opportunistic species such as the maximum growth temperature that allows a better risk assessment of species with unclear medical importance detected in clinical samples. The example of the Mucorales shows, that a close cooperation of medical mycologist, physicians and taxonomists is of benefit for all parties.

Session 2 - Host-Fungal Interactions I

S02-01

Fungal morphology predefines the triggered immune response

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The polymorphic yeast *Candida albicans* uses its virulence trait to grow as hyphae to invade epithelial barriers and infect predisposed individuals. Most *in vitro* models studying antifungal immunity do not consider long hyphae as the main morphology encountered by our innate

immune cells early during infection. Macrophages are tissue-resident sentinels, among the first to sense invading pathogens. Therefore, we investigated, how the early interaction of human macrophages with either the hyphal or yeast morphology of *C. albicans* predefines downstream antifungal immunity. We used supernatants of macrophages treated with different fungal morphotypes as stimuli for neutrophils representing key early recruited effector cells and measured their activation. We observed that a prior interaction of macrophages with hyphae instigated massive neutrophil activation indicated by activation marker expression, ROS and chemotaxis, while macrophage interaction with yeast cells, only elicited moderate responses in neutrophils. By cytokine ELISAs, proteomics and live cell imaging, we found that interaction with hyphae triggered inflammatory cell death in macrophages, explaining the strong neutrophil activation. Using cell death inhibitors and genetically modified macrophages, we observed that this cell death did not mirror classical apoptosis, pyroptosis, necroptosis, nor was it due to passive candidalysin intoxication. Notably, this cell death phenotype was macrophage specific and did not depend on hyphal viability. We characterized the required signaling events and found Dectin-1, Mincle, PI3K, and ROS crucial for inflammatory macrophage death induction. In summary, we propose a novel form of regulated macrophage death, driving strong neutrophil activation and being triggered by the fungal hyphal morphology. Understanding how to control this cell death process could help improving therapy of (necrotizing) invasive fungal infections, especially in neutropenic patients.

S02-02

Multi-reporter cell lines: fluorescent reporter for detection of cell death types in infection

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The yeast *Candida albicans* is the most important causative agent of candidiasis. It usually resides harmlessly on the host"s mucosal surfaces as part of the normal microbiota. When the balance between the host, the microbiota, and the pathogen is disrupted, *C. albicans* may cause superficial or even lethal systemic infections. As a consequence of such *C. albicans* infections, epithelial cells and macrophages experience different types of cell death, e.g., pyroptosisor apoptosis, depending on the stage and type of infection.

Therefore, we are creating fluorescent reporters for different cellular stress and death pathways which allow real-time detection by fluorescent microscopy. Cell lines are created via lentiviral transduction, which allows multiple transductions into a single cell line to create multi-pathway reporters. We have created fluorescent reporters for redox status (stress), using a roGFP2 fluorophore, and for apoptosis (cell death), using a switch-on fluorophore for live detection of caspase 3/7 activation. Another reporter using a bimolecular fluorescence complementation (BiFC) system for live detection of pyroptosis is being validated. As all reporters work with non-overlapping spectra, we aim to create a single "multi-reporter", which will enable us to detect simultaneously different types of cell stress and death pathways. To leverage our ability for spatial detection of cell death in infection, we will implement reporter cell lines in a gut-on-chip system, a 3D *in vitro* model that mimics the dynamic and physiological conditions of the human gut.

Our reporter cell lines will allow to monitor cellular stress and differentiate the different host cell death pathways that are active during *C. albicans* infection. Importantly, it makes it possible to follow the state of the host cells in a temporally and spatially resolved manner. Our new reporter will thereby help to answer many of the open questions on host-pathogen interactions during infection.

S02-03

The fungus-carcinoma axis: is *Malassezia* able to survive in pancreas and to contribute to Pancreatic Ductal Adenocarcinoma

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Introduction

Recent publications hypothesize that the yeast *Malassezia* can migrate from human gut into the pancreas where it represents a relevant trigger for the progression of pancreatic ductal adenocarcinoma (PDAC). A prerequisite for such an effect is the fungal survival and proliferation in the tumor environment.

Objectives

We aimed: (1) to confirm the augmented presence of *Malassezia* in tumor tissue of PDAC patients; (2) to study the capacity of *Malassezia* to survive and proliferate in the pancreas with its digestive enzymes and in the hypoxic tumor environment.

Materials and Methods

The presence of *Malassezia* in formalin-fixed paraffin-embedded pancreatic cancer tissue was evaluated by PCR analysis. *Malassezia* survival and proliferation in tumor environment were investigated by cultivation in presence/absence of homogenized murine pancreas, human pancreatic juice or under hypoxic conditions.

Results

A small pilot study confirmed the increased presence of *Malassezia* in PDAC tissue compared to tissue samples derived from patients with other pancreatic diseases. The fungal presence was not caused by contamination during pre-surgical interventions such as endoscopic retrograde cholangiopancreatography.

Incubation of *Malassezia* with homogenized murine pancreas or human pancreatic juice demonstrated that the yeast can survive in presence of pancreatic enzymes and even exploit the nutrients for proliferation. This could be confirmed by inoculation of non-homogenized whole murine pancreas with *Malassezia*, followed by fungal staining over time. Hypoxic conditions, which are typical for solid tumor environment, did not interfere with fungal survival.

Conclusion

Confirmation of increased fungal detection in tumor tissue in our study makes a contribution of *Malassezia* to PDAC progression more likely. Furthermore, *Malassezia* is able to survive and grow in pancreatic tumor environment, a prerequisite for its putative role as a pro-carcinogenic trigger.

S02-04

Antibiotic-induced changes in the microbiota affect susceptibility and immune responses to systemic candidiasis

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Prolonged treatment with antibiotics is a risk factor for systemic candidiasis. By affecting overall bacterial burden in the gut and depletion of antagonistic bacteria, antibiotics reduce colonization resistance and thereby promotes fungal overgrowth. As the gut is an important source for translocation of *Candida* into the blood stream, high intestinal fungal burden increases the likelihood of translocation and subsequent dissemination. We hypothesized that antibiotic treatment might additionally affect host susceptibility by impacting tissue physiology or immune responses to infection.

To test this hypothesis, we systemically infected mice with *C. albicans*. Pretreatment with a combination of antibiotics led to significantly reduced survival in specific-pathogen-free (SPF) mice, indicating that antibiotic treatment indeed affects host susceptibility. This effect was reproducible across SPF mice that differed in their microbiome composition prior to treatment. However, antibiotics did not affect the susceptibility of germ-free mice, suggesting that changes in the microbiota, rather than a direct impact on host cell physiology, are responsible for the negative impact of antibiotics on host resistance.

Dysbiosis was associated with altered hematopoietic and progenitor cell composition and, maturation and function of neutrophils. Additionally, peritoneal macrophage function was reduced. This was accompanied by excessive proinflammatory cytokines in the blood and kidney. Combined, these changes likely contributed to impaired fungal control and exacerbated immune-pathology. *C. albicans* gut colonization in the absence of antibiotic treatment selectively enhanced systemic fungicidal immune responses and reduced proinflammatory cytokine production. The combination of antibiotic treatment and colonization lead to stronger Th17 responses and increased *C. albicans*-specific IgG. This could lead to increased immunopathology in hosts that are not able to clear the infection.

Session 3 - Dermatomykosen

S03-01
Update on *Trichophyton indotineae* (*Trichophyton mentagrophytes* ITS genotype VIII) in Germany

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Introduction

Trichophyton (*T.*) *indotineae* is the source of severe dermatophytoses in Asia.

Methods

Isolates from patients in Germany with tinea caused by *T. indotineae* have been analysed since 2018. Dermatophytes were detected by culture and RT-PCR. For confirmation, sequencing of the ITS region of the rDNA was performed for all isolates. A breakpoint method

was used for susceptibility testing against terbinafine and itraconazole. In addition, sequencing of the squalene epoxidase gene (SQLE) was performed. The DermaGenius® Resistance RT-PCR Kit (PathoNostics®, Netherlands) was used for selected isolates.

Results

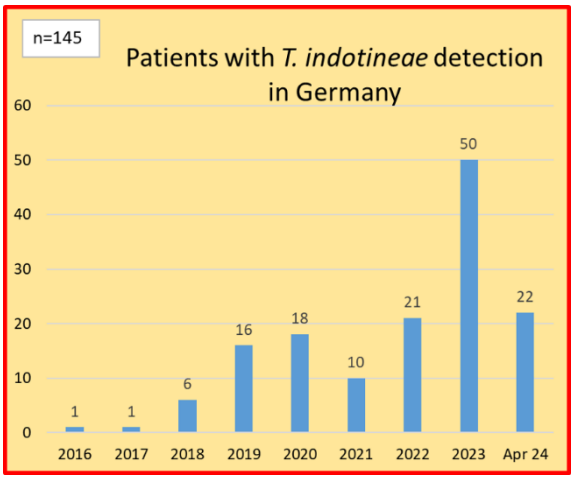
In the period from 2016 to April 2024, 145 strains of *T. indotineae* were identified in patients in Germany. At the beginning - in 2016 and 2017 - there was only one isolate per year retrospectively. There was a significant increase in 2023 with 50 patients. By April 2024 alone, 22 patients had already been diagnosed with *T. indotineae* (Fig.). Almost all of those affected by the dermatophytoses had a migration background. The countries of origin included India, Bangladesh, Pakistan, Bahrain, Saudi Arabia, Iran, Iraq, Libya and Afghanistan. In addition to the predominant tinea cruris, tinea corporis was particularly common and affected the abdomen, mons pubis, buttocks and thighs. *T. indotineae* was found throughout Germany. Terbinafine resistance was detected in 92 (65%) of 141 T. indotineae strains. In vitro, 10 (7 %) of 141 strains were resistant to itraconazole, two isolates were intermediate susceptible. The data from long-term observations will be particularly important, such as in a patient with evidence of *T. indotineae* in 2018, 2022 and 2024.

Conclusion

Very good data are available on the occurrence of *T. indotineae* in Germany. Patients with a *T. indotineae* infection are characterised by the clinical picture - an extensive spread of dermatophytosis accompanied by excruciating itching. Other family members are often also affected.

Fig. Summary

Figure 1



S03-02

The multiple azole resistant *Trichophyton indotineae erg1*^{Ala448Thr} strain constitutively expresses high levels of sterol 14-α demethylase *Erg11B* mRNA

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Trichophyton indotineae is an emerging pathogen[1, 2] that causes recalcitrant skin infections due to multiple resistances to azoles and allylamines. Squalene epoxidase *erg1*^{Ala448Thr} mutants often show association with multiple azole resistance,[2, 3] whereas terbinafine resistance is mainly the result of a point mutation in other *Erg1* amino acid positions for example 393 and 397.[1,2,4] Point mutations in *Erg11B* associated with amino acid exchanges have also been found in *T. indotineae* isolates,[3] which are similar to those of phytopathogenic fungi.[5]

Objectives

RT-PCR expression analyses help to elucidate the connection between ergosterol biosynthesis regulation and efflux control through activation of multiple drug resistance (MDR) and major facilitator superfamily (MFS1) transporters as well as heat shock (HSP) proteins.

Results

The azole resistant *erg1*^{Ala448Thr} mutant UKJ 476/21 showed exceptionally high transcript levels of the sterol 14-α demethylase *Erg11B.* Additionally, unlike all other *T. indotineae* isolates *HSP60* and *HSP90* expression of the same mutant failed to respond to increasing growth temperatures. Interestingly, several other *T. indotineae* isolates demonstrated a heat-dependent increase of *Erg11B* transcripts combined with down regulation of *Erg1,* suggesting a protective role for *Erg11B* regulation persistent upregulation of *MFS1* was also observed in some *T. indotineae* isolates*.*

Conclusion

The observed overexpression of *Erg11B* could explain the multiple resistance mechanism of the *erg1*^{Ala448Thr} mutant strain.

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S03-03

Ein Garten als Habitat rarer keratinophiler Pilze – relevant für Mensch und Tier

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Erreger einer Tinea beim Menschen sind keratinophile Pilze, und zwar ganz überwiegend Dermatophyten. Deren anthropophile Vertreter werden am häufigsten nachgewiesen, seltener aber auch zoophile und geophile Spezies aus der Ordnung *Onygenales*, wie im hier präsentierten Fall.

Eine 55-jährige Patientin stellte sich mit einer typischen Tinea am Oberschenkel vor. Bei ihrem Hund waren gleichzeitig kahle Stellen am Bauch aufgefallen. Es erfolgten eine erweiterte Anamnese, Entnahme von Schuppenmaterial, Nativpräparate, Kulturen und Mikrokulturen auf

Dermatophyten-Agar, PCR (EUROArray Dermatomycosis®), Sequenzierung (Prof. Gräser, Charité, Berlin) und Untersuchung von Bodenproben mittels Haarködermethode.

Bei Patientin und Hund gelang der Nachweis von *Nannizzia (N.) persicolor*. Die Anamnese enthüllte einen Wühlmausbefall im Garten der Patientin. Aus Erde eines Wühlmausbaueingangs wurde mittels der Haarködermethode *Arthroderma uncinatum* (alte Bezeichnung: *Trichophyton ajelloi*) angezüchtet, aus Erde eines Mausbaus *Aphanoascus fulvescens* (alter Name einer vermutlichen anamorphen Form: *Chrysosporium keratinophilum)*. Patientin und Hund wurden erfolgreich mit SUBA-Itrakonazol p.o. und lokal mit Ciclopiroxolamincreme behandelt. Die Wohnung wurde desinfiziert, die Wühlmausgänge aufgegraben und mit Zement verfüllt.

N.persicolor ist ein zoophiler/geophiler Dermatophyt (Familie *Arthrodermataceae*), dessen Wirtstiere überwiegend Mausarten sind. Im Erdboden aus dem Mäuseumfeld ließen sich jedoch überraschend 2 weitere geophile keratinabbauende Pilze nachweisen: *Arthroderma uncinatum* (Familie *Arthrodermataceae*) und *Aphanoascus fulvescens* (Familie *Onygenaceae*); auch diese beiden Erreger können beim Menschen eine Tinea auslösen.

Dermatologen sollten bei einer Tinea an Gärten als mögliches Habitat (Tiere/Erde) von Dermatophyten und anderen keratinophilen *Onygenales* denken und ggf. Sanierungsmaßnahmen empfehlen.

S03-04

News from dermatophyte diagnostics of routine samples, current statistics from 2023 in Germany

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Introduction

Species-specific pathogen identification is essential for the targeted treatment of dermatophytoses. The pathogen reservoir and a distribution pattern can often be assigned to each dermatophyte species.

Materials and methods

Over the period from January to December 2023, routine samples from nail, skin, swabs and hair were mycologically analysed for suspected dermatomycoses and onychomycoses. The analysis was carried out microscopically, culturally and molecularly. If the PCR was negative or the dermatophyte species could not be clearly identified, further differentiation was carried out by sequencing the ITS rDNA.

Results

Dermatophyte infection was detectable in 9769 (36.78 %) of 26561 patient samples. The most common anthropophilic dermatophytes were *Trichophyton (T.) rubrum* in 7829 (80.14 %) of 9769 patients (pat), followed by *T. interdigitale*, *T. tonsurans* in 349 (3.57 %) pat, *T. violaceum* 56 pat, *T. indotineae* 50 pat, *Epidermophyton floccosum* 24 pat, *T. mentagrophytes* VII 18 pat, *Microsporum (M.) audouinii* 8 pat and *T. soudanense* 5 pat. Among the zoophilic dermatophytes, *T. benhamiae* 126 pat, *M. canis* 105 pat, *T. mentagrophytes*, *T. verrucosum* 52 pat, *T. quinckeanum* 49 pat and *T. erinacei* 7 pat were detectable. The most common geophilic dermatophytes were *Nannizzia* (*N.*) *gypsea* 18 pat, *N. fulva* 7 pat, *N. persicolor* 6 pat,

N. praecox 2 pat and *N. incurvata* 2 pat. Dermatophytes of the genus *Arthroderma (A.)* were detectable in 11 patients. These included *A. quadrifidum*, *A. insingulare*, *A. chiloniense*, *A. crocatum* and *A. onychocola*.

Conclusion

In the routine diagnostics of dermatophytoses, a high sensitivity and specificity could be achieved by combining cultural and molecular methods. Among the anthropophilic dermatophytes, *T. rubrum* was detected by far the most common and there was a significant increase due to *T. tonsurans* in tinea capitis. The zoophilic dermatophytes remain at a high level. The increase in *T. verrucosum* infections was new.

Session 4 - Immunology of fungal infections

S04-01

Immunometabolism during mucosal fungal recognition

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Cells of the innate immune system undergo long-term epigenetic and metabolic changes in response to pathogen-associated molecular patterns (PAMPs), resulting in enhanced immune responsiveness, termed trained immunity or innate immune memory. This mechanism occurs in responses to isolated fungal cell wall components such as β-glucan (a cell wall polysaccharide) or in complex mixtures (heat-killed *Candida albicans*). Repetitive encounters of phagocytic cells with β-glucan cause shifts from oxidative phosphorylation to glycolysis, and some of the resulting epigenetic changes mediate protective innate memory. Oral epithelial cells provide a barrier against commensal fungi and the first line of host defense against invading pathogens. These encounters result in β-glucan exposure and recognition of epithelial cells. Thus, we asked if β-glucan induces programmed memory in oral epithelial cells. We established an in vitro model of epithelial β-glucan programming, referred to as BGP-OECs (β-glucan programmed oral epithelial cells). We found that β-glucan priming induces epigenetic remodeling via reduction in DNA methylation in i*n vitro*. Using metabolic approaches, we identified that β-glucan recognition induces proline catabolism via proline dehydrogenase (Prodh) to sustain the TCA cycle and mitochondrial oxidative functions. Proline catabolism was, in part, required for innate memory since inhibition of Prodh during β-glucan stimulation decreased expression of distinct cytokines during reinfection with C. albicans. Moreover, exposure of *C. albicans* to oral mucosa tissue induces proline catabolism and increases the TCA cycle intermediates, while enhancing fungal clearance during acute mucosal reinfection. Collectively, our findings reveal that β-glucan-induced proline catabolism is crucial for innate immune memory in oral epithelial cells.

S04-03

Role of DNAM-1 in the NK cell-fungus interaction

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Natural killer (NK) cells are crucial in the defense against fungal infection in neutropenic patients e.g suffering from invasive pulmonary aspergillosis (IPA). Understanding the interactions between NK cells and the causative agent of IPA - *Aspergillus fumigatus* therefore is of high importance. Surface proteins play a pivotal role in immune cell-target interactions, including neural cell adhesion molecule 1 (NCAM-1; CD56) and DNAX accessory molecule 1 (DNAM-1; CD226). While NCAM-1 has been shown to play a key role in NK cell-mediated recognition and killing of invading fungi, the role of DNAM-1 in the interaction of NK cells and fungus is yet unclear. However, DNAM-1 dysfunctions in humans have been linked to various diseases, from autoimmune to viral infections and cancer. Here, we investigate the involvement of DNAM-1 in the interaction of NK cells with fungal hyphae using fluorescence microscopy techniques. Whereas DNAM-1 is displaced towards the immunological synapse upon contact with the fungus, single molecule analysis (dSTORM) of DNAM-1 in NK cells exposed to fungal hyphae did not reveal a similar behavior. However, purified and fluorescently tagged DNAM-1 protein stained the *A. fumigatus* hyphae cell wall, indicating a direct interaction between DNAM-1 and the fungal surface. Bioinformatic analysis suggested the cell-wall proteases opsB in *A. fumigatus* and Sap10 in *C. albicans* as potential target proteins for DNAM-1. Indeed, direct interaction of Sap10 and DNAM-1 was shown by fluorescence correlation spectroscopy (FCS). Furthermore, binding of purified surface-associated protease (Sap10) from *C. albicans* was only observed in primary NK cells and not in ΔDNAM-1 cells. NK cells treated with Sap10 exhibited an increase in protein and chemokine secretion, suggesting a stimulatory role of Sap10 in NK cells.

Session 5 - Mykosen in der Pädiatrie

S05-01

Invasive fungal infections in pediatric patients with ALL: Analysis of a prospective multinational study

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Background

In children with acute lymphoblastic leukemia (ALL), specific risk groups and periods of risk for invasive fungal disease (IFD) with need for antifungal prophylaxis are not well characterized. In addition, with the advent of new antifungal compounds, current data on outcome are scarce.

Patients and Methods

We screened prospectively captured severe adverse event reports of children enrolled in the international, multi-center clinical trial AIEOP-BFM ALL2009 (EudraCT 2007-004270-43) for proven, probable, and possible IFD, defined according to the updated EORTC/MSG consensus definitions.

Results

In a total of 6136 children (42.3% female, median age 5.2 years), 148 possible and 224 proven/probable IFDs (65 yeast and 159 mold) were reported. By logistic regression, the risk for proven/probable IFDs was significantly increased in children ≥12 years and in those with a

blast count of more than 10% in the bone marrow on day 15. Proven/probable IFDs significantly prolonged intensive chemotherapy, and 6-week and 12-week mortality were 10.7% and 11.2%, respectively. In the multivariate analysis, the hazard ratio for event-free and overall survival was significant for proven/probable IFD, age ≥12 years, and insufficient response to therapy (P<0.001, each).

Conclusions

Although the overall incidence rate of IFD in pediatric ALL is relatively low, it is significantly higher in older children and in those with insufficient treatment-response. These patients may benefit from targeted antifungal prophylaxis, as proven/probable IFD is an independent risk factor for event-free and overall survival.

S05-02

Pneumocystis jirovecii pneumonia in children with ALL

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Background

Pneumocystis jirovecii can cause life-threatening pneumonia (PjP), and patients with hematological malignancies are at high risk of this infection. Prophylactic measures have significantly decreased morbidity and mortality, but there is a paucity of contemporary data on the incidence and clinical course of PjP in well-defined and homogenous patient populations such as children suffering from acute lymphoblastic leukemia (ALL).

Patients and Methods

PjP was prospectively captured as severe adverse event in children enrolled in the international, multi-center clinical trial AIEOP-BFM ALL2009 (EudraCT 2007-004270-43). PjP was categorized as proven or probable according to recently published and updated definitions of *Pneumocystis jirovecii* disease in individuals without Human Immunodeficiency Virus infection and severity according to a grading system reported in the European Conference on Infections in Leukemia (ECIL) guidelines.

Results

In the multi-international trial AIEOP-BFM ALL2009 enrolling a total of 6136 children, PjP was diagnosed in six children (incidence 1/1000) and was associated with insufficient prophylaxis in five of them. Although none of the patients died of PjP, the long-term impact of the infection is unclear.

Discussion

Based on the results, current recommendations of PjP diagnosis and prophylaxis will be discussed.

S05-03

Detecting the often undetectable: Mucorales infection of a 11-year-old girl

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Introduction

Mucormycosis is an uncommon and deadly invasive fungal infection, being especially rare in pediatric patients. The main risk factors for mucormycosis in children are hematological malignancies and solid organ transplantation. Histopathology and culture are the diagnostic gold standard, but new molecular methods can play an important role for this difficult to diagnose disease.

Objectives

We attempt to illustrate the difficulties of a timely diagnosis of mucormycosis due to the unspecific clinical presentation and the difficulties arising from conventional diagnostic methods and elucidate the benefits of newly molecular diagnostic methods.

Patient and Methods

We present a severe and fatal case of rhino-orbito-cerebral mucormycosis in an 11-year-old child suffering from a recurrence of acute lymphatic leukemia treated with chemotherapy and corticosteroids who was admitted to the ICU due to severe neurological deterioration after initially presenting with abducens nerve palsy and anisocoria. There was an extensive diagnostic work-up including clinical examination, radiological imaging, cytology, clinical chemistry and microbiology trying to cover possible differential diagnosis. The patient died promptly after ICU admission without a final diagnosis.

Results

An autopsy revealed severe clotting of the internal carotid and cerebral arteries. Histopathological examination showed the presence of invasive hyphae in the thrombi, cerebral cortex and meninges, morphologically suspecting mucormycosis. The subsequent molecular analysis of the histopathological slides was not able to find the causative pathogen. Only reanalysis of ante-mortem obtained serum using molecular methods was able to identify the causative pathogen as *Lichtheimia corymbifera.*

Conclusion

Diagnosing mucormycosis remains a difficult task. Awareness of this differential diagnosis as well as newly molecular methods may help to achieve a promptly diagnosis in this time sensitive disease.

S05-04

EQUAL Candida Score: A tool for measuring the quality of candidemia management in Pediatrics

Andreas Groll (Münster/DE)

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Candida bloodstream infection in children and neonates is associated with high morbidity and mortality. Standards for management are available by international guidelines of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the European Conference on Infections in Leukemia (ECIL), and the Infectious Diseases Society of America (IDSA). While the management of candidemia is broadly similar across all patient groups, certain aspects of Candida bloodstream infection are specific to the neonatal and pediatric population.

A simplified guideline-driven point-based validation tool approach for the management of candidemia in adults has been published by the European Confederation of Clinical Mycology (ECMM). The ECMM Quality (EQUAL) Candida score has been validated and used in a variety of settings and is available as a smartphone application. The EQUAL Candida score captures the diagnostic and therapeutic aspects of the management of candidemia and provides a tool that can be utilized for the evaluation of guideline adherence. However, it is not specifically designed for use in neonates and children.

Here we review the current efforts to design a points-based pediatric EQUAL (ped-EQUAL) Score tool for management of candidemia and discuss the preliminary proposal for the scores for neonates and children beyond the neonatal period. This ped-EQUAL tool shall be used as future guidance to improve management and outcomes in pediatric patients with Candida bloodstream infections.

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Session 6 - Strategies for novel therapeutic approaches

S06-01

Human-like models for anti-biofilm activity studies of antifungal compounds

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In the past decade, azole-resistant *A. fumigatus* isolates (ARAF) have started to become more prevalent in clinical settings, representing a significant threat to the healthcare systems worldwide. A well-known factor that contributes to their decreased susceptibility against antifungal compounds is their biofilm formation capability. Several recent *in vitro* studies have shown the anti-biofilm effect of novel and traditional antifungal drugs.

However, it is not uncommon that *in vitro* results cannot be replicated in *in vivo* settings. Here, two distinct human-like models have been applied to test the activity of voriconazole (VCZ), amphotericin B (AMB), as well as the imidiazole luliconazole (LLCZ), against ARAF biofilms.

For this purpose, an *ex vivo* model with precision cut lung slices (PCLS) (N= 10 ARAF strains) and a biofilm co-culture model with human epithelial cells (A549) (N= 20 ARAF strains) have been used. The metabolic activity of the formed biofilm was quantified via an XTT assay at different biofilm developmental stages. The anti-biofilm effect was visualized via confocal laser scanning microscopy.

In the *ex vivo* PCLS model, biofilm formation in the early development stages was decreased by 52 % for LLCZ, 68 % for VCZ and 93 % for AMB, whereas the decrease in metabolic activity was only 20 % (LLCZ), 29 % (VCZ) or 25 % (AMB) for mature biofilm. A comparable strong inhibition of immature (2 h) biofilm (78% LLCZ, 75 % VCZ and 71 % AMB) was recorded in the co-culture model, while mature biofilm (24 h) was not significantly inhibited (13 % LLCZ, 15 % VCZ and 16 % AMPB).

This study is the first to use a PCLS *ex vivo* model to study antifungal compounds and their effect against *Aspergillus* biofilm formation. It could also contribute towards bridging the knowledge gap between *in vitro* and *in vivo* antifungal testing.

S06-02

Nanoparticles for the treatment of fungal infections

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Introduction

The treatment of life-threatening fungal infections is limited and needs urgent improvement. The discovery of novel antifungals with new mode of actions is not a limiting factor, rather than the pharmacological properties of these compounds like low solubility, high toxicity or low bioavailability. The use of nanoparticles (NPs) can overcome these limitations, as the pharmacological characteristics of these formulations are dominated by the polymer and not by the substance itself. However, the mechanism, how polymeric NPs deliver encapsulated substances into pathogenic fungi, is not fully understood.

Objectives

The aim of the study was the investigation of the interaction of polymeric NPs with several pathogenic fungi.

Materials & Methods

4 different polymers labelled with 3 different covalently attached fluorescent dyes were used to prepare the NPs. In addition, a fluorescent dye was encapsulated to track the NP and the cargo simultaneously. The interaction of the fluorescently labelled NPs with the molds *Aspergillus fumigatus*, *A. nidulans*, and *A. terreus*, or the yeasts *Cryptococcus neoformans* and *Candida albicans* was investigated by confocal laser scanning microscopy. Furthermore, the antifungal effect of the low water-soluble drug Itraconazole encapsulated in these NPs was tested.

Results

Irrespective of the applied conditions, none of the used NPs reached the fungal cytosol, but adhered to the fungal surface. In-depth characterization revealed that the NPs cross the fungal cell wall, but remain in invaginations of the cytoplasmic membrane. Nevertheless, encapsulating a fluorescent dye or Itraconazole lead to an accumulation of the fluorescent dye in the fungal lumen or a lower minimal inhibitory concentration compared to the pristine drug, respectively.

Conclusion

Although NPs are not internalized by human pathogenic fungi, the delivery of substances like antifungals into these microorganisms with the help of NPs is possible and effective.

S06-03

Host variability influences success of interferon-γ to augment *Candida albicans* killing by human PBMCs

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Invasive candidiasis in immunocompromised patients is a major nosocomial infection in Intensive Care Units (ICUs). Despite the available antifungal treatments, the mortality rate of candidiasis remains high. Therefore, therapies addressing the compromised immune status of these patients are considered an attractive approach that can improve the outcome of invasive candidiasis. Specifically, interferon-γ (IFNγ) immunotherapy has shown promising results in individual cases and small case series. However, little is known about how inter-individual differences impact the effectiveness of IFNγ to augment antifungal immunity. In our study, we

investigated how IFNγ affects the fungal killing capacity and cytokine responses in a cohort of over 150 healthy blood donors. IFNγ differentially impacted the ability of peripheral blood mononuclear cells (PBMCs) to kill *Candida albicans*. Interindividual differences such as age and gender did not correlate with the differential effects of IFNγ. Yet genetic variation in the immunomodulatory gene *LY86* and the autophagy-related gene *IRGM* were associated with a distinctive efficiency of IFNγ to augment *C. albicans* killing. Further, our data suggest a beneficial role of autophagy and detrimental effects of apoptosis, necroptosis and TNF-signaling on IFNγ-augmented fungal clearance. Overall, our study highlights the importance of interindividual differences in host-directed medicine against invasive candidiasis and provides targets for additional modulation to improve efficacy of IFNγ.

S06-04

Molecular characterization of *Candida albicans* attributes during translocation and dissemination and the effects of immunotherapy

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Translocation across the intestinal barrier into the bloodstream by intestine-colonizing *Candida albicans* cells serves as an important source of disseminated candidiasis. This process occurs under predisposing host conditions, like compromised innate immunity and a dysfunctional intestinal barrier. We have showed that fungal translocation across intestinal epithelial cells (IECs) requires the fungal peptide toxin candidalysin (CaL) and the resulting tissue damage. Other host and fungal factors that contribute to this process and the effect of immunotherapy strategies like interferon gamma (IFN-γ) treatment are not well-understood.

In vitro IEC infection models and dual-species RNA sequencing were used to investigate these processes. We found that zinc acquisition from the host promotes fungal growth and facilitates host-cell damage, but that CaL-mediated damage is also required to access zinc from the host. On the host side, IECs up-regulated inflammatory pathways involving NFκB signaling, which limited epithelial cytotoxicity and barrier breakdown. Conversely, IFNγ treatment increased fungal translocation. Our data suggest this is due to altered expression of junction proteins and disorganization of tight junctions. Together, our results show host-nutrient acquisition promotes *C. albicans* pathogenicity and required fungal-mediated cytotoxicity. IECs help to resist *C. albicans* infection by maintaining the intestinal barrier integrity and limiting damage. Though this can be counteracted by inflammatory cytokines like IFNγ.

Currently, we are working to identify specific fungal processes that mediate dissemination from the intestine and to determine the mechanisms by which NFκB activation limits *C. albicans* pathogenicity. We are also employing an intestine-on-chip infection model to further evaluate the impact of immunotherapy strategies on *C. albicans* infection of the intestinal barrier.

Session 7 - Pilzinfektionen in der Hämatologie

S07-01

Diagnose von Mukormykosen mittels PCR aus Blut

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Fragestellung

Fadenpilze der Ordnung Mucorales verursachen bei Immunsupprimierten lebensbedrohliche Infektionen ("Mukormykose"). Da nach wie vor keine Biomarker für den Nachweis im Blut verfügbar sind, kann die Diagnose meist nur anhand von invasiv gewonnenem Probenmaterial gestellt werden. Die Diagnose wird häufig zu spät oder bei fulminantem Verlauf gar nicht gestellt, so dass bei immunsupprimierten Patienten die Letalität sehr hoch ist. Neuere Publikationen beschreiben PCR-Verfahren aus Blut, die den DNA-Nachweis zu einem frühen Infektionszeitpunkt ohne invasive Probenentnahme ermöglichen könnten. Ziel dieser Arbeit war es, retrospektiv bei Patienten mit gesicherter Mukormykose die Wertigkeit eines PCR-Nachweises aus Serum oder Plasma für die Diagnostik zu untersuchen.

Methoden

Retrospektiv wurden in einem 10-Jahreszeitraum insgesamt 13 hämato-onkologische Patienten mit gesicherter Mukormykose (radiologischer Befund, Blankophorpräparat, Kultur und DNA-Nachweis aus Gewebe/respiratorischem Material oder Histologie) identifiziert. Als Tag 0 wurde der Zeitpunkt der Diagnosestellung mit den bisher angewandten Verfahren gewertet. Asservierte Seren und Plasmen dieser Patienten mit Entnahme sowohl vor als auch nach diesem Zeitpunkt wurden mittels einer Mucorales-spezifischen Multiplex Real-Time-PCR untersucht.

Ergebnisse

Bei 9 von 13 Patienten konnte mittels PCR aus Serum bzw. Plasma der Nachweis von Mucorales-DNA zwischen 3 und 29 Tagen vor dem Zeitpunkt 0 geführt werden.

Schlussfolgerungen

Diese Ergebnisse zeigen, dass eine frühe Diagnose dieser Infektion möglich wäre. In Zukunft scheint ein Screening von Hochrisikopatienten denkbar.

S07-02

Molecular diagnostic strategies in cancer patients with suspected respiratory mold infections

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Background

Invasive fungal infections (IFI), most notably invasive aspergillosis are serious complications in cancer patients. Additional fungi including the mucorales, and polymicrobial infections are a concern, but optimal fungal detection strategies have not been defined.

Methods

In a prospective multicenter study, we compared fungal detection by culture, broadrange and specific qPCR assays and fluorescence *in situ* hybridization (FISH) targeting *Aspergillus* and mucorales from bronchoalveolar lavage fluid (BALF) of adult cancer patients with suspected fungal pneumonia. Comparison of culture and molecular tests for detection of molds was primarily done for proven/probable episodes. In subgroups of patients, fungal DNA was concomitantly amplified from serum, as well as CMV, and respiratory viruses from BALF.

Results

We included 210 IFI episodes classified according to EORTC/MSG criteria (proven: 3, probable: 72, possible: 107, unclassifiable: 28). Broadrange PCR was terminated due to frequent amplification of colonizing/contaminating fungi. Specific qPCR assays demonstrated higher sensitivity for detection of molds (*Aspergillus*, Mucorales) than culture (47% vs 21%; p=0.05). Of note, mucorales DNA was detected in 6 of 7 BALF together with *Aspergillus* DNA. Aspergillus qPCR in serum was less likely than in BALF to identify mold DNA (27/50 (54%) vs. 10/50 (20%); p=0.0001). Detection of *Aspergillus* or yeasts by FISH was a predictor for subsequent cultivation of *Aspergillus* and *Candida* (p<0.0001). CMV was detected in 12%, respiratory viruses in 11% of BALF samples. Detection of CMV or respiratory viruses was not associated with proven/probable mold infection.

Conclusions

Specific qPCRs from BALF is superior to BALF culture and serum PCR to identify molds in cancer patients with suspected mold pneumonia. Molecular tests restricted to *Aspergillus* may underestimate the presence of mucorales. FISH may predict culturability and appears to be an indicator of recent exposure rather than invasive infection.

S07-03

Mucormycosis after allogeneic hematopoietic stem cell transplantation and the rationale for isavuconazole therapeutic drug monitoring

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Introduction

Mucormycosis is an acute and aggressive invasive fungal disease (IFD) usually - but not exclusively - occurring in immunocompromised patients. The diagnosis has to be made by combining patient's risk factors, the clinical presentation, and mycological findings. Due to the high morbidity and mortality, prompt initiation of antifungal treatment is crucial, e. g. with liposomal amphotericin B (LAMB) or isavuconazole (ISA). Moreover, a multidisciplinary therapeutic approach - including surgery - is essential to ensure the most favourable outcome.

Objective

Our case highlights the pitfalls in diagnosis and treatment of mucormycosis and illustrates the need for ISA therapeutic drug monitoring (TDM) under special clinical circumstances.

Case Report

We report a case of rhino-orbital-cerebral mucormycosis in a 74 year old male patient with relapsed acute myeloid leukaemia (AML) after allogeneic hematopoietic stem cell transplantation (HSCT). The patient was admitted with febrile neutropenia, initially presented with symptoms of an upper respiratory infection, and broad-spectrum antibiotics were immediately initiated. Furthermore, the patients was already on antifungal prophylaxis due to his relapsed AML. As his condition did not improve, further diagnostic was initiated and an IFD was suspected. Therefore, we started high dose LAMB and the patient first stabilized. In parallel, rhino-orbital biopsies revealed *Rizopus* spp. as the causing pathogen. Due to nephrotoxicity, antifungal treatment was switched to ISA. However, the patients rapidly deteriorated and died due to intracranial complications. Afterwards, drug levels of ISA were found to be significantly lower as expected.

Conclusion

Our case proofs again, that early diagnosis, and a multidisciplinary treatment approach are essential to achieve a favourable outcome. Moreover, it also highlights the need for ISA TDM also in immunocompromised patients - e. g. after HSCT - as already reported for ICU patients.

S07-04

Gastrointestinal mucormycosis in adults: an analysis of 319 adults from the FungiScope® registry and literature

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Background

Gastrointestinal mucormycosis (GIM) presents diagnostic challenges due to its nonspecific symptoms, often leading to delayed treatment. This study aimed to analyze the clinical characteristics, treatment, and outcomes of GIM.

Methods

An analysis was conducted on patients with GIM recorded in the FungiScope® registry and cases reported in the literature from January 2003 to December 2023. The study included patients aged 18 and older.

Results

A total of 319 invasive GIM infections were identified across 46 countries. Species identification was possible in 48.6% of cases, with *Rhizopus* (22.3%), *Mucor* (11.0%), *Rhizomucor* (6.3%), and *Lichtheimia* (4.7%) being the most common. Major predisposing factors included haemato-oncological malignancies (45.5%), diabetes mellitus (19.4%), and ICU admission (21.9%). Frequent symptoms were fever (47.6%), abdominal pain (53.6%), gastrointestinal bleeding (29.2%), and gastrointestinal perforation (17.6%). The stomach (33.5%), liver (26.6%), large intestine (26.3%), and small intestine (24.1%) were commonly affected, with an overall dissemination rate of 36.3%. Diagnosis was achieved via histopathologic examination (90%), culture (32.3%), and molecular methods (23.8%), with 20.4% diagnosed postmortem. Systemic antifungal therapy was administered in 85% of cases, predominantly liposomal amphotericin B (51.1%), and surgical interventions were performed in 59.6% of cases. Overall mortality was 58.9%, whereby localized disease was associated with a significant better outcome compared to multifocal and disseminated infection.

Conclusion

GIM diagnosis is difficult, often occurring postmortem, highlighting a systematic underrecognition. Prompt diagnosis and immediate treatment, including surgery, are vital for improving patient outcomes.

Session 8 - Fungal Genomes & Genome Analysis

S08-01

Evolution of *Candida albicans* in the modern human host – Analysis and comparison of strains from unusual sources

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In modern Western societies, the yeast *Candida albicans* is frequently found as a commensal member of the gut microbiota, which serves as a reservoir for severe infections. The human gut microbiota has changed significantly due to industrialization. Interestingly, a study of a mostly isolated, non-Western human population found little indications of *Candida* colonization. Furthermore, *C. albicans* strains from potential environmental reservoirs are still rarely found and little is known about them.

We aim to understand the evolution of *C. albicans* as it adapted to the human host and the effects the changes in human lifestyle and diet had during industrialization on this member of the human microbiome.

Studying over 30 strains from diverse sources, including isolated human populations, animals, and the environment, we conducted metabolic, genomic, antifungal sensitivity, and virulence assays. With laboratory evolution experiments, we explored how *C. albicans* adapted to life in the modern Western gut.

Human-derived strains exhibited a generally higher virulence, damaging epithelial cells more than environmental strains. Some of these commensal human isolates were even more damaging than clinical isolates. In general, the metabolic spectrum of environmental strains was narrower than that of human-derived strains, especially for sugars. Several non-clinical strains showed resistance to typical antifungal agents. For the experimental evolution, non-

human adapted strains were continuously cultured for months in media containing different dietary sugars. The resulting strains were specialized to grow better on the dietary sugar provided, but worse on other sugars. A systematic genome-level comparison of the adapted strains, their environmental progenitors, and human isolates is underway.

Our findings reveal metabolic and virulence disparities between human-derived and environmental *C. albicans* strains and suggest an evolutionary trade-off in adapting to dietary sugars.

S08-02

Host adaptive microevolutionary processes in *Microsporum canis*

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Introduction

Microsporum canis is the predominant pathogen causing dermatophytosis in companion animals (dogs, cats, rodents), it can cause low-high inflammatory infection in the human hosts. Our preliminary research found that the species is undergoing a transition from a truly zoophilic entity (*M. canis*) to two sister anthropophiles (*M. audouinii* and *M. ferrugineum*). Still, the precise mechanisms underlying this evolutionary process remain elusive.

Methods

we conducted comparative genomic analysis and transcript-level validation of *Microsporum* in five different mammal hosts from various geographic locations, characterising nuclear and mitochondrial genome evolution processes.

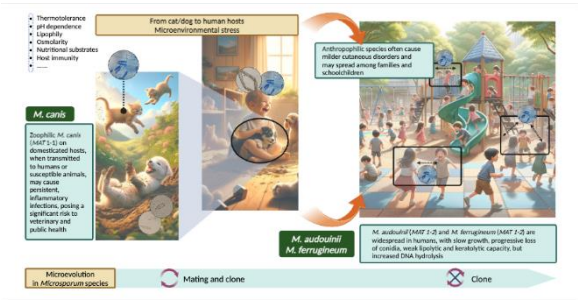
Results

Isolates of the same species from different hosts display minor nucleotide diversity, yet significant disparities in protein function are evident across the spectrum, from zoophiles to two anthropophilic. Furthermore, aspects of carbohydrate metabolism are implicated in the host shift, signal transduction regulation, and post-translational modifications contribute to microevolution and host specificity. Among the differential genes influencing host fitness, emphasis is placed on proteases affecting phenotype (*Arb2, PRB1*), sporulation (*Arb2*), endoproteases (*SUB1, SUB7*), lipases (*Glip2*), and those involved in pH-adaptive regulation (*SedA*). Lipophily, Osmolarity and pH in the Microenvironment are important factors affecting the transcriptional levels of key endoproteases Subs. Additionally, we identified highly interconnected block regions in coding protease and CAZy genes and conducted an analysis of the relationship between SNP site mutations and haplotypes.

Conclusions

This study analyses the mechanisms involved in changes in microenvironmental adaptation of *Microsporum* species from companion animals to human hosts, providing a model for understanding dermatophyte adaptation to host studies.

Figure 1



S08-03

The proteomic response of *Aspergillus fumigatus* to Amphotericin B (AmB) reveals the involvement of the RTA-like protein RtaA in AmB resistance

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The opportunistic human pathogen *Aspergillus fumigatus* poses a significant threat by causing mycoses, which can be fatal especially in immunocompromised individuals. Due to the increase of azole resistance in *A. fumigatus*, treatment options are often limited to amphotericin B (AmB), a member of the polyene family of antifungals that has well known side effects. A rising number of resistant isolates against AmB as well as limited knowledge about resistance and compensatory mechanisms give rise to concerns. To elucidate the effects of AMB on the fungal proteome, we conducted liquid chromatography-tandem mass spectrometry analyses to identify changes in the proteomic profiles of *A. fumigatus* treated with sublethal concentrations of AmB and its liposomal formulation. Selected proteins with significant increase in abundance upon AmB exposure were then characterized.By comparison of the proteomic response of AmB-treated samples and untreated controls, we found significant increases in the abundance of proteins belonging to secondary metabolite biosynthesis gene clusters, proteins anchored to the membrane, involved in catabolic processes or aromatic acid degradation. One of the proteins with the highest increase in abundance was RtaA, a fungal Rta1-like family protein. While deletion of *rtaA* led to increased sensitivity against AmB, overexpression resulted in a two-fold increase of resistance. Interestingly, only treatment with AmB and nystatin led to a rise of *rtaA* transcript levels, which hints towards a specific protection mechanism against polyenes. Deletion of *rtaA* did not significantly change the content in ergosterol and intracellular lipid droplets of *A. fumigatus.* While not being crucial for the virulence of *A. fumigatus* itself, RtaA is most likely involved in the resistance against AmB by maintaining lipid homeostasis and membrane stability. These findings reveal a novel polyene resistance mechanism.

S08-04

Analysis of intra-species diversity of *Candida albicans*

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Introduction

The yeast *Candida albicans* is a member of the human microbiota that can cause serious infections in immunocompromised hosts, earning it a place on the WHO priority pathogens list. Past research on *C. albicans* has mainly focused on the reference strain SC5314, which has helped elucidate many key aspects of the infection process. However, it has recently become clear that *C. albicans* is a heterogeneous species both regarding phenotype and genotype.

Aim

Investigate intraspecies variability with a focus on genotypic and phenotypic features that impact the fungal cell wall.

Methods

Complete genomes of *C. albicans* isolates were sequenced and aligned, then SNPs were called, and mutation pressure was calculated. For phenotypic analyses, 218 *C. albicans* isolates were characterised using a high-throughput approach that assessed each strain"s ability to form biofilms and grow efficiently under conditions relevant to life in the human host, including exposure to cell wall-perturbing chemicals.

Results

The biggest driver of intraspecies variability were strain-dependent differences rather than clade-dependent differences. Strains did not cluster according to their geographic or isolation site origin. Clustering by clade was only observed for Clade 13 (also known as *C. africana*) which significantly underperformed compared to other clades under many of the stress conditions tested. By combining genotypic and phenotypic data, several variants were identified that influence cell wall variability in *C. albicans* clinical isolates.

Conclusion

Our study highlights significant intra-species variability of the WHO priority pathogen *C. albicans*, emphasizing the need to include strains from diverse genetic backgrounds in future studies.

Session 9 - Updates aus der Diagnostik

S09-01

Exploring Invasive Fungal Infection Diagnosis and Treatment: A Cross-Continental Comparison between Brazil and Europe

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Background

Brazil's tropical climate and a higher prevalence of immunocompromised cases, such as those with HIV/AIDS, contribute to increased invasive fungal infection (IFI) rates like histoplasmosis and paracoccidioidomycosis. In Europe, vulnerable populations undergoing immunosuppressive therapies or organ transplants face elevated risks of IFIs such as candidiasis and aspergillosis.

Methods

An online survey engaged mycologists across Europe and Brazil to assess IFI diagnostic capacities. The survey covered institutional characteristics, IFI perspectives, and the use of diagnostic techniques like microscopy, culture, serology, molecular testing, and therapeutic drug monitoring.

Results

As of 2023, the survey included 484 centers, with 96 in Brazil and 388 in Europe. In Brazil, 45% had on-site mycological diagnostics, while in Europe, it was higher at 58% ($p<0.001$). In Brazil, microscopy was available in 96% of centers, culture in 95%, antigen detection in 63%, antibody detection in 47%, and molecular tests in 19%. In Europe, these figures were: culture 99%, microscopy 97%, antigen detection 94%, molecular tests 85%, and antibody detection 82%. Significant disparities were observed in antigen, molecular, and antibody detection ($p<0.001$). Regarding antifungal treatment, in Brazil, at least one form of amphotericin B was used in 72% of centers, one echinocandin in 55%, fluconazole in 93%, and itraconazole in 66%. In Europe, these figures were: amphotericin B 87%, echinocandin 89%, fluconazole 93%, and itraconazole 89%. Access to all antifungals differed significantly ($p<0.001$).

Conclusions

Brazil's healthcare disparities and limited advanced care access impact IFI diagnosis and management, while Europe's established healthcare infrastructure aids in early detection and treatment. Improving capabilities in Brazil is crucial.

S09-02

Is the T2MR Candida Panel a suitable method for the rapid diagnosis of candidemia in routine clinical practice?

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The diagnosis of invasive *Candida* infection remains challenging due to tests with slow turnaround times or mediocre performance. T2MR is a new diagnostic tool. We investigated the diagnostic accuracy of the T2Candida® panel (T2) in comparison to blood culture (BC) and the SeptiFast® (SF) for the detection of five different *Candida* species among high-risk ICU patients with suspected candidemia.

Material and Methods

We analysed blood samples collected from patients with suspected candidemia (177 samples from 138 patients) from August 2018 to April 2020. Blood samples were collected and analysed concurrently by BC, SF and T2Candida®. Subsequently – based on clinical and microbiological findings – patient samples were assigned to specific risk categories (proven, probable, no candidemia). As a sequelae of our study the performance of T2MR in daily routine diagnostics was also performed.

Results

Twenty-two samples from 17 patients were classified as proven candidemia, and 15 samples from 14 patients were classified as probable candidemia. A sensitivity of 68.2% was observed for the BC and the SF, and a sensitivity of 63.6% was observed for the T2 when only cases with proven candidemia were evaluated. For proven & probable candidemia, the sensitivity was 40.5% for BC, 81.1% for SF and 73.0% for T2.

Conclusions

The diagnostic performance of SF and T2 was similar. For samples with proven/probable candidemia, SF and T2 had a higher sensitivity compared to BC. Used in conjunction with other diagnostic methods, T2 can replace the no longer available SF for the diagnosis of candidemia, enabling the timely initiation of a targeted antifungal therapy.

S09-03

Identification of filamentous fungi: an evaluation of three MALDI-TOF mass spectrometry systems

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Background

Identification of filamentous fungi still poses a major challenge to laboratories. Matrix assisted laser desorption / ionization time of flight mass spectrometry (MALDI-TOF MS) is a promising tool, since it offers cheap and fast results. Different MALDI-TOF MS systems are available for

routine laboratories. This is a comprehensive head-to-head comparison of three devices and the respective reference spectrum databases.

Materials and methods

A set of 79 pre-characterized isolates of filamentous fungi was measured (in duplicates) parallelly with three MALDI-TOF MS systems after 24 h, 48 h, and 72 h of incubation: 1) Biotyper smart ("BT", Bruker Daltonics), 2) EXS2600 ("EXS", Zybio), and 3) VITEK MS PRIME ("VITEK", bioMérieux)

Results

82 %, 77 %, and 86 % of isolates yielded valid (green score) measurements using BT, EXS, and VITEK over all three measurement timepoints. Depending on the MALDI-TOF MS system, validity rates ranged from 58-82 % for the different timepoints. Correct results (green score) were obtained for 82 %, 73 %, and 81 % of isolates by BT, EXS, and VITEK. BT was the system to require the most duplicate measurements. EXS displayed the highest rate of misidentification events. VITEK tended to provide valid and correct measurement results particularly after 48 h incubation.

Conclusion

Based on our experience, all three devices proved to be suitable for routine diagnostics. The timepoint of measurement had a major impact on the quality of analysis and should be considered by the user. Laboratories should be aware of the limitations of the applied system.

S09-04

Diagnostic performance of three lateral flow assays for measurement of (1–3)-β-D-glucan, mannan antigen and anti-mannan IgG antibodies in patients with candidemia: a prospective case-control study

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Introduction

Candidemia is the fourth most common blood stream infection on ICU and is associated with a high mortality. Rapid diagnosis is essential for timely initiation of antifungal therapy.

Objectives

We aimed to analyse the performance of three lateral flow assays (LFA) for the measurement of (1–3)-β-D-glucan, mannan antigen and anti-mannan IgG antibodies in sera of patients with and without candidemia.

Methods

Serum samples of 248 patients with blood culture-proven candidemia and 198 controls were tested with the TECO® Fast Fungus (1-3)-ß-D-Glucan LFA (BDG-LFA), the Fast *Candida* Mannan Antigen LFA (Mannan-LFA) and the Fast *Candida* IgG Antibody LFA (anti-Mannan-

LFA), respectively. The assays have a turn-around-time of approximately 15 min (BDG-LFA, anti-Mannan-LFA) and 40 min (Mannan-LFA). The control sera were taken from hospitalized patients with negative blood cultures (n=138) and bacteremia (n=60), respectively. The readings were performed with the TECO FIC-Q100N immunofluorescence analyzer.

Results

Candidemia was caused by *Candida* (*C.*) *albicans* (46.4%), *C. glabrata* (27.4%), *C. parapsilosis* complex (10.1%), *C. tropicalis* (5.2%), *C. krusei* (4.8%), and other *Candida* spp (2.8%). A mixed candidemia occurred in 3.2% of patients. The sensitivity, specificity and area under the ROC-curve (AUROC) was 51.2%, 80.7% and 0.660 for the BDG-LFA; 45.6%, 86.9% and 0.662 for the Mannan-LFA; and 44.8%, 75.6% and 0.602 for the anti-Mannan-LFA. Diagnostic performance of all LFAs alone and in combination is shown in table 1.

Conclusion

All three LFAs offer rapid and cost-efficient testing of individual samples and show a comparable diagnostic performance. In terms of time-to-result, the LFA format is superior to classic EIA and LAL tests if only small sample numbers are to be analysed. A combination of LFAs can improve the performance, with the highest sensitivity for BDG-LFA + anti-Mannan-LFA and the highest AUROC for BDG-LFA + Mannan-LFA.

Figure 1

Lateral Flow Assay	Sensitivity [%] (95%-CI)	Specificity [%] (95%-CI)	AUROC (95%-CI)
BDG-LFA	51.2 (44.8 - 57.6)	80.7 (74.5 - 86.0)	0.660 (0.614 - 0.704)
Mannan-LFA	45.6 (39.3 - 52.0)	86.9 (81.3 - 91.2)	0.662 (0.616 - 0.706)
anti-Mannan-LFA	44.8 (38.5 - 51.2)	75.6 (69.0 - 81.5)	0.602 (0.555 - 0.648)
Mannan-LFA and anti-Mannan-LFA	65.3 (59.0 - 71.2)	65.5 (58.4 - 72.1)	0.654 (0.608 - 0.698)
BDG-LFA and Mannan-LFA	65.3 (59.0 - 71.2)	71.1 (64.2 – 77.3)	0.682 (0.636 – 0.725)
BDG-LFA and anti-Mannan-LFA	72.6 (66.6 – 78.0)	60.9 (53.7 – 67.8)	0.667 (0.622 – 0.711)

Table 1: Diagnostic performance of the three LFAs alone and in combination.

Session 10 - Systems Biology of Fungal Infections

Siehe Kapitel “Lectures and Poster”

Session 11 - Resistenzen und Neue Wirkstoffe

S11-01

Bacterial-fungal interactions impact intestinal colonization and host immune maturation in germ-free and monocolonized mice

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Dysbiosis of the mucosal microbiota correlates with fungal overgrowth, in particular *Candida*, which is a risk factor for disseminated candidiasis. During dysbiosis, a parallel expansion of distinct bacteria, such as *Enterococcus faecalis*, is observed in patients. Probiotic bacteria that confer protective mucosal immunity, such as *Bacteroides vulgatus* mpk, might be useful as prophylactic therapy. Unpublished data show that the enterococcal virulence factor cytolysin is crucial for synergistic damage with *C. albicans in vitro* and in a model of oropharyngeal candidiasis. *B. vulgatus* is able to mitigate this synergistic damage *in vitro*. Here, we focus on the role of cytolysin and prior colonization with *B. vulgatus* mpk in bacterial-fungal interactions and the resulting consequences for intestinal colonization and host immune maturation.

To investigate isolated interactions of fungi and bacteria with the host, we colonized germ-free or *B. vulgatus* mpk-colonized C57BL/5NTac mice with *C. albicans* alone or in combination with different *E. faecalis* strains with and without cytolysin. Temporal and spatial changes in microbial burden were assessed in feces and intestinal organs. Bacterial burden in feces was similar for both enterococcal strains, stable over time, and not affected by *C. albicans*. Early, but not late, colonization levels of *C. albicans* were lower in the presence of enterococci. Preliminary analysis of host responses to colonization by flow cytometry, comprehensive cytokine and oxylipin profiling revealed differences depending on the type of colonization.

Thus, *B. vulgatus* mpk does not reduce intestinal fungal colonization in gnotobiotic mice but might affect immune responses.

S11-02

Investigating potential links between the unfolded protein response and triazole resistance in *Aspergillus fumigatus*

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Introduction

The opportunistic human-pathogenic fungus *Aspergillus fumigatus* naturally secretes large amounts of extracellular enzymes when growing on complex substrates, and intense protein secretion can also be induced by stress factors or antifungal agents. Increased secretion levels involve activation of the unfolded protein response (UPR) via the transcription factor HacA, which promotes endoplasmic reticulum (ER) stress adaptation and ER homeostasis. A recent transcriptomics study revealed that triazole-resistant *A. fumigatus* isolates with tandem repeat (TR_{34} or TR_{46}) mutations in the promoter of the *cyp51A* gene, which result in overexpression

of the ER transmembrane protein and azole target CYP51A, show increased expression of UPR-related genes compared to susceptible isolates (Hokken *et al.*, 2023, *J. Fungi*, 9(8):807).

Objectives

We are investigating whether increased levels of CYP51A cause ER stress, which could render triazole-resistant isolates more dependent on the UPR.

Material and Methods

Mycelial growth tests and microtiter plate assays were used to compare the susceptibility profiles of triazole-susceptible vs. resistant isolates to ER stress compounds. We also assessed the impact of combining triazoles with the UPR inhibitor 4µ8C on resistant isolates. Moreover, we are testing the effect of artificially inducing *cyp51A* levels in the UPR-deficient Δ*hacA* mutant.

Results

Triazole-resistant isolates with TR_{46}, but not TR_{34}, *cyp51A* genotypes were more growth-impaired than triazole-susceptible isolates when exposed to the ER stressor dithiothreitol. Treatment of triazole-resistant isolates with 4µ8C increased the minimum inhibitory concentrations of triazole drugs, indicating drug antagonism.

Conclusion

Investigating the UPR in the context of antifungal resistance in *A. fumigatus* can uncover differential adaptations in triazole-resistant isolates, which might inform antifungal strategies that exploit secretion stress.

S11-03

The zinc cluster transcription factor Znc1 regulates Rta3-dependent miltefosine resistance in *Candida albicans*

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Zinc cluster transcription factors (ZCFs) are a family of transcription regulators that are almost exclusively found in the fungal kingdom. Activating mutations in the ZCFs Mrr1, Tac1, and Upc2 are a frequent cause of acquired resistance to the widely used antifungal drug fluconazole in the pathogenic yeast *Candida albicans*. Similar to a hyperactive Tac1, a constitutively active form of the ZCF Znc1 causes increased fluconazole resistance by upregulating the multidrug efflux pump-encoding gene *CDR1*. Hyperactive forms of both Tac1 and Znc1 also cause overexpression of *RTA3*, which encodes a 7-transmembrane receptor protein involved in the regulation of asymmetric lipid distribution in the plasma membrane. *RTA3* expression is also upregulated by miltefosine, an antiparasitic drug that is active against fungal pathogens and considered for treatment of invasive candidiasis, and *rta3*Δ mutants are hypersensitive to miltefosine. We found that activated forms of both Tac1 and Znc1 confer increased miltefosine resistance, which was dependent on *RTA3* whereas *CDR1* was dispensable. Intriguingly, the induction of *RTA3* expression by miltefosine depended on Znc1, but not Tac1, in contrast to the known Tac1-dependent *RTA3* upregulation by fluphenazine. In

line with this observation, *znc1*Δ mutants were hypersensitive to miltefosine, whereas *tac1*Δ mutants showed wild-type tolerance. Forced expression of *RTA3* reverted the hypersensitivity of *znc1*Δ mutants, demonstrating that the hypersensitivity was caused by the inability of the mutants to upregulate *RTA3* in response to the drug. These findings establish Znc1 as a key regulator of miltefosine-induced *RTA3* expression that is important for wild-type miltefosine tolerance.

S11-04

Structural stratification of *cyp51A* polymorphisms and their effects on azole susceptibility in *Aspergillus fumigatus*

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Environmental spread of azole resistant *Aspergillus fumigatus* has emerged as a global public health problem concerning critically ill patients, stem cell recipients, or those suffering from cancer or pulmonary stresses such as COVID19. Mostly, clinical azole resistance in *A. fumigatus* can be attributed to mutations in the *cyp51A* gene, dominantly to those with changes in the promotor (TR_{34}/L98H and TR_{46}/Y121F/T289A). However, numerous rarer substitutions are described whose functional relevance is unclear.

Compilation of MIC values from the literature associated with individual substitutions shows heterogeneous distribution between intermediate and resistance categories in several cases. To address phylogenetic differences among those isolates, 210 publicly available *A. fumigatus* genome sequences were analyzed: isolates with TR_{34}/L98H, N248K, and G138C cyp51A-alleles formed phylogenetic clusters, each indicating a similar ancestor, however, other substitutions were not yet sufficiently represented. MIC values of isolates with TR_{34}/L98H alleles also aligned along the genetic marker *csp1*. Together, this indicates an influence of the strain background of susceptibility values in addition to the *cyp51A* substitutions themselves.

We then created a collection of individually constructed strains containing each of the known *cyp51A* polymorphisms, promotor variants, and frequent combinations thereof in the same genetic background and measured MIC values towards a panel of clinical drugs as well as agricultural fungicides according to the EUCAST protocol. This allows differentiation of structural groups of substitutions that have no effect at all, those effecting only on long-chained or those effecting mainly short-chained azoles. For several clinical relevant resistance substitutions, we observed increased susceptibility against agricultural fungicides, making environmental selection unlikely. In addition, several mutations present mainly among strains from environmental samples conveyed decreased susceptibility towards several fungicides only.

Session 12 - Aspergillus Infection Biology

S12-01

Ablation of Mod5-dependent tRNA isopentenylation modulates antifungal resistance and gene expression

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Human fungal pathogens cause over a billion infections annually worldwide making them a global cause of concern. Rapidly increasing occurrence of antifungal drug resistance is a major challenge associated with these infections. Resistance can be achieved by different mechanisms including genetic mutations and/or epimutations, but understanding RNA modifications in the context of resistance development is only in its infancy in critical fungal pathogens. RNA modifications, summarized in the epitranscriptome, are naturally found alone or in combination on several RNA species. Here, we assessed the consequences of deleting tRNA-modifying isopentenyl transferase ortholog Mod5 of *Aspergillus fumigatus*, resulting in altered stress responses and unexpectedly, resistance against the antifungal drug 5-fluorocytosine (5-FC). Combination of transcriptomic and proteomic approaches revealed comparable adaptation to 5-FC stress. Additionally, the knockout displayed premature activation of cross-pathway control (CPC) genes, which was further increased after antifungal treatment. Associations of codon usage patterns with proteomics abundances showed negative correlations with the number of $tRNA^{Tyr}_{G\Psi A}$-decoded codons in the knockout, indicative of modification-tuneable transcripts. Subsequent rescuing approaches by $tRNA^{Tyr}_{G\Psi A}$ overexpression in Δ*mod5* reversed select phenotypes but the 5-FC resistance remained. In conclusion, 5-FC resistance caused by *mod5* deletion is multifaceted, with an altered transcriptome and translatome driving *A. fumigatus* towards starvation responses over optimal growth. This uncovers a potential mechanism that results in transient antifungal resistance depending on RNA modification.

S12-02

Unveiling the Potential of Complement-Based Therapy in Fungal Infections: Promising Results in a Murine Model of Invasive Pulmonary Aspergillosis

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Introduction

Complement bridges innate with adaptive immunity and serves as a first-line defense against non-self structures like fungi. We showed the binding of MASP-1, a complement serine protease, to *Aspergillus fumigatus*, which results in the activation of complement and induction of phagocytosis. Since there is an urgent need for new antimycotics due to the increasing prevalence of invasive pulmonary aspergillosis (IPA) and the emergence of antifungal-resistant strains, an antibody-like structure called peptibody was designed as a new antifungal drug candidate consisting of a human IgG1 Fc region, a hinge region, and, instead of the Fab region, a 30 amino acid long peptide derived from MASP-1.

Objectives

Investigate the efficacy of a new complement-based therapeutic approach against fungal infections in a murine model of IPA.

Methods

Cyclophosphamide-treated mice were intranasally infected with *Aspergillus fumigatus* conidia and either treated with three selected peptibody dosages (0.6, 6, or 60 mg/kg mouse) or, as a control, "mock-treated" with PBS at 1 h, 8 h, and 24 h post-infection. Survival was monitored for 14 days, Bronchoalveolar lavage (BAL) fluid and organs were collected and fungal load was determined.

Results

When intranasally infected with *Aspergillus fumigatus*, the resulting IPA led to a lethal outcome for "mock-treated" animals within 8 days. The intranasal administration of 0.6 mg/kg peptibody rescued 33.3 % of the animals. The survival improved when using 6 mg/kg and 60 mg/kg to 83.3 % and 100 % respectively. Additionally, the fungal load in BAL fluid and lung tissue was also reduced in the peptibody-treated animals compared to the "mock-treated" ones.

Conclusion

In conclusion, the study shows a decisive role of the peptibody in fungal clearance and survival of the treated animals thereby demonstrating the impressive potential of this new antifungal drug candidate as a salvage therapy for IPA in immunosuppressed patients.

S12-03

The SOFT orthologue of *Aspergillus fumigatus* impacts membrane integrity and pathogenicity

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Introduction

A hallmark of *Aspergillus fumigatus* is its high stress adaptability, which promotes its survival in both the natural and host environment. Among the molecular factors that facilitate stress adaptation, a fungal-specific signalling protein widely known as SOFT, which is studied primarily during cell fusion, is also linked to stress responses. In addition to promoting mycelial growth and conidiation, SOFT orthologues mediate the response to hyphal damage and thermal stress. Although SOFT also contributes to the pathogenic potential of several phytopathogenic fungi, the role of this multifunctional protein in the major mould infecting humans remains unknown.

Objectives

This study explores whether the previously uncharacterized SOFT orthologue in *A. fumigatus*, which we call AfSO, has a role in stress adaptation and pathogenicity.

Materials & Methods

We created Δ*AfSO* gene deletion mutants to analyse loss-of-function phenotypes under *in vitro* stress conditions and in the *Galleria mellonella* larvae infection model.

Results

Loss of AfSO did not affect mycelial growth on minimal or complex media, and the mutants grew normally under thermal stress. However, the Δ*AfSO* mutants displayed defects in sporulation on minimal medium, particularly in the centre of the mycelium. When exposed to membrane-disrupting compounds in RPMI medium, the mutants were more susceptible to a polyene and a saponin, but not to an azole drug. Surprisingly, larvae infected with the Δ*AfSO* mutants tended to succumb more quickly than those infected with the control strain. Consistent with these observations, a *soft* gene mutation in the background of a mildly pathogenic conidial colour mutant of *Aspergillus nidulans* causes hypervirulence in *Galleria* larvae.

Conclusion

The AfSO protein in *A. fumigatus* facilitates the adaptation to direct membrane stress, and SOFT orthologues in Aspergilli might regulate fungal signalling processes that shape the outcome of host-pathogen interactions.

Session 13 - Co-Infektionen

S13-01

Posaconazole for Prevention of COVID-19 Associated Pulmonary Aspergillosis in Critically Ill patients: a prospective case-control study

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Purpose

The relative number of COVID-19 patients developing COVID-19 associated pulmonary aspergillosis (CAPA) has been increasing within the vaccination area and mortality is still high. This study investigated the impact of posaconazole (POSA) prophylaxis in COVID-19 patients with acute respiratory failure receiving corticosteroids on the risk for development of CAPA.

Methods

The primary aim of this prospective, multicenter, case-control study was to assess whether application of POSA prophylaxis in mechanically ventilated COVID-19 patients reduces the risk for CAPA development. All consecutive patients from center 1 (cases) who received POSA prophylaxis as standard-of-care were matched to one subject from center 2 and center 3 who did not receive any antifungal prophylaxis, using propensity score matching for the following variables: (i) age; (ii) sex; (iii) treatment with tocilizumab; (iv) time at risk.

Results

Eighty-three consecutive patients receiving POSA were identified at center 1 and matched to 166 controls. The pre-matching CAPA incidence rates were 1.69 CAPA/1000 ICU days in center 1, 1.42 CAPA/1000 ICU days in center 2 and 9.53 CAPA/1000 ICU days in center 3. The CAPA incidence rate ratio before matching was 2.38 (95% CI 0.87–9.08; p = 0.072) for those not receiving prophylaxis versus those who did. In post-matching multivariable logistic regression, presence of an EORTC/MSG risk factor at ICU admission (OR 4.35) and Center (Center 3 versus 1: OR 6.07; 95% CI 1.76 – 20.91; p = 0.004; Center 2 versus 1: not significant) were associated with CAPA development.

Conclusion

The impact of POSA prophylaxis depends on the baseline CAPA incidence rate, which varies widely between centers and underlying individual patient risk factors. Future trials should therefore investigate targeted antifungal prophylaxis in COVID-19 patients.

S13-02

Mycobiome Dynamics in Cystic Fibrosis: Impact of Antibiotics on Short-term and Cumulative Treatment

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Introduction

Cystic fibrosis (CF) is marked by recurrent bacterial respiratory infections, yet fungal colonization's impact, particularly under extensive antibiotic use, remains understudied. Recent sequencing studies reveal complex fungal microbiota in CF airways, suggesting antibiotics and corticosteroids may promote fungal colonization.

Methods

A pilot study recruited 12 CF patients, collecting nasal lavage and stool samples before and after acute exacerbation and during a stable phase. Antibiotics were administered orally, intravenously, or via inhalation. Fungal DNA was quantified using qPCR targeting the ITS1 region, followed by ITS1 rRNA amplicon sequencing. Bioinformatic processing involved QIIME2, with taxonomy assignment from the UNITE database. Differential abundance and diversity analyses used 'ancombc' and 'phyloseq' R packages. Cumulative antibiotic doses over three years were converted into Antibiotic Equivalent Dose per patient.

Results

CF patients showed significantly higher fungal loads in nasal lavage and stool samples. Alpha diversity decreased in nasal lavage, with Candida predominance. Augmented fungal abundance correlated with reduced Shannon index as cumulative antibiotic exposure increased. Intravenous or inhalation administration correlated with Candida predominance in nasal lavage. Candida predominance exceeding 50% correlated with increased Antibiotic

Equivalent Dose in nasal lavage. Short-term antibiotic treatment also resulted in increased Candida abundance. Notably, Candida predominance was absent in stool samples.

Conclusion

Our study unveils fungal colonization dynamics in CF patients under antibiotic treatment, with Candida predominance observed in nasal lavage but not in stool samples. These findings suggest a distinct response of the fungal microbiome to antibiotics between respiratory and gastrointestinal tracts, emphasizing the need for further mechanistic exploration.

Session 14 - Host-Fungal Interactions II

S14-01

Dynamics of neutrophil migration and antifungal activity in an invasive aspergillosis-on-chip model

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Invasive fungal infections by the mould *Aspergillus fumigatus (A.f.)* in immunocompromised patients are associated with high mortality rates but limited treatment options. Infection occurs in the lung where fungal conidia germinate and grow into filamentous hyphae if they are not controlled by innate immune cells such as alveolar macrophages and neutrophils immigrating from the blood stream. The microfluidic "invasive aspergillosis-on-chip" (IAC) model closely mimics infection progression by addition of conidia to an "alveolar side" at an air-liquid-interface and a "blood side" of perfused neutrophils in medium.

In previous work we showed that neutrophils, in the presence of macrophages, are essential for preventing *A.f.* hyphal growth. Here, we study the recruitment of neutrophils, physical interactions of neutrophils with *A.f.* and/or macrophages during infection in the IAC model.

Live cell imaging of infected IAC models using a confocal laser scanning microscope was performed immediately upon addition of neutrophils to the blood side for up to 24 hours. Macrophages and neutrophils were pre-labelled with orange or red cytosolic dyes, respectively, while a GFP-carrying *A.f.* mutant strain was used.

Neutrophil migration from the blood to the alveolar side was observed within 30 minutes of cell addition, regardless of *A.f.* presence, and continued throughout imaging. Upon infection, incoming neutrophil numbers increased up to four times within the first hour. Neutrophils moved fast initially (0.1µm/sec) and slowed down gradually after phagocytosis of several conidia. Hyphae were attacked mainly at their tip. Cell death of neutrophils started at about 12

hours into imaging and occurred mainly in the vicinity of macrophages which took up released conidia as well as dead neutrophils.

This research underlines the necessity of neutrophil recruitment to the site of infection and sheds light on modes of action inhibiting fungal growth.

S14-03

Experimental preclinical imaging-compatible animal models of mucormycosis

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Introduction

Although infections by mucormycetes are a serious threat in clinical settings, due to fast progression and limited treatment options, little is known about pathogenesis mechanisms.

Aim

Generation of luciferase expressing Mucorales strains to be used for non-invasive monitoring of mucormycosis infection over time in different animal models -especially to establish imaging-compatible preclinical insect and mouse models.

Methods

Codon-optimized firefly luciferase without the peroxisomal target sequence, under the control of two different promoters was cloned into auxotrophic *M. lusitanicus* recipient strains, including gene deletion mutants. Positive, now prototroph, transformants were checked for gene integration by PCR and Southern Blot. Subsequently, growth pattern and light emission under various conditions were determined by luminometer. Selected strains were used for *in vivo* validation in Galleria infection assays and in a neutropenic mouse model and fungal infection was determined by BLI imaging analysis.

Results

Firefly luciferase, with a single integration was successfully expressed in *M. lusitanicus.* Light emission could be measured by luminometer and visualized in animal models. High light signal was obtained in infected *Galleria* larvae 48h after infection but decreased at 96h in those still alive. Likewise, light signals were detected in mice since day 1 post-infection which increased until day 3. On day 4, light signals decreased in tandem with recovery of weight and immunosuppression. Overall, the strains are usable for real-time, non-invasive infection monitoring which could be potentially used for antifungal efficacy assessment by means other than survival.

Conclusion

The successful visualization of *M. lusitanicus* infection by a non-invasive method in insect and murine models, offers new ways to study mucormycosis. Extending this approach to other species will provide valuable insights into the pathogenesis of Mucorales infections.

S14-05

CD56-mediated activation of human natural killer cells is triggered by *Aspergillus fumigatus* galactosaminogalactan

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Introduction

Invasive *Aspergillus (A.) fumigatus* infections are a major cause of severe complications in immunocompromised patients. Delayed natural killer (NK) cell reconstitution in allogenic hematopoietic cell transplant recipients is associated with higher susceptibility to invasive pulmonary aspergillosis, indicating that NK cells are indispensable for fungal clearance. NK cells recognize *A. fumigatus* by their receptor CD56, which is required for their antifungal activity. However, the underlying cellular mechanisms and the fungal ligand of CD56 are still unknown.

The objectives of this A2 project are to identify the fungal ligand interacting with CD56 on NK cells and to elucidate the cellular mechanism of this interplay.

Materials & Methods

We used a combination of purified cell wall components, biochemical treatments, and *A. fumigatus* mutants with altered cell wall composition and investigated their relevance for the interaction of *A. fumigatus* with NK cells by flow cytometry, microscopy and ELISA. Furthermore, PI3K and Pyk2 inhibitors were used to evaluate their involvement in the signalling pathway of *A. fumigatus*-induced NK-cell activation. Moreover, we tested whether GAG-primed NK-cell supernatants may lead to activation and engagement of other immune cells into the antifungal immune response.

Results

A. fumigatus cell wall galactosaminogalactan (GAG) showed binding to CD56, and especially deacetylated residues of GAG play a role in interaction with CD56 and triggered strong NK-cell activation, along with potent release of cytotoxic effectors and immune-enhancing chemokines. Inhibition of PI3K and Pyk2 decreased *A. fumigatus*/ GAG-mediated activation of NK cells. Supernatants of GAG-stimulated NK cells engage PMNs and enhance their anti-*Aspergillus* activity.

Conclusion

Our data suggest that *A. fumigatus* GAG is a ligand of CD56 on human primary NK cells and stimulates potent antifungal effector responses under the involvement of PI3K and Pyk2.

Session 15 - Leitlinien/Best Practice/Drug Monitoring

S15-01

Mortality attributable to candidemia: Results from the European Confederation of Medical Mycology Multinational Observational Cohort Study Candida III

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Background

Despite advances in antifungals, Candida infections still have a high mortality rate of up to 40%. The ECMM Candida III study in Europe investigated the changing epidemiology and outcomes, highlighting the need to understand and manage these infections.

Methods

In this observational cohort study, participating hospitals enrolled the first ten consecutive adults with confirmed candidemia. Data collected included patient demographics, risk factors, hospital stay length (with a 90-day follow-up), diagnostic procedures, Candida species, treatment details, and outcome. Controls were matched in a 1:1 ratio from the same hospitals, ensuring similarity in age, underlying illness, ICU versus normal ward stay, and recent major surgery. The study described overall and attributable mortality and assessed survival probability for both cases and controls.

Findings

The study included 171 pairs consisting of patients with candidemia and matched controls from 28 institutions. In those with candidemia, overall mortality was 40.4%. The attributable mortality was 18.1% overall but differed among the causative Candida species (7.7% for Candida albicans, 23.7% for Candida glabrata, 7.7% for Candida parapsilosis, and 63.6% for Candida tropicalis). Regarding risk factors, the presence of central venous catheter, total parenteral nutrition, and acute or chronic kidney disease were significantly more common in cases versus

controls. Length of hospitalization and ICU stay were significantly longer in candidemia cases (20 days (IQR 10-33) vs. 15 days (IQR 7-28); p=0.004).

Interpretation

Although overall mortality remains high in this matched case/control analysis, attributable mortality has decreased compared to historical cohorts. This may be due to a better prognosis for candidemia caused by Candida albicans, which has an attributable mortality of 7.7%, while candidemia cases caused by non-albicans Candida exhibit higher attributable mortality.

S15-02

AWMF Guideline for Medical Clinical Diagnostics for Indoor Mould Exposure – Update 2023

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Introduction

In 2016 in Germany, the AWMF Guideline of Medical Clinical Diagnostics for Indoor Mould Exposure was introduced.

Objectives

Guidline update in 2023.

Materials and Methods

A completely new systematic literature search was carried out for the guideline update. Search results were refined by abstract screening and, if necessary, evidence-based full text evaluation. Topic-related guidelines were included.

Results

Medically rational diagnostics are based on individual risk factors and include recording of the medical history, physical examination, and allergy diagnostics including provocation tests if necessary; sometimes cellular test systems are recommended. For invasive mould infections, reference was made to the specific guidelines. There are currently no useful and validated test procedures for mycotoxins. Indoor measurements In most cases, indoor measurements of mould species are not useful from a medical point of view. Measurements of MVOC or mycotoxins in indoor air or house dust are not indicated.

Conclusion

In case of visible mould infestation, quantitative and qualitative determination of mould species is unnecessary. Instead of carrying out measurements, the causes and the mould infestation should be eliminated as quickly as possible. For the medical assessment, it is important that the doctors think of mould at all. If mould exposure is suspected or exists, patients should be examined for specific risk factors.

Keywords

Mould, indoor, AWMF guideline, recommendations, key messages, diagnostic methods, diagnostic algorithm

S15-03

The Cologne ECMM Excellence Center: A Comprehensive Evaluation of its External Consultation Service for Invasive Fungal Infections over Two Years

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Introduction

The increasing prevalence of invasive fungal infections (IFIs) spurred the establishment of the European Confederation of Medical Mycology (ECMM) in 1993. Recognizing IFIs' growing public health impact, the ECMM initiated the ECMM Excellence Centers in 2016. These centers aim to direct physicians and patients to specialized institutions known for exceptional achievements in medical mycology. This article provides a detailed overview of the external consulting service offered by one such center, the Cologne ECMM Excellence Center. It has played a crucial role in providing swift expert guidance for IFI management.

Methods

The Cologne ECMM Excellence Center, attaining Diamond status for scientific engagement and coordinating multicenter publications, offers specialized IFI consultation. This service provides free expert advice via email and phone, prioritizing life-threatening cases, with a commitment to respond within 24 hours. It collects essential data, including inquiry origin, fungal pathogen, infection details, and imaging usage.

Results

Over two years, the Cologne ECMM Excellence Center provided crucial guidance, receiving 189 consultation requests from healthcare professionals across 17 countries. The majority were from Germany, comprising 85%. Commonly consulted fungal pathogens included Aspergillus spp., Mucorales, and Candida spp., reflecting the diverse challenges in IFI management. Additionally, 4% of cases involved fungal mixed infections, underscoring their complexity. Within Germany, consultation requests were highest from regions like North Rhine-Westphalia, Bavaria, and Baden-Württemberg.

Conclusion

The Cologne ECMM Excellence Center is a key player in advancing medical mycology. Providing expert guidance to healthcare professionals dealing with IFIs, it enhances patient

care and contributes to mycology research. With the global concern of IFIs persisting, ongoing efforts to improve accessibility will amplify the center's global impact.

Session 16 - Fungi & Mucosa

S16-02

Comparative analysis of *Debaryomyces hansenii* strains and their immunostimulatory potential

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Introduction

The common food colonizer *Debaryomyces hansenii* belongs to the taxonomic group of *Candida* CTG species and is regularly identified in intestinal mycobiome analyses. Although the yeast shows some probiotic properties, it was also found in inflamed mucosa of Crohn´s disease patients and enriched in abundance in metabolic dysfunction-associated steatotic liver disease patients with severe fibrosis.

Objectives

The aim of this study was to characterize the adaption of *D. hansenii* isolates to human gastrointestinal tract (GIT) conditions and their possible immunostimulatory potential targeting the Th-17 signalling pathway.

Methods

Multiple *D. hansenii* strains, with and without the capability to grow at 37°C, were investigated for genomic and transcriptomic differences to explain their different growth behaviour. The possible immunostimulatory potential of these *D. hansenii* isolates was investigated in a human T cell stimulation assay, measuring cytokine secretion following stimulation with fungal lysates.

Results

Whole genome sequencing revealed two clusters of *D. hansenii* isolates corresponding to their growth ability at 37°C. Interestingly, all isolates able to grow at 37°C were patient-associated whereas the cluster comprising isolates that were not able to grow at 37°C also included environment-associated isolates. Additionally, some *D. hansenii* isolates elicited a strong IL-17A secretion after *ex vivo* human T cell stimulation comparable to the *C. albicans* response.

Conclusion

*D. hansenii´*s ability to grow at 37°C as well as its immunostimulatory potential is highly isolate-specific and could explain the yeasts probiotic features in contrast to its involvement in chronic intestinal and liver associated diseases with inflammatory properties. Ongoing RNA

sequencing analysis should reveal isolate-specific differences in transcriptional responses to GIT conditions and help clarify *D. hansenii´*s contrastive role in human health and disease.

S16-03

Using a human airway organoid-derived monolayer co-culture model to study *Cryptococcus* uptake

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Introduction

Cryptococci are environmental fungi that cause localized or disseminated infections typically after uptake via the respiratory tract. Studies on pathogenicity are based on animal models, cancer or immune cells. However, to understand the pathomechanisms from pulmonary uptake to dissemination, complementary models are required. We are using a human lung model to investigate parameters of early cryptococcal interaction with respiratory tissue.

Methods

Human airway organoid-derived monolayers (ODMs) on transwell filters were co-cultured with *C. neoformans* (H99) and *C. gattii* (R265). Barrier function of ODMs was probed by measuring the transepithelial electrical resistance (TEER). We visualized internalization of fungi into respiratory cells by microscopy and quantified fungal viability by cultivation. Fungal morphology was assessed using ink stainings.

Results

Human ODMs could be co-cultured with cryptococci and TEER measurements revealed no breakdown of barrier function. Rare events of fungal internalization by airway epithelial cells were detected. Fungi remained viable during co-culture and able to further proliferate. Quantification of cryptococcal morphology revealed a shift from a uniform cell population towards distinct strain-specific phenotypes, including micro- and giant cells.

Discussion

The established co-culture model provides a suitable *in vitro* model to study cryptococcal interactions with the respiratory epithelium. Quantification of TEER indicates no impact of different cryptococcal strains on the barrier function of ODMs, despite the fact that both strains show different disease phenotypes. However, since fungal cell morphology may predispose to clinical disease manifestations, this advanced human cell culture model may provide insights into fungal traits and interactions with host cells determining clinical disease.

S16-04

The IL-1 family cytokines of the IL-36 subfamily may drive inflammation during RVVC

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Vulvovaginal candidiasis (VVC) is an infection of vaginal mucosa caused by deregulated cytokine signaling driven hyperinflammation. As a leading infectious cause of vaginal and vulvar inflammation, VVC impacts the quality of life of millions of women worldwide, approximately 75% of women. 7-9% of them even suffer from recurrent VVC (RVVC) portrayed by at least four episodes annually. The cytokines interleukin (IL)-1α and IL-1β are key drivers of the inflammatory pathogenesis of VVC. These cytokines are part of a family of 10 cytokines, some of which share the same downstream signaling pathway. Particularly, IL-36 subfamily cytokines exhibit very similar proinflammatory characteristics and can similarly activate neutrophil-mediated inflammation.

When assessing a hypothesis-driven cytokine panel in vaginal washes of RVVC patients, we found significantly higher IL-36β (IL-1F8) levels in women with RVVC compared to healthy women. While vaginal epithelial cells (VECs), the first barrier of vaginal defense, demonstrated increased *IL36G* (IL-1F9) expression upon infection with *Candida spp.*, release of IL-36 family cytokines was below or close to detection limit on protein level. Moreover, monocyte-derived macrophages did not release IL-36 family cytokines upon encountering *Candida albicans*. We are systematically exploring various cell types of the vaginal niche to identify the source of IL-36 cytokines during RVVC. To shed light on their role during VVC, VECs were infected with *C. albicans* in the presence of IL-36β or IL-36γ. We found that these cytokines potentiated the release of the alarmin IL-1α only upon *C. albicans* infection, whereas neutrophil chemoattractant IL-8 and survival factor GM-CSF were induced by IL-36β/γ irrespective of infection.

These data shed a first light on a potentially important role of IL-36 family cytokines in amplifying inflammatory responses during RVVC.

S16-05

Pra1: a molecular determinant of fungal immunopathology and immune evasion.

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Zinc is an essential mineral required by all organisms. We originally reported that *Candida albicans* releases a zinc-scavenging molecule (the Pra1 "zincophore") to sequester this mineral during infection of human cells. We have recently shown that *C. albicans* Pra1 drives neutrophil recruitment and the immunopathological symptoms of vaginal candidiasis (PMID: 38055800).

Interestingly, *Candida glabrata* (which is the second most common cause of vaginal *Candida* infections) has lost the *PRA1* gene and fails to recruit neutrophils *ex vivo*, *in vivo* and in the clinic. Heterologous expression of *PRA1* by *C. glabrata* establishes neutrophil recognition whilst deletion of *PRA1* in *C. albicans* prevents recognition.

As Pra1 is directly targeted by neutrophils, we predicted that *PRA1* gene loss in the fungi may represent a novel form of immune evasion. Bioinformatics analysis of various environmental mycobiomes indicates that approximately three quarters of the fungal kingdom have maintained the *PRA1* gene. In contrast, of those species which can cause life-threatening infections in humans, >76% have undergone zincophore locus rearrangement or lost the *PRA1* gene entirely.

Given the dynamic evolutionary trajectory of the zincophore locus in the fungi, and its strong impact on host-pathogen interactions, we are now exploring the nature of the Pra1 protein in greater detail. Here I will present recent findings on Pra1"s regulatory control and zinc-binding

activity, its novel hexameric structure and its role in governing inflammatory responses against pathogenic fungi.

Session 17 - Seltene Mykosen und besondere Fälle

S17-01

Virulence of *Cryptococcus gattii* VGI isolated in Germany in a *Galleria mellonella* infection model

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Background

Cryptococcus gattii (*Cg*) species complex have been primarily cultivated as infectious agents in tropical countries causing infections in non-immunocompromised hosts. Molecular typing distinguishes six clades (VGI-VGVI). Infections caused by VG II have gained interest due to an outbreak of animal and human infections on the North American Westcoast. VGI have been first isolated in Australia and are the most prevalent reported in Europe.

Methods

Retrospective review of animal and human *Cg* VGI cases identified 2009-2023 at the German reference laboratory for cryptococcosis. Isolates were identified by pheno-, and genotypic test. Virulence was evaluated in a *Galleria mellonella* (Gm) infection model. Larvae (250-600mg) were inoculated with 20µl of physiologic saline with 10^8 fungi per ml, incubated at 37°C and checked daily for survival until day 10. Median larval survival was compared to infection with a highly virulent clinical reference strain (VGII, R265) and environmental reference strains representing VGII (CBS7500) and VGI (WM276).

Results

Human VG I infections were documented in two males with CD4 lymphopenia and without underlying disease, both with pulmonary and central nervous system involvement. Two further strains were isolated from nasal swaps of Koalas kept in captivity at two Zoos, one infected with nasal lesion and positive serum antigen, one with nasal colonization.

Virulence testing showed rapid larval killing (median survival day 6) for the clinical reference strain R265. Human isolates from disseminated infections were not statistically different from the VGI and VGII reference isolates (median survival day 7-8). Nasal isolates from Koalas were significantly less virulent with median survival of > 10 days.

Conclusion

Cryptococcosis caused by *Cg* (VGI) is rarely diagnosed in Germany. Animal hosts may serve as a reservoir or vehicle for spread of *Cg*. Fungal factors contributing to reduced virulence in *Galleria* need further study.

S17-02

Isolation einer neuen *Onygenales*-Spezies von menschlicher Haut

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Aus Schuppenmaterial einer Patientin wurde ein nicht einzuordnender dermatophytenähnlicher Pilz (Stamm 724-23 = DSM 116806) angezüchtet, der anschließend genau charakterisiert wurde.

Auf Dermatophyten-Agar zeigte sich ein flacher weißer Thallus mit samtiger Oberfläche und zentral schwach orangener Pigmentierung der Unterseite. KD-Agar stimulierte eine gelbliche Färbung der Oberseite. Mikroskopisch ließ sich die Bildung polymorpher Chlamydosporen erkennen, Mikro- und Makrokonidien fehlten. In älteren Kulturen bildeten sich Bündel dicker Hyphen und Hyphenwirbel, außerdem auf manchen Nährböden Myzelbällchen auf der Oberseite. 724-23 wuchs gut bei 26 °C auf Sabouraud-Agar, Kimmig-Agar und weiteren Nährböden, sehr langsam bei 5 °C, jedoch nicht bei 37 °C und zeigte deutliche keratinolytische und proteolytische Aktivität sowie Wachstum auf pflanzlichen Substraten. ITS- und partielle LSU-Sequenzen ergaben geringe Ähnlichkeiten mit Referenzstämmen. Die Ähnlichkeit der ITS-Sequenzen lag bei 93% zu verschiedenen *Malbranchea*-Arten. Die LSU-Sequenzen zeigten 95-93% Ähnlichkeit zu *Amauroascus*, *Arthroderma*, *Auxarthronopsis* und *Malbranchea*. Nach der phylogenetischen Analyse (ITS- und LSU-Genbereiche) war 724-23 als *Onygenales*-Spezies in Clade 8 (incertae sedis) mit *Amauroascus aureus* CBS 593.71 (T) und *Diploospora rosea* DAOM 250100 (ET) nach [1] einzuordnen. Die Art wurde weit von anderen Spezies verwandter Gattungen und den Familien *Malbrancheaceae* (Clade 6, [1]) und *Neogymnomycetaceae* (Clade 7, [1]) platziert.

Danach handelt es sich bei 724-23 um eine bislang nicht beschriebene *Onygenales*-Spezies, die keiner bekannten Gattung oder Familie dieser Ordnung angehört. Ergänzende Untersuchungen laufen noch bei Abstrakteinreichung. Der Stamm und die bestimmten Gensequenzen sind bereits hinterlegt.

1. Kandemir H. et al. Phylogenetic and ecological reevaluation of the order *Onygenales*. Fungal Diversity 2022; 115: 1-72.

S17-03

Human Coccidioidomycosis in Germany: Retrospective Analysis of cases diagnosed between 2011 and 2023

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Introduction

Coccidioidomycosis (cocci), caused by *Coccidioides posadasii* (*C.p.*) and *Coccidioides immitis (C.i.)*, is a fungal infection found in semi-arid regions in the Americas. Diagnosis relies on histopathology, cultivation and antibody testing.

Objective

Describe imported cocci in Germany, including epidemiology, clinical presentation and diagnostic.

Methods

Studied proven cocci cases at the German reference laboratory for rare systemic fungal infections. Data collection involved patient records. Isolates genotyped via proline-rich antigen gene PRA2. Serum antibodies detected using lateral flow assay (lfa) at diagnosis.

Results

Fifteen cases were identified (female 33.3%, male 66.7%), aged 23 to 82, from Germany (40%), USA (20%), other/unknown (40%). Underlying diseases were documented in two patients (20%), i.e. rheumatoid arthritis and hepatitis C infection. Seven patients reported as healthy (46.7%), while underlying diseases for six patients not reported (40%).

Nine patients had pulmonary disease (60%), two patients with intracerebral abscess (13.3%). Disseminated infection diagnosed in three patients with lung disease together with mucocutaneous (n=2, 13.3%) or bone (n=1, 6.7%) involvement. Relapsed infection was documented in two patients (13.3%), occurring months to years after the initial diagnosis.

Cases were linked to endemic regions, notably Arizona (n=6, 40%), Texas (n=2, 13.3%), California (n=2, 13.3%) or unspecified US states (n=5, 30%), 4 months (median) after exposure. Diagnosis by cultivation (n=12, 80%; *C.p.* (n=10), *C.i.* (n=2)) and/or histopathology (n=11, 73.3%). Antibody testing with lfa at the time of diagnosis yielded positive results in all six patients tested.

Conclusion

Cocci is rarely imported to Germany from the US. Diagnosis is primarily established by cultivation. Diagnostic laboratories should be informed on suspected cases to prevent laboratory exposure. Antibody detection by lfa seems to be a sensitive screening test.

Session 18 - Fungal Virulence

S18-01

Fungal effector interactome analysis identifies mechanisms for fungal evasion of phagosome killing

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Introduction

An essential host defense mechanism against pathogens is their elimination by phagocytosis. *Aspergillus fumigatus* (Af) is a fungal pathogen capable of manipulating phagosome maturation, surviving phagosome killing, and causing life-threatening invasive infections in

individuals with impaired immunity. HscA, on the surface of Af, is an effector protein that interferes with phagosome maturation by anchoring host p11 protein on phagosomes.

Objectives

Treatment of fungal infections is challenging. Understanding how pathogens interfere with phagosomal maturation by studying fungal-host protein interactions addresses fundamental questions in cell biology of phagocytosis and facilitates the development of targeted therapeutic approaches to eradicate pathogens within phagosomes.

Patients & Methods or Materials & Methods

Proximity-dependent biotin identification (BioID) was employed to identify host cell proteins targeted by the fungal HscA protein. Af strains expressing HscA fused with miniTurboID biotin ligase were generated and used to infect A549 lung epithelial cells. Proteins labelled by BioID were identified using proteomics methods. Candidates with potential roles in vesicular trafficking and exo- or endocytosis pathways were verified for their function in phagosome maturation by immunostaining of A549 cells infected with wild-type (WT) or D*hscA* conidia.

Results

After successfully generating of Af strains expressing hscA-miniTurbo, hscADC-miniTurbo, or miniTurbo-hscA and performing proteomics analysis, immunostaining revealed recruitment of multiple BioID-identified proteins on phagosomes or phagocytotic cups containing Af conidia. For several proteins, the intensities of immunofluorescence were higher on phagosomes containing WT conidia than those containing D*hscA* conidia.

Conclusion

HscA interacts with multiple host proteins that play roles in phagocytosis and could potentially serve as targets for new treatment approaches.

S18-02

The role of rhizoferrin in growth and virulence of *Rhizopus microsporus*

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Introduction

Mucormycosis, an invasive fungal infection caused by Mucorales fungi, presents a significant threat particularly for patients with conditions such as uncontrolled diabetes, hematological malignancies, and COVID-19 co-infections. Iron acquisition is vital for Mucorales pathogenicity, with elevated serum free iron levels intensifying their virulence. Investigating virulence factors to potentially find new drug targets is urgently needed for this group of fungi.

Aim of the study

Fungi secrete siderophores to enable chelation and uptake of ferric iron. For clinically relevant mucormycetes it has been shown that a polycarboxylate siderophore, rhizoferrin, is secreted. This study aims to elucidate the role of the rhizoferrin synthetase-encoding gene (rfs) and its product rhizoferrin, in the growth and virulence potential of *Rhizopus microsporus*.

Material and Methods

Assessment of rhizoferrin production via chrome azurol S (CAS)-assays and High-performance liquid chromatography (HPLC), growth assays under varying iron or (xeno-)siderophore availabilities were conducted for both *R. microsporus* wild type and rfs-deletion mutants. *Galleria mellonella* larvae were utilized to study virulence potential of wt versus deletion strains with or without addition of (xeno-)siderophores or co-infection with *Pseudomonas aeruginosa*.

Results

Rfs deletion resulted in non-detectable amounts of rhizoferrin and significantly reduced virulence potential in the *Galleria* mellonella infection model. Further, rfs deletion strains were unable to form hyphae within Galleria larvae and growth reduction/inability under low iron conditions was evident. Currently investigations are carried out to determine if virulence and germination can be restored in the presence of iron or (xeno-)siderophores, applied directly or vi co-incubation with *Pseudomonas*.

Conclusion
Rhizoferrin production is inevitable for virulence of *R. microsporus*.

S18-03

Host albumin metabolically unlocks an alternative oxylipin-mediated pathogenicity strategy of *Candida albicans*

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The pathogenicity of the polymorphic yeast *Candida albicans* is associated with filamentation, adhesion, invasion, and production of the toxin candidalysin. Interestingly, many clinical isolates and other *Candida spp*., can cause infection independent of filamentation or candidalysin production. Consequently, these strains and species are often avirulent in *in vitro* infection models, yet this does not correlate with their potential to cause infection in patients. We hypothesized that specific host factors, which trigger pathogenicity, are absent in *in vitro* models, and thereby these models do not reflect the situation in the host.

We determined the impact of albumin, the most abundant protein in the human body, on infection of vaginal epithelial cells with different *C. albicans* strains. The presence of albumin increased the pathogenic potential of otherwise non-damaging and non-filamentous clinical isolates. Albumin even rescued the pathogenicity of avirulent deletion mutants deficient in filamentation, Als3 adhesin/invasin, or candidalysin production. The host protein induced transcriptional changes and reprogrammed *C. albicans* metabolism in the direction of biofilm formation and the production of the oxylipins such as 13-HODE. Mechanistically we show that the accumulation of 13-HODE triggers vaginal epithelial cell cytotoxicity.

Collectively, our study uncovered a pathogenicity mechanism by which *C. albicans* causes epithelial damage independent of adhesion, invasion, filamentation, and toxin production. This alternative pathogenicity strategy helps to explain why clinical isolates often seem avirulent *in vitro*, when they are out of the context of the host environment.

Poster

Poster session I

PI-01

In vitro whole leukocyte infection model and detection of hydrophobic surface-binding protein A (HsbA) in *L. corymbifera*

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Introduction

Mucormycosis, a fungal infection caused by members of the order Mucorales, is primarily attributed to *Rhizopus arrhizus (ex: R. oryzae), Mucor circinelloides,* and *Lichtheimia corymbifera.*

Objectives

We present an in vitro whole leukocyte infection model and the detection of hydrophobic surface-binding protein A (HsbA) through liquid chromatography-mass spectrometry analysis (LC-MS/MS).

Patients & Methods

We identified the predominant spore coat protein (CotH) and HsbA on the surface of L. corymbifera sporangiospores. HsbA secretion was detected, and subsequent coinfection (MOI 1:2) of leukocytes with *L. corymbifera* spores allowed the detection of HsbA in the supernatant after 72 hours by LC-MS/MS. Furthermore, we investigated the binding of HsbA to monocytes and macrophages and assessed its antiproliferative and apoptosis-inducing effects on MH-S cells.

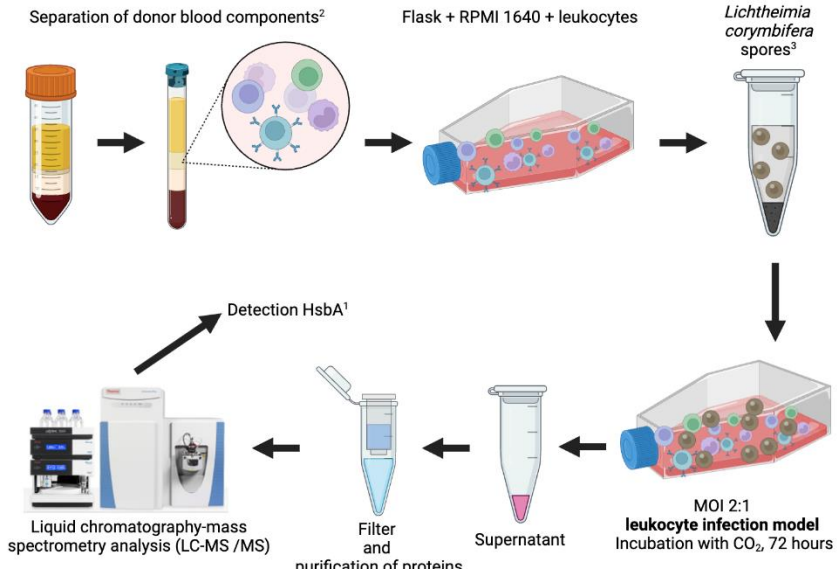
Results

Our findings suggest that HsbA plays a significant role in the interaction with the immune system, shedding light on the mechanisms underlying the interaction between *L. corymbifera* and leukocytes.

Conclusion

Our study provides insights into the role of HsbA in the immune response to *L. corymbifera infection,* furthering our understanding of the pathogenesis of mucormycosis.

Figure 1



PI-03

Characterization of T cell immunity in the human tongue

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The human tongue is a multifunctional muscular organ primarily involved in the processes of taste, speech and food processing. The tongue also houses a diverse microbiome and mycobiome that needs to be kept in check by immune cells embedded in its mucosal tissue, forming a barrier against pathogens. It can therefore be assumed that the tongue plays a significant role in the immune system of the oral cavity and thus also in anti-fungal host protection.

We conducted comprehensive analysis of human tongue tissue samples using advanced cutting-edge technologies such as single-cell RNA-seq and single-cell proteomics to investigate the cellular composition of the tongue tissue in depth on the single-cell level. In particular, we focused on T cells within the human tongue and compared their transcriptomic identity to that of T cells from multiple other matched human tissues from the same organ donor. Surprisingly, we found that T cells from tongues displayed the highest expression of Th17 cell associated genes such as IL17A, IL17F and IL22 among all investigated organs. Furthermore, we found a unique expression pattern of genes associated with tissue repair and with antimicrobial genes. Given frequent colonization of the human tongue with C. albicans, which we showed previously to be regulated by Th17 cells, this indicates an anti-fungal specialization pattern of tongue-resident T cells that exceeds anti-fungal activities of any other human organ including the gut and skin.

Our work provides novel insights into anti-fungal immune regulation in a so far overlooked immune organ, the human tongue. Future work will further dissect the intricacies of T cell functions and T cell regulation in the tongue in immune homeostasis as well as the implications for host defense against infections.

PI-07

Variations in the bacterial and fungal composition of laboratory mice and the impact on *Candida albicans* colonization

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The gut microbiota plays a key role in health and disease of the host. One main function is the mediation of colonization resistance against pathobionts, such as the fungus *Candida albicans*.Consequently, microbial dysbiosis due to antibiotic treatment represents a major risk factor for candidiasis.

To identify bacterial candidates mediating colonization resistance against *C. albicans*, we investigated natural microbiota variation in laboratory mice originated from various breeding facilities. Fecal samples from 20 C57BL/6 colonies were used for 16S and ITS1 sequencing. Based on differences in taxonomic composition and quantitative microbial burden, five colonies were selected for colonization experiments. After antibiotic administration and oral inoculation with *C. albicans*, fecal samples were collected at different time points to monitor fungal burden in the gut and changes of the intestinal microbiome.

Despite variations in microbiome composition, all five tested colonies displayed similar colonization patterns. Antibiotic treatment led to an increase of fungal colonization.

Surprisingly, sucrose supplementation of drinking water was sufficient to support substantial and stable *C. albicans* colonization.

These results reveal that considerable microbiota variation in breeding colonies of laboratory mice does not necessarily affect colonization resistance. Nevertheless, the fecal microbiome analysis identified changes in bacterial composition associated with *C. albicans* colonization, housing conditions or administration of sucrose-supplemented drinking water. The existing dataset will be used for further analysis and to identify bacterial candidates for colonization resistance.

PI-09

Mycobiome analysis of wild rodents in Thuringia

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The significance of the gastrointestinal microbiota composition towards the balance between human health or disease has been demonstrated in numerous studies over the last decades. Most research focused on bacteria as the most numerous component of the intestinal microbiota. More recently, however, fungal colonization has been shown to have a specific and profound impact on development and function of the immune system.

As functional studies in humans are often not possible due to ethical and practical constraints, laboratory mice are commonly used to investigate the impact of the microbiota and fungal colonization on the host. However, laboratory mice are usually maintained in a highly standardized environment with strict hygiene regimens. Compared to wild mice, they exhibit less immune activation and lower microbial diversity due to the lack of environmental exposure to a wider range of microbes, including fungi.

In order to identify fungi that can colonize the gut of wild rodents, and to determine the possible impact of host-specific factors and the environment, we analysed over 300 gut samples from different mouse species captured in different habitats in Thuringia. Overall abundance of fungal DNA determined by qPCR confirmed the expected higher abundance of fungal DNA in wild mice compared to specific pathogen free mice. ITS-sequencing and phylogenetic analyses are ongoing, and the results will be presented at the conference. This study provides not only additional information on the microbiome of wild rodents, but might also identify fungi that colonize a range of host species in different environments, or as specialists are associated with specific host species or habitats.

PI-11

Unraveling the role of LncRNAs in antifungal drug resistance: A comprehensive RNA bioinformatics approach

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Fungal pathogens within the *Aspergillus* genus, notably *Aspergillus fumigatus*, pose substantial threats to both human health and food production. Current treatments for systemic infections rely on four primary drug classes, but their efficacy is undermined by escalating resistance and notable toxicity. Globally, resistance rates range from 3-10%, rising to over 30% in specific regions. Despite advancements in understanding antifungal resistance

mechanisms, a crucial knowledge gap exists concerning the role of long non-coding RNAs (lncRNAs) in this context. Emerging evidence suggests lncRNAs serve as influential regulators in various biological processes, potentially playing pivotal roles in antifungal resistance. Our study introduces a comprehensive methodology for discovering and characterizing lncRNAs within *Aspergillus* species. By conducting an in-depth analysis of the entire lncRNA landscape, we aim to address this knowledge gap, identifying their distinct features and putative target genes. To support our research, we utilized a comprehensive bioinformatic workflow to generate complete transcriptome assemblies for both pathogenic and non-pathogenic *Aspergillus* species available in the NCBI SRA. Employing two distinct machine learning algorithms, CPC2 and Feelnc, we predicted coding potential and identified lncRNAs, taking into account various features such as sequence homology, motifs, and secondary structure. Furthermore, we conducted a comparative analysis between lncRNAs and protein-coding genes, examining transcript lengths, expression levels, and GC content. In conclusion, our multifaceted approach, encompassing data mining, transcriptome assembly, and coding potential prediction, defines lncRNAs in *Aspergillus* species. This serves as a project proof of concept, enhancing our comprehension of the non-coding genome and laying the groundwork for exploring potential regulatory roles in the pathogenic transition, particularly affecting human health.

PI-13

An Automated *ilastik*-based Workflow for Fungal Cell Morphology Analysis, Counting and Data Analysis: Enhancing Throughput and Accuracy with *caactus* (cell analysis and counting Tool using *ilastik* software)

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Introduction

Traditional methods for quantifying different (fungal) cell morphologies in liquid media via microscopy are labor-intensive and subject to user-dependent variability. This study addresses these limitations by introducing a streamlined workflow for fungal cell analysis, leveraging *ilastik* software and Python scripting.

Aim of the Study

The aim is to provide a user-friendly method for counting and analyzing fungal cell morphologies, accessible to those with minimal programming experience. *caactus* integrates with *ilastik*, simplifying image segmentation, data preparation, and statistical modeling.

Materials and Methods

Fungal spores were cultivated in a 96-well plate, with images captured at various time points using a Zeiss Axio Observer microscope. For identifying cells in the images, segmentation and object classification was realized by chaining three *ilastik* workflows: Pixel Classification for semantic segmentation of cell boundaries, instance segmentation based on the boundary images in Multicut, and Object Classification. Python scripting enabled image pre- and post-processing, data analysis, and statistical modeling, complementing the *ilastik* workflow. Manual counting of printed images served as a control for accuracy assessment. Time from image acquisition to receiving count data was recorded for both manual counting and the *ilastik*-based workflow.

Results

The workflow significantly reduced time from image acquisition to determining cell development stages and enabled rapid statistical analysis compared to manual counting. It also minimized variability compared to manual counting across users.

Conclusion

Caactus provides a high-throughput solution for automated cell analysis, integrating *ilastik* software with image processing and statistical modeling. This approach promises to accelerate research and enhance data consistency in biological studies.

PI-15

***Candida albicans* uses a host damage indicator for adaptation to high fever temperatures**

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Microbial success and persistence during infection requires appropriate adaptation to the host environment. *Candida albicans* is an opportunistic pathogen that can cause life-threatening infections in immunocompromised individuals. Yet, its existence as a common commensal yeast on human mucosal surfaces fostered the evolution of adaptation strategies that allow it to evade, escape, and counteract the host immune responses. In line with this, we have shown that *C. albicans* growth at high fever temperatures is increased when cultured in supernatants from activated human macrophages.

C. albicans adaptations to cope with the host inflammatory environment were evaluated using our inflammation-adaptation model. In brief, monocyte-derived macrophages (MDM)s are stimulated with inactivated *C. albicans* morphotypes, which culminates in the release of a complex mixture of immune mediators into the supernatant (SN). The protein composition of the different activated MDM SNs revealed several factors that could potentially be sensed by the fungus to trigger fungal adaptations. This include the cytoplasmic enzyme lactate dehydrogenase (LDH), which from epithelial cells is frequently used as a readout for cell damage.

These MDM supernatants are then used to culture *C. albicans,* and we specifically examined the effect of LDH on *C. albicans* growth potential under different physiologically relevant stress conditions. The addition of LDH to the culture specifically improved *C. albicans* growth at high fever temperatures (40°C), but not at 37°C.

Our results suggest that the differential composition of the host environment determines *C. albicans* potential to adapt to host-imposed stresses like fever. To further validate this idea, the effect of other proteins identified in the supernatants will be investigated.

PI-17

Malassezia and Pancreatic Ductal Adenocarcinoma: fungal opsonization as a relevant process in cancer progression?

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Background

Growing evidence hypothesizes a pro-carcinogenic role of *Malassezia* in pancreatic ductal adenocarcinoma (PDAC). According to the model, *Malassezia* migrates from gut into pancreas and activates local complement, thereby inducing inflammatory processes fueling tumor progression.

Objectives

(1) To investigate whether complement recognizes *Malassezia* as foreign (2) to test if pancreatic complement levels are sufficient for opsonization (3) to study the influence of pancreatic environment with its digestive enzymes on *Malassezia* opsonization.

Material Methods

Opsonization of *Malassezia* was measured by incubation with serum, followed by flow cytometry with complement-specific antibodies. Pancreatic environment was simulated by homogenized pancreatic tissue.

Results

Despite the unique thick lipid layer of *Malassezia*, complement recognized the fungal surface as foreign with C3 deposition on *Malassezia* and thus release of proinflammatory anaphylatoxins. Complement levels in non-inflamed pancreas were not sufficient to achieve significant opsonization; however, upregulation of complement expression was detected in PDAC tissue.

Pre-incubation of *Malassezia* with pancreas homogenate interfered with complement deposition; usage of protease or lipase inhibitor cocktails did not rescue significant opsonization, implying that masking by pancreatic components is more likely than surface modification.

Pancreatic enzymes are able to degrade deposited complement factors on the fungal surface, presumably by proteolytic processes. This mechanism might enable fungal survival in the presence of complement activation, without interfering with release of pro-inflammatory factors that can trigger tumor progression.

Conclusions

Basic levels of complement-driven inflammation can be triggered by the presence of *Malassezia* and may contribute to tumor progression. Consequently, antimycotics or complement inhibitors might represent supplementary therapeutic regimens against PDAC.

PI-19

T cell Gasdermin E – a novel player in immune interactions

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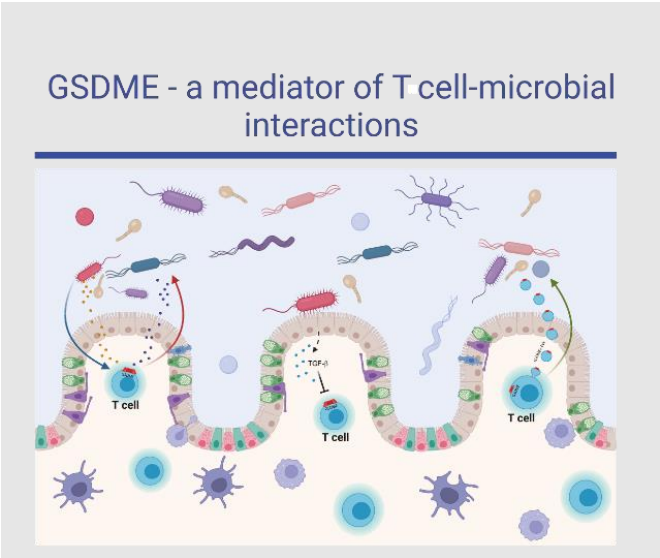
Gasdermins, a family of pore-forming proteins, have traditionally been associated with innate immune responses and pyroptotic cell death. However, this concept has been challenged by a recent observation of Gasdermin E expression in human Th17 cells, where it was found to be repurposed as a pore for cytokine secretion in the absence of cell death. This previously overlooked property equipped Th17 cells with enhanced anti-fungal functions.

We now report that Gasdermin E expression is also a property of CD8+ cytotoxic T cells. We show that cleaved Gasdermin E (N-GSDME), the pore forming unit of Gasdermin E, can be released from CD8+ T cells via extracellular vesicles (EVs). CD8+ T cell derived EVs shuttle gasdermin E pores to other cells and potentially also to microbial organisms, where they execute cell killing by membrane rupture. We furthermore found that TGF-b can regulate the cleavage of Gadermin E in T cells, thus modulating pore formation and the release of the membrane disruptive cell-killing pores into the local tissue environment. Interestingly, T cells were protected from autocrine cell death through membrane repair mechanisms induced by T cell activation and calcium signalling, a mechanism that was absent in EV target cells that were vulnerable to cell death through Gasdermin E incorporation.

Overall, we demonstrate a novel mode of T cell mediated cell killing that involves the generation Gasdermin E pores and their EV mediated transfer to target cells or host or microbial origin.

Figure 1



PI-21

Exploring the role of *Candida albicans* hyphal-specific toxin in promoting fungal gut commensalism

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The opportunistic fungal pathogen *Candida albicans*, poses a significant risk to human health by causing very frequently superficial infections, but also severe systemic infections under certain predisposing conditions. The current dogma of *C. albicans* commensalism is that the yeast morphology is the preferred growth form during gut colonization, while hyphae are detrimental for commensal growth in the gut and rather required for pathogenesis. Hypha formation is associated with a strong induction of the gene *ECE1,* encoding candidalysin (CaL) - the first (ribosomal) peptide toxin identified in a human pathogenic fungus – and seven additional non-candidalysin Ece1 peptides (NCEPs). While CaL directly inflicts damage to human cells, we propose that Ece1 may also act on bacteria of the human microbiota during commensalism.

Using *in vivo* competition models, we confirmed that the yeast morphology in fact favors murine gut colonization in models using antibiotics to remove antagonistic bacteria. However, hypha formation plays a crucial role to facilitate *C. albicans* colonization in hosts with either an undisturbed gut microbiota or carrying specific bacterial populations. In these niches, hyphal competent *C. albicans* outcompeted yeast-locked mutant cells. This phenotype was primarily attributed to the expression of *ECE1*.

We explored the effects of Ece1 on co-colonizing bacteria by assessing the susceptibility of selected bacteria from different body sites to Ece1 peptides. Our screening revealed that CaL influences the growth of several members of the microbiota and modulates bacterial properties. We are currently analyzing the transcriptional response of gut bacteria to CaL stimulation to understand the mechanisms of *Candida*-bacteria cross-kingdom interactions.

Our study provides evidence that CaL has evolved to improve fungal fitness during gut-commensalism or polymicrobial infections through inter-kingdom competition.

PI-29

Diagnostic peptides differentiating *Lichtheimia Corymbifera* infection from Mucorales and *Aspergillus*(B1 Project, Würzburg University)

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Introduction

Treating mucormycosis is challenging due to varying resistance among species to antifungal drugs. Accurate and rapid diagnosis is crucial for patient outcomes, and while sequence-based methods show promise in distinguishing Mucorales species, clinical diagnosis faces challenges in specificity and sensitivity.

Objective

Enhance mucoromycosis diagnosis by identifying diagnostic peptides specific to *L.corymbifera* through targeting the Hydrophobic Surface Binding Protein A (HsbA), thereby improving taxon-specific diagnosis.

Materials and Methods

1. Collected annotated genome assemblies of Mucorales and *Aspergillus.*
2. Identified the HsbA gene family and prepared multiple sequence alignments.
3. Calculated sensitivity and specificity of each residue[1].
4. Determined surface accessibility of HsbA protein[2].
5. Predicted B-cell epitopes[3].
6.

Results

The alignment of 209 HsbA sequences revealed over 30 potential diagnostic regions. Evaluation of surface accessibility revealed 20 of them were inaccessible for antibody detection. B-cell epitopes analysis confirmed 10 identified regions are antigenic determinants.

Inference

Predicted sites combined with surface data pinpoint potential seroprevalence regions. Amino acid changes in diagnostic peptide mainly occur on HsbA's surface, leading to minor structural variations. Consequently, cross-reactive antibodies are anticipated to bind to these regions. However, the adaptive humoral response is expected to produce polyclonal antibodies capable of differentiating between regions specific to *L. corymbifera* and those shared by Mucorales and *Aspergillus*[1].

Validation

Ongoing work derives neutralizing antibodies according to predicted peptides and will test them in vitro and in vivo for their protective capabilities.

Reference

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PI-31

Proteomic insights into the *Aspergillus fumigatus* response to antifungals

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Treatment of critical *Aspergillus fumigatus* infections are limited to only a few therapeutic drug options in the clinics. This is aggravated by the fact that antifungal resistance is emerging in *A. fumigatus*. Promising new drug candidates exhibit ideally new molecular targets to counteract existing resistances in *A. fumigatus*. However, determining unknown mode of actions is difficult to achieve due to the variety of processes involved in fungal stress responses and compensation strategies against antifungals. The analysis of global proteome responses of the fungus treated with different antifungal substances using mass spectrometry is an unbiased and fast approach which enables us to uncover specific as well as general fungal responses. We have defined signature proteins and processes for a range of different antifungal compounds with different known mode of actions, respectively. This knowledge enables us to compare fungal protein profiles of yet undiscovered compounds with our in-house library and thereby provide valuable insights into the molecular mechanism of newly discovered antifungal compounds. With our approach, we aim to speed up the process of drug discovery by preventing de-replication of compounds with already existing mode of actions.

PI-33

Immuno-Metabolomics of Invasive Aspergillosis: Nutritional Determinants of *Aspergillus fumigatus.*

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Aspergillus fumigatus is a saprophytic filamentous fungus that causes the majority of invasive aspergillosis cases in immunocompromised patients. Its omnipresent, small, and hydrophobic conidia serve as infectious agents. These dormant spores reflect the nutritional conditions supplied by the environment in which they were produced. Multiple factors determine the virulence of *A. fumigatus* in the host, among them its metabolic versatility, but how the nutritional status of this fungal pathogen impacts its interaction with cells of host immunity is rather unexplored. To elucidate the effect of varying sources of carbon and nitrogen on the fitness of conidia and their interaction with immune cells, we nutritionally programmed the spores by growing an *A. fumigatus* wild-type isolate on culture media with defined combinations of C- and N-sources and harvesting the resulting asexual spores. Such adapted conidia were tested for fitness under stressful and clinically relevant conditions such as iron limitation, elevated temperature, presence of reactive oxygen species or agents interrupting cell wall integrity, and antifungals. Spores corresponding to C/N combinations that resulted in significant phenotypes were monitored for their recognition and phagocytosis by immune effector cells, such as macrophages. Our experimental efforts offer the perspective to gain insights about the influence of metabolic pathways affecting conidial fitness and the cell wall, which serves as dominant PAMP in fungal pathogen/host interactions.

PI-35

Pathogenicity of different *Fusarium* Keratitis isolates

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Introduction

Fusarium species, especially those of the *Fusarium solani* species complex (FSSC) are amongst the most prevalent fungal keratitis pathogens. Due to late diagnosis and limited treatment options, the outcome of the disease is frequently unfavorable, leading to corneal transplantation or even enucleation of the eye.

Objectives

In this work, we aim to identify different pathogenicity pattern of the common *Fusarium* keratitis pathogens *F. falciforme, F. petroliphilum and F. keratoplasticum* in a 2D as well as a 3D corneal infection model.

Methods

2D monolayers of immortalized hTCEpi cells and a 3D hemi cornea model, consisting of a stromal and a multilayer epithelium, were infected with conidia of FSSC keratitis isolates for up to 48 h at 34 °C, 5 % CO2. Transmission electron, fluorescence microscopy and histology were used to visualize fungal invasion. Host cell damage was measured by lactate dehydrogenase (LDH) release.

Results

In the 2D monolayer model the examined FSSC species *F. keratoplasticum, F. petroliphilum* and *F. falciforme* were able to adhere to and invade into host cells and to cause cell damage but to a lesser degree than the major human fungal pathogen *Candida albicans*. Interestingly, *F. keratoplasticum* hyphae were also able to disseminate between host cells by initiating the formation of trans cellular tunnels which was so far only described for *C. albicans*. We established the 3D hemi cornea model as a more complex infection model for *Fusarium* species and were able to confirm the findings from the 2D monolayer model.

Conclusion

We have established 2D and 3D human corneal infection models to study the virulence attributes of Fusarium keratitis isolates. In our settings *F. keratoplasticum* turned out to be more virulent than *F. petroliphilum* and *F. falciforme*. However, further experiments, including additional genomic and transcriptomic analyses, are necessary to elucidate if this increased virulence is species or only strain specific.

PI-37

Characterisation of MAIT cell-induced antifungal immune response in a microfluidic invasive aspergillosis-on-chip disease model

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Invasive aspergillosis (IA) is a life-threatening infectious disease, mainly caused by the filamentous mould *Aspergillus fumigatus (A.f.)*. Whereas *A.f.* conidia are efficiently cleared by pulmonary immunity in immunocompetent hosts, fungal conidia are able to persist and morph into hyphae in immunocompromised individuals. Recently, an innate-like subset of T cells, mucosal-associated invariant T (MAIT) cells, was identified as a further player in the immune response against moulds. To further elucidate the antifungal potential of MAIT cells, we employed an *in vitro* infection assay and the novel "invasive aspergillosis-on-chip" (IAC) model.

Germination and hyphal length of *A.f.* was analysed by confocal microscopy in the 2D infection model after coincubation with either macrophages alone, macrophages and MAIT cells or macrophages and conventional CD8+ T cells. Similarly, fungal growth was investigated in the IAC model resembling features of a human alveolus in a 3D perfused microenvironment containing human alveolar epithelial cells, monocyte-derived macrophages as well as endothelial cells. Sorted human MAIT cells from peripheral blood were applied in the alveolar epithelium and/ or perfused in the endothelial cell compartment.

MAIT cells were activated by *A.f.* in *in vivo*-like conditions, followed by strong reduction of hyphal growth both in the IAC model and *in vitro*. In addition, MAIT cells elicited enhanced antifungal effects in comparison to conventional CD8+ T cells *in vitro*. Whereas MAIT cells potently reduced hyphal growth, they did not affect the germination rate of *A.f.* Furthermore, it was observed that MAIT cell incubation on both the lung, and blood side of the IAC model resulted in shorter hyphae by trend and further limited *A.f.* invasive growth.

In conclusion, MAIT cells contribute to antifungal immunity and therefore this cell type is a promising therapeutic tool for treatment of IA in the future.

PI-39

Responses of macrophages against *A. fumigatus* – do they know it's a fungus?

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Patients with weakened immune systems, e.g. during the process of hematopoietic stem-cell transplantation (HSCT), are particularly vulnerable to severe infection and potentially mortal outcome upon infection with *A. fumigatus*, highlighting the critical risk of fungal pathogens. As first line defense, alveolar immune cells, especially macrophages, are of great importance for recognition, initiation of further response and elimination of fungal spores. To clarify the interaction of healthy and post-HSCT macrophages with *A. fumigatus*, we infected them with *A. fumigatus* and analyzed their responses by RNA sequencing, live or confocal imaging and western blotting.

Interestingly, mainly expression of genes related to antiviral immunity, as ISG15 or MX1, was elevated. Especially upregulation of IRF1 and its increased translocation to the nucleus, resulting in higher interferon expression in antiviral responses, was of great interest. This increase of IRF1 was shown on transcriptional as well as protein level. In HSCT-patients' monocytes decreased levels of IRF1 were measured, which could be one possible link to reduced responsiveness against fungal pathogens.

In known pathways IRF1 is upregulated by nucleic acid recognizing receptors, as RIG-I or MDA5. Therefore, we investigated the response of macrophages, generated from bone marrow of mice with either Mda5 or Rig-I knockout. Both knockouts showed impaired reaction to infection with *A. fumigatus* compared to gene expression in wild-type controls. Whether impaired recognition by these receptors also plays a role in patients, needs to be proven in subsequent experiments. In addition, we could ask, if macrophages are able to discriminate between fungal pathogens and viruses or whether the immune signaling in macrophages is more universal than we thought.

In summary our data showed, that upregulation and translocation of IRF1 as well as the signaling by nucleic acid receptors is important for the response against *A. fumigatus.*

PI-41

Mimicry of the antifungal response of immunosuppressed human neutrophils in microfluidic invasive aspergillosis-on-chip infection models

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Introduction

Glucocorticoids (GCs) have been used for several decades to suppress the immune response in recipients of organ transplants and cancer patients. At the same time, immunosuppressed patients are predisposed to pulmonary invasive fungal infections, particularly by the

opportunistic mould *Aspergillus fumigatus (A.f.).* The fully human microfluidic "invasive aspergillosis-on-chip (IAC)" model mimics *A.f.* infection in the physiological environment, the alveolar epithelium.

Objective

We aimed to study the effect of impairment by GCs on the neutrophilic antifungal response as seen in immunocompromised patients in a fully human physiologically relevant model.

Materials & Methods

Here we perfused pre-labeled freshly isolated neutrophils from healthy donors on the blood side of our "invasive aspergillosis-on-chip" model. Neutrophils were treated with either Dexamethasone or Prednisolone for two hours before perfusion. Infection was done for 90 minutes by adding *Aspergillus fumigatus* conidia at the epithelium resembling the alveolus.

Results

Impaired neutrophils in the IAC setting were not able to inhibit fungal growth. The resulting hyphae similarly invaded the blood side as with untreated neutrophils in the IAC model but more frequently than in the setting without any neutrophils.

Conclusion

This study will help to understand the influence of glucocorticosteroids on immunocompromised patients and, in the long run, help to provide treatment for these patients.

PI-43

Establishment of a 3D lung infection models to study *A. fumigatus* infection

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Aspergillus fumigatus is an opportunistic fungal pathogen and one of the most common causes of invasive pulmonary aspergillosis in solid-organ transplant (SOT) recipients, allogenic hematopoietic stem cell (HSC) recipients and leukemia patients. The prognosis is poor with mortality rates of 40-50 %. While neutrophils have been identified as the most important effector cell, the role of the epithelium in *A. fumigatus* infection is poorly understood. The reasons for this are the use of 2D cell culture approaches, which do not recapitulate several important characteristics of the lung, like cell-to-cell interactions, the heterogeneity of cell types or proximal to distal patterning. On the other hand, the translation of animal models to humans is often affected by species-specific differences between mice and men and the use of laboratory animals raises ethical concerns. To address these limitations and reduce the number of used laboratory animals, in accordance with the 3R principles (refine, reduce, replace), we sought to establish the use of murine precision-cut lung slices (PCLS) and a human epithelial lung organoid model to investigate the interaction of *A. fumigatus* with the

epithelium. PCLS were viable for up to seven days and retained the complex 3D architecture of the lung, irrespective of the experimental treatment. Furthermore, PCLS were immunocompetent and showed a strong cytokine production upon treatment with different TLR agonists. In summary, murine PCLS represent a scalable tool that retain the complex 3D architecture cellular heterogeneity of the lung. This makes them suitable to analyze the spatial interaction of pathogens and host cells in a near-native environment.

PI-49

Novel, Fast and Cost-effective real-time PCR based method for the differential diagnosis of dermatophytes in veterinary medicine

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Dermatophyte infections, particularly those affecting animals, present a significant challenge in veterinary medicine. Traditional diagnostic methods for dermatophytosis often lack specificity and sensitivity, leading to misdiagnosis and ineffective treatment. In response to this need, we propose the development of a novel molecular diagnostic tool utilizing quantitative polymerase chain reaction (qPCR) for precise identification of dermatophytes infecting animals.

Our study focuses on designing species-specific qPCR primers targeting one-third of known dermatophytes, which are zoophilic in nature. Through meticulous primer design and validation processes, we aim to create a robust qPCR assay capable of accurately detecting and quantifying dermatophyte DNA in clinical samples. The development of such a tool promises to revolutionize the diagnosis of dermatophytosis in animals, enabling early detection and targeted treatment strategies.

The implementation of our qPCR tool will be structured into five distinct modules, each addressing key aspects of the diagnostic process, including sample collection, DNA extraction, primer optimization, qPCR assay development, and validation. By incorporating these modules, we ensure a comprehensive approach to the development and implementation of our molecular diagnostic tool.

Our research not only addresses the urgent need for improved diagnostic methods in veterinary dermatology but also contributes to the advancement of molecular biology techniques for infectious disease diagnostics. The successful implementation of this qPCR tool has the potential to enhance animal health outcomes, minimize zoonotic transmission, and optimize treatment strategies for dermatophyte infections in both domestic and wild animals.

Fig. Legend: Planned experimental design of the modular qPCR.

References

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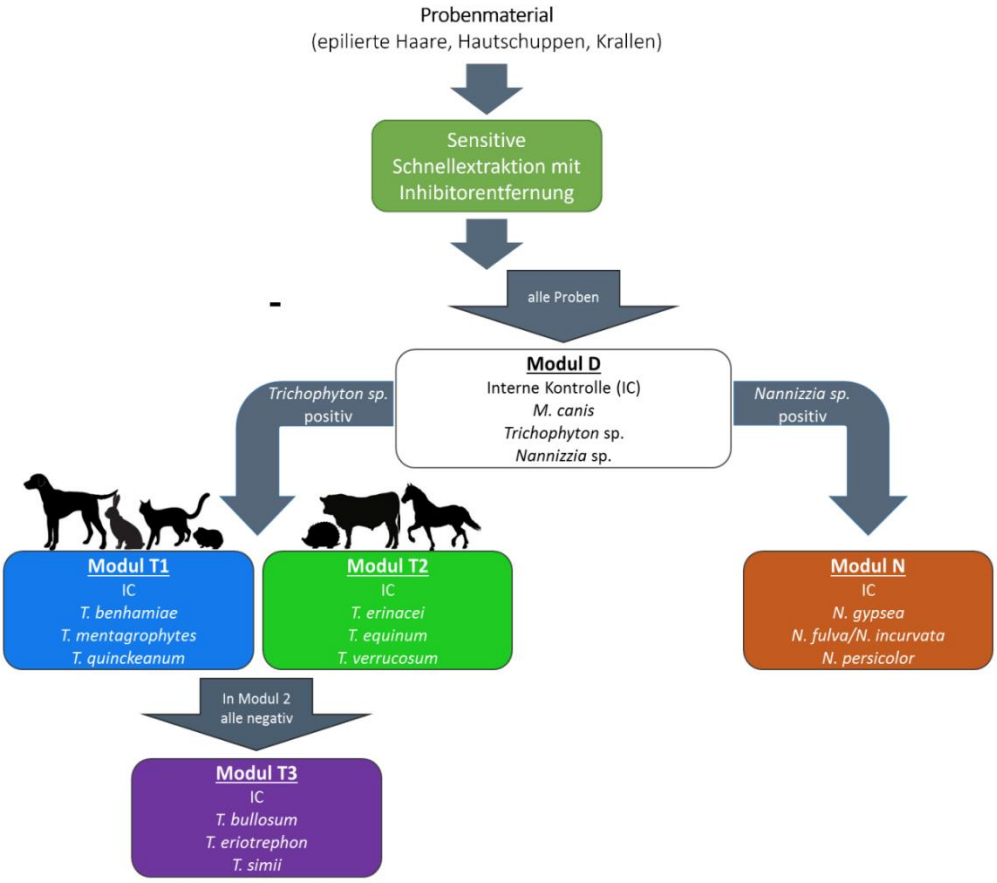
Figure 1



PI-51

Comparison of TGS-based and culture-based identification and quantification of bacteria and fungi from human stool specimen

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The microbial intestinal flora plays a key role in human health as bacteria and fungi that colonize the human gut may have health promoting or adverse effects. Therefore, incorrect colonization and shifts in the microbial balance, so-called dysbiosis, are associated with various diseases. Still, the analysis of the human mycobiome has long been scientifically neglected. In addition, there is a lack of standardized molecular methods for the simultaneous detection of bacteria and fungi in the human intestinal flora. The aim of this work is the development and validation of third-generation sequencing (TGS)-based microbiome/mycobiome diagnostics, which enables the simultaneous identification and quantification of bacterial and fungal DNA from human stool specimen. At the outset of the study, we will optimize DNA extraction, test pan-fungal universal primers, establish and optimize the TGS pipeline, with a focus on microbiome/mycobiome quantification, and optimize

bioinformatic evaluation criteria and database analyses. In the next step, the established TGS-based methodology will be compared to the currently used cultural method. For this purpose, 25 human stool samples are cultivated on various common culture media and the isolated bacterial and fungal populations are quantified and identified based on the colony-forming unit counts. The same stool samples are then analyzed using the established TGS technology. In this way, the weaknesses and strengths of both methods will be compared. The results obtained, and the newly established pipeline should promote the progressive optimization and standardization of microbiome analyses and diagnostics.

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PI-53

Evaluation of a novel quadruplex pan-*Mucorales*, pan-*Aspergillus* and section *Terrei* specific qPCR-assay, targeting the mitochondrial genome

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Introduction

Invasive fungal infections pose an increasing threat, partly due to rising numbers of at-risk patients and the emerging of drug-resistant pathogens. Early diagnosis and targeted therapy are essential for a positive outcome. Due to different therapeutic management of aspergillosis, and infections caused by *Aspergillus terreus*, differentiation of these pathogens is crucial.

Aim

This study aimed to evaluate the pan-Mucorales, pan-Aspergillus, and *Aspergillus terreus* section of a novel quadruplex diagnostic qPCR assay based on the MIQE guidelines.

Methods

Sensitivity, specificity, limit of detection (LoD), amplification efficiency, and cross-reactivity were evaluated. For the LoD, one strain each of *Mucor lusitanicus*, *Lichtheimia corymbifera*, *L. ramosa*, *L. hyalospora*, *Rhizopus microspores var. Microsporus*, *A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus* were tested, focusing on stages with high concentrations of mitochondrial DNA. LoDs were compared with and without human background (0.1ng/reaction), utilizing symmetrical and asymmetrical setups. The finalized quadruplex qPCR assay was then assessed in 35 clinical samples, including 17 with confirmed fungal infections.

Results

The LoD for the quadruplex qPCR assay ranged from 39.67 fg to 23,762 fg (0.05 to 250 genome copies) in the symmetrical setup without human background, 537.6 fg to 59,405 fg (2 to 250 genome copies) in the symmetrical setup with human background, and 84.95 fg to 59,405 fg (0.05 to 250 genome copies) in the asymmetrical setup with human background. Therefore, the asymmetrical setup was employed for specificity testing. Of the 16 clinical samples with confirmed aspergillosis, 11 were detected (68.75%).

Conclusions

Based on our initial clinical evaluations, our qPCR assay successfully detected a diverse range of clinically relevant mucormycetes and *Aspergillus* species. However, further clinical validation is required.

PI-55

Evaluation of DNA extraction methods for recovering clinically relevant fungal pathogens from German soil

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Introduction

The presence of pathogenic fungi in soils, such as *Cryptococcus* spp., poses a significant public health concern. This study aimed to assess the efficacy of various commercially available DNA extraction kits, including soil-specific options, in recovering fungal pathogens from diverse soil types representative of the Berlin region. Specifically, our focus was on *Cryptococcus neoformans* utilizing yeast cells for soil inoculation.

Objectives

The objectives included evaluating the utility of soil-specific DNA extraction kits, such as the Qiagen DNeasy® PowerSoil® Pro Kit (QIA) and MP Biomedicals FastDNA™ SPIN Kit for Soil (MPB), in comparison to MPY, a kit used in clinical diagnostics.

Methods

Sampling involved collecting diverse soil types from Berlin, followed by spiking the soils with *C. neoformans* and subsequent DNA extraction using the designated kits. Fungal detection was conducted using a specific qPCR assay designed to target ribosomal DNA. Further, we evaluated PCR inhibition using an internal amplification control and assessed DNA quality and quantity.

Results

QIA exhibited superior extraction of amplifiable *C. neoformans* DNA compared to MPY and MPB, showing higher DNA yield and better removal of PCR inhibitors, with an efficiency of around 20%. However, PCR inhibition was detected with all extraction kits. Protocol optimization, such as additional dilutions to mitigate PCR inhibition, is necessary for all explored extraction kits.

Conclusion

Overall, commercially available DNA extraction kits demonstrated effectiveness in obtaining PCR-quality DNA from yeast cells in soil. These findings underscore the importance of considering soil characteristics when selecting extraction kits for downstream applications. This study significantly contributes to assessing fungal pathogen risks in local soils and emphasizes the need for accurate detection methods in surveillance and management strategies.

PI-57

Unexpected perseverance – A prolonged case of Tinea corporis

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Emerging multiple resistance of dermatophyte spp. pose a threat to antimycotic treatment and may prolong the course of disease or jeopardize its complete cure. We hereby present the case of a patient with tinea corporis who tested positive for *Trichophyton indotineae* and had a prolonged course of disease despite guideline-based treatment with terbinafine. We report this case to highlight the importance of quantitative Real-Time PCR (qPCR) for assessment of gene expression of multiple-drug-resistance transporters, heat shock proteins (HSP) and ergosterol (Erg) biosynthesis genes.

Our patient presented with persistent margin accentuated scaly plaques all over his body that had existed for about 6 months. Topical antibiotic and antimycotic treatment combined with glucocorticosteroids showed no effect. Mycological diagnostic was positive for *T. indotineae* in both ITS-PCR and culture. We initiated oral systemic treatment with terbinafine for one month and ongoing topical treatment with ciclopiroxolamine. However, persisting and new lesions followed the initial reduction of the lesions. After renewed cultivation of *T. indotineae* we repeated systemic treatment with terbinafine for another cycle. New lesions reappeared 6 months later. Comparison of initial and relapse isolates showed high basal levels of MDR3 transporter and heat shock proteins HSP60 and HSP90 in relapse isolate in the qPCR analysis.

Oral systemic administration of itraconazole for 30 days and topical treatment with miconazolenitrate resulted in full clearance both proven clinically and through mycological findings. The resistance of *T. indotineae* seems to be part based on efflux mechanisms due to activation of the MDR transporter, as also reported for other fungi. Our case shows the importance of PCR-based species identification combined with qPCR assays to evaluate the different resistance mechanisms conferred by Erg1 and Erg11B mutations, namely overexpression and efflux control.

PI-59

Saccharomyces cerevisiae fungemias in hospitalized patients

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Introduction

Saccharomyces cerevisiae is widely used in the alcohol and bakery industries. It is also commonly used as a probiotic in the prevention and treatment of digestive system disorders. *S. cerevisiae* is a rare cause of infections in humans, however invasive mycoses have been reported before.

Objectives

The aim of the study was to present *S. cerevisiae* case reports from blood samples of hospitalized patients.

Patients & Methods

A retrospective study of the results of mycological cultures in patients hospitalized at the University Hospital in Krakow, Poland in 2021-2022 was performed. The cases of *S. cerevisiae* isolation from blood were analyzed in detail, with clinical and laboratory data available in the medical records. For blood cultures BacT/ALERT (bioMerieux) system was used. *S. cerevisiae* strains were isolated on Sabouraud glucose agar with antibiotics and identified with MALDI-TOF MS (VITEK, bioMerieux). Antifungal susceptibility was tested with the automatic system (VITEK, bioMerieux).

Results

In total, 7 cases of *S. cerevisiae* from blood were found. Most patients had central venous catheter. Only two patients received *Saccharomyces* probiotic (Enterol®, Biocodex) during hospital stay. Two patients received fluconazole (FCZ) for treatment. Four patients died.

All *S. cerevisiae* strains were tested for susceptibility to amphotericin B (AMB), FCZ, and voriconazole (VCZ). MIC values ranged: 0.008-0.023 mg/L, 8-12 mg/L, 0.094-0.64 mg/L, for AMB, FCZ, VCZ, respectively.

Conclusions

Invasive *S. cerevisie* infections may occur in hospitalized patients with various risk factors who do not necessarily receive probiotic *Saccharomyces* strain. There is no established drug of choice for the treatment. Antifungal drugs breakpoints are lacking and formal categorising of the susceptibility is not possible. According to EUCAST guidance for rare yeast without breakpoints (ver. 05.03.2024) AMB may be considered for therapy.

PI-61

Trichophyton tonsurans und der Barbershop – Epidemiologie, Klinik und die Infektionsquelle

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Infektionen mit *Trichophyton (T.) tonsurans* in Form einer Tinea capitis (TC) nach Besuchen beim Barbershop wurden bereits in der Vergangenheit berichtet. In den letzten Jahren hat sich allerdings eine Häufung der Infektionen mit dem anthropophilen Dermatophyten gezeigt. Im Sommer 2023 stellten sich in unserer Ambulanz innerhalb eines Monats drei junge Männer mit dem klinischen Bild einer TC vor. Bei allen konnte *T. tonsurans* als Erreger nachgewiesen werden. Es stellte sich heraus, dass die Patienten wenige Wochen vorher Kunden im gleichen Barbershop gewesen waren.

Nach weiterer Häufung von Fällen wurde das örtliche Gesundheitsamt informiert und es erfolgte schließlich eine Begehung des Barbershops mit einer Beprobung der Oberflächen und Rasierapparate für eine mykologische Diagnostik. Es wurden insgesamt 10 Proben

entnommen: sowohl in einer Schublade, in der die Schneidegeräte aufbewahrt werden, als auch an einem Rasierer konnte *T. tonsurans* nachgewiesen werden. Die Inhaber des Barbershops wurden darüber in Kenntnis gesetzt und die Einhaltung der Hygienemaßnahmen wurden überprüft. Insbesondere die hygienische Aufbereitung der Haarschneidegeräte war mangelhaft. Im Verlauf konnte jedoch nach Unterweisung bei einer erneuten Kontrolluntersuchung der Erreger nicht mehr detektiert werden.

Die Häufung der Fälle hat das Gesundheitsamt zum Anlass genommen, die Hygieneauflagen für Barbershops zu spezifizieren und verstärkt zu kommunizieren.

Anhand der verschiedenen Fälle werden das klinische Spektrum von *T. tonsurans* und die Epidemiologie des Erregers demonstriert. Zudem werden die aktualisierten (in unserem Fall wirksamen) Hygieneauflagen für Barbershops erläutert.

PI-65

Comparison of olorofim and voriconazole activity against *Aspergillus fumigatus* using a calorimetric assay

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One of the most reported and clinically relevant fungi is the mold *Aspergillus fumigatus*, increasingly recognized for its acquired resistances towards azoles. Olorofim is one of the novel antifungal agents in the pipeline, awaiting FDA approval. We here assessed the activity of olorofim and compared it with voriconazole against *A. fumigatus*, including azole-resistant strains using microcalorimetry.

A. fumigatus (N = 5), including one strain with a TR34, one with a TR46, and one with a G54R mutation, as well as two wild type strains were analysed on their susceptibility towards olorofim (0.5 mg/L) and voriconazole (1 mg/L). The inoculum was set to 3 x 10^4 cells/mL in RPMI medium. Strains were incubated in presence and absence of the agents over 72 h at 37°C. Calorimetric data were collected using the CalScreener™ (Symcel, Stockholm, Sweden). Additionally, after 72 h of incubation, the cultures were analysed on formed biofilm using a standard XTT assay and microscopy.

The heat flow of the incubated samples differed widely between olorofim treated and non-treated conditions, resulting in almost no heat flow (µW) in all treated samples. The activity of olorofim was additionally found in significantly lower total heat (0-0.6 J) compared to the control (1.2-1.3 J). For the azole resistant strains treated with voriconazole, the heat flow and total heat showed similar results as the non-treated control with slight differences in time to peak whereas the susceptible strains showed flat curves when treated with voriconazole. A principal component analysis revealed differences in calorimetric profiles of azole-susceptible and resistance strains. Further, voriconazole treated samples showed significantly higher amount of biofilm formed after 72 h compared to olorofim treated samples.

We here demonstrated the activity of olorofim, also against formation of biofilms, in *A. fumigatus.* These data suggest calorimetry as a suitable method for susceptibility testing in molds.

PI-67

Can accumulating toxic sterols be effluxed under azole treatment in *Aspergillus fumigatus?*

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Objectives

Ergosterol plays a key role in sustaining membrane integrity, permeability, and fluidity of fungal cells. Under azole treatment fungistatic or fungitoxic sterols accumulate like lanosterol, eburicol, and 14-methylergosta-8,24(28)-diene-3β,6α-diol. As a mode of antifungal drug resistance azoles can be actively exported *via* efflux pumps. In fact, several genes coding for efflux pumps were shown to be upregulated in azole resistant clinical isolates [1,2]. Therefore, the question arises as a mechanism of detoxification whether not only azoles are extruded *via* efflux pumps but also fungistatic or fungitoxic sterols.

Methods

Ergosterol biosynthesis intermediates of *Aspergillus fumigatus* were analyzed in a targeted metabolomics fashion by GC-MS [3]. Sterol content and sterol composition of lyophilized mycelia and medium were compared to untreated samples and samples treated with sublethal doses of voriconazole. Besides sterol analysis we monitored the intracellular concentration of voriconazole in treated samples by LC-MS.

Results

Under voriconazole treatment fungistatic or fungitoxic sterols were not only detectable in the mycelia but also in the medium. This result indicates that sterols are actively effluxed.

Conclusion

Further studies are needed to investigate the contribution of effluxed fungistatic or fungitoxic sterols to the detoxification process under azole treatment.

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PI-69

Amphotericin B resistance in clinical *Candida glabrata* isolates

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Introduction

Multidrug resistance is an emerging problem in the treatment of systemic *Candida glabrata* infections. Administration of echinocandins is considered first- line therapy as resistance is less frequent compared to azoles. However, the number of isolates showing reduced susceptibility to both drug classes is increasing. An option is such cases is amphotericin B which targets ergosterol found in fungal cell membranes. Resistance to this drug is rare and its underlying mechanisms are still not fully understood.

Objectives

In this study we analyzed the biology of an amphotericin B resistant *C. glabrata* strain to understand resistance development and the consequences for fungal fitness.

Material and Methods

We performed a variety of experiments including whole genome sequencing, RNA-seq, sterol composition analysis and infection assays in a *Galleria mellonella* model to characterize the biology of a clinical resistant isolate.

Results

We found that loss of function mutations in the genes *ERG3* and *ERG4* correlated with ergosterol depletion in the fungal cell membrane diminishing the polyene`s target. Surprisingly, the strain did not display growth defects, even under stress conditions. Transcriptionally, the resistant isolate was well adapted to environmental stress, e.g. by up-regulation of ergosterol biosynthesis and drug efflux genes. The strain displayed an additional resistance to azoles which diminished after deletion of *PDR1*. This increased susceptibility to azoles could be linked to a down-regulation of *CDR1* and *PDH1*. In an *in vivo* infection model using *Galleria mellonella* larvae, the strain was as virulent as CBS138 and effectively killed infected larvae under amphotericin B treatment.

Conclusion

Our results illustrate that amphotericin B resistance does not necessarily impair the fitness nor the virulence of clinical *C. glabrata* strains but could be even trigger additional adaptation processes which initiate further evasion strategies to other antifungals.

PI-71

Geringe Azolempfindlichkeit von *Trichophyton rubrum* – Isolaten: Analysen zum Verständnis von Resistenzen

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Resistenzen gegenüber Antimykotika werden seit einigen Jahren bei Dermatophyten, sowohl gegenüber Azolen als auch Terbinafin, beobachtet. Die Resistenzmechanismen sind noch nicht gänzlich verstanden, zudem wird eine vereinfachte Durchführung von

Resistenztestungen gegenüber Antimykotika für die Routine relevanter. Für dieses Projekt standen uns zum Vergleich *Trichophyton (T.) rubrum*-Stämme aus Deutschland zur Verfügung, die vor > 10 Jahren isoliert wurden, und Stämme aus den aktuellen Jahren. Bei den Isolaten handelte es sich um Schuppen- und Nagelmaterial von Tinea-Patienten aus den Zeiträumen 2003-2012 und 2022-2023. Für alle Isolate wurde die minimale Hemmkonzentration (MHK90) mittels der Empfindlichkeitstestung nach EUCAST E.Def 11.0 ermittelt, und zwar für Amorolfin und Ciclopirox im Testbereich 0,016 mg/L – 8 mg/L, für Fluconazol im Testbereich 0,5 mg/L – 256 mg/L und für Itraconazol und Terbinafin im Testbereich 0,008 mg/L – 4 mg/L. Insbesondere für Itraconazol wurden bei 36 von 42 Isolaten hohe MHK90-Werte (oberhalb der Testbereichsgrenze von 4 mg/L) ermittelt (1).

Es folgte eine Kultivierung der Stämme sowohl auf Itraconazol- (1mg/l) und Terbinafin- (0,016 mg/l und 0,125mg/l)haltigem Agar (nach einem modifizierten Protokoll (EUCAST DEFINITIVE DOCUMENT E.Def 10.2)). Erste Ergebnisse zeigen einzelne Abweichungen zwischen den Ergebnissen nach der EUCAST-Methode und dem Antimykotika-haltigen Agar. Weiterhin werden die Stämme hinsichtlich ihrer Genexpression der Arzneimitteltransporter MDR 1,2 und 4 untersucht, da diesen eine Rolle in der Resistenz von Dermatophyten gegenüber Azolen zugeschrieben wird.

1. Überraschend geringe Azolempfindlichkeit von *Trichophyton rubrum* - sowohl bei Isolaten aus den Jahren 2003 - 2012 als auch aus 2022 – 2023; Lea Helm et al. Abstracts of the 57th Scientific Conference of the German speaking Mycological Society (DMykG) e.V. 27-29 September 2023, Frankfurt am Main, Germany. 2023 Sep;66 Suppl 1:3-42.

PI-73

Are polymeric particles eligible drug delivery systems to reach intracellular pathogens?

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Introduction and objectives

Conidia of *Aspergillus fumigatus* can avoid their ellimination inside the phagolysosomes of alveolar macrophages and cause invasive aspergillosis in immunocompromised patients. the treatment of intracellular persisting pathogens is challenging because the utilized drugs have to cross two membranes, the cytoplasmic and the phagolysosomal membrane. Drug delivery systems bear the potential to alter the internalization route of antifungals and thus, reach intracellular persistent microorganisms more efficiently compared to the pristine drug. The study was aiming to elucidate whether polymeric particles (PPs) can target intracellular persistent pathogens.

Methods

Dye-labeled PPs were formulated *via* the single emulsion technique to generate PP sizes large enough for their phagocytosis by macrophages. The sizes of PPs were characterized by dynamic light scattering and scanning electron microscopy. Internalization of PPs into RAW

264.7 macrophages was analyzed by imaging flow cytometry. Intracellular localization of PPs was confirmed microscopically by immunofluorescent staining and transmission electron microscopy.

Results

RAW 264.7 macrophagesrevealed an efficient internalization of the PPs. Addition of PPs to prior infected macrophages confirmed co-localization of PPs and conidia in the same phagolysosome due to fusion of phagolysosomes containing PPs with phagolysosomes containing conidia. The number of phagolysosomes containing both conidia and PPs was increased at elevated PP concentrations or after utilization of the fusion enhancer Vacuolin-1.

Conclusion

The fusion of conidia- and PP-containing phagolysosomes was proposed as the putative mechanism for PPs reaching intracellular conidia. Certain methods were identified for increasing fusion events between conidia- and PP-containing phagolysosomes. These results represent the requisite for the development of advanced delivery systems reaching intracellular persistent pathogens.

Poster Session II

PII-2

Establishing Reporter Systems for monitoring RNA Transfer from Neutrophils to *Aspergillus fumigatus*

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Aspergillus fumigatus is a filamentous fungus and an opportunistic human pathogen. The healthy immune system clears *A. fumigatus* infections quickly from the lungs; however, in immune-compromised patients, the fungus can germinate and cause various diseases. These range from allergic reactions to severe aspergillosis, a lung disease with a mortality rate between 40% to 90%. Due to a limiting number of anti-mycotics and a growing rate of resistance, new treatment options must be explored. Here, we build on a report showing that infection-derived extracellular vesicles (EVs) from neutrophils can inhibit hyphal growth of *A. fumigatus*. In plant-fungal interactions both partners can send RNA-based-messages in association with EVs to influence the transcription of the receiver. On this basis, we wanted to know if cross-kingdom-RNA-transfer also happens between humans and *A. fumigatus.*

For this we infected neutrophils with conidia for 2 h, isolated EVs, and performed sRNA-seq. We found that the main population of EV-associated sRNAs are members of the let-7 miRNA family. To prove let-7 miRNA uptake and functionality we have established several reporter systems. The first one is an artificial system with two fluorescent proteins (FP) expressed in the fungus. One FP has a known let-7 binding site in its 3'UTR and should be transcriptionally downregulated, while the other FP has no binding site and should stay constant upon EV-treatment. Additionally, let-7 miRNA-based target gene prediction and RT-qPCR showed downregulation of additional fungal genes. With this knowledge, we created an improved system by swapping the target sequence with the predicted one. In another set-up we myc-tagged a predicted target gene to monitor translational effects by horizontal RNA transfer.

In conclusion, we have improved our molecular toolkit for the study of the diverse population of potentially functional extracellular sRNAs involved in host fungal pathogenesis.

PII-4

Strategies to treat vulvovaginal candidiasis: neutralizing candidalysin and augmenting the protective potential of probiotic lactobacilli to reduce *Candida albicans* pathogenicity

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While *Candida albicans* is normally a harmless colonizer of mucosal surfaces, this fungus can cause vulvovaginal candidiasis (VVC) under certain conditions. This infection affects millions of women worldwide and significantly impacts quality of life. During infection, the *C. albicans* peptide toxin candidalysin drives host cell damage and activation of immune responses. Unlike other mucosal *C. albicans* infections, neutrophils that are recruited during VVC do not clear the infection and cause hyperinflammation and symptomatic disease. VVC is difficult to treat since the cause of infection is often unknown, infections are recurrent, and antifungal resistance is increasing. We therefore evaluated different treatment strategies to reduce *C. albicans* pathogenicity. We investigated anti-candidalysin nanobodies for their potential to prevent candidalysin-induced epithelial damage and metabolic supplementation to improve the anti-*C. albicans* activity of probiotic lactobacilli as an indirect strategy.

We showed that anti-candidalysin nanobodies reduced vaginal epithelial cell (VEC) damage during *C. albicans* infection *in vitro*. The nanobodies reached candidalysin within the invasion pocket of hyphae invading VECs. The presence of nanobodies, dampened proinflammatory cytokine release by infected VECs leading to decreased neutrophil activation. Candidalysin-neutralizing nanobodies can thus reduce epithelial cell damage and inflammation responsible for VVC symptoms. Further, by using untargeted metabolomics, we identified metabolites that support lactobacilli growth to improve their protective potential against *C. albicans*. This data set also gives us insight into the antagonistic mechanisms of lactobacilli and helps us to identify anti-*Candida* metabolites. In conclusion, we describe direct and indirect treatment strategies to reduce *C. albicans* pathogenicity during VVC by neutralizing candidalysin or metabolically enhancing the protective potential of probiotic lactobacilli.

PII-6

Small RNA regulation in *Aspergillus fumigatus*

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The RNA interference (RNAi) pathway has evolved numerous functionalities in eukaryotes, with many on display in Kingdom Fungi. RNAi can regulate gene expression, facilitate drug resistance, or even be altogether lost to improve growth potential in some fungal pathogens. Fungi infect over 1 billion people annually and kill over 1.5 million per year. In the WHO fungal priority pathogen, *Aspergillus fumigatus*, the RNAi system is known to be intact and functional. To extend our limited understanding of *A. fumigatus* RNAi, we used endogenously expressed inverted-repeat transgenes complementary to a conditionally essential gene (*pabA*) or a nonessential gene (*pksP*), we determined that a subset of the RNAi componentry is active in inverted-repeat transgene silencing in asexual spores (conidia) and mycelium. Analysis of mRNA-seq data from RNAi double-knockout strains linked the *A. fumigatus* dicer-like enzymes (DclA/B) and RNA-dependent RNA polymerases (RrpA/B) to regulation of conidial ribosome biogenesis genes; however, surprisingly few endogenous small RNAs were identified in wild-type conidia that could explain this broad change. A larger matched set of small RNA-seq data from the knockouts revealed few candidate small RNAs reminiscent of microRNAs or short-interfering RNAs, but instead revealed an abundance of rRNA and tRNA fragments differentially regulated across development. Biogenesis of these tRNA fragments appeared to be primarily independent of the RNAi machinery, but select fragments were shown to be developmentally regulated. Although RNAi was not clearly linked to growth or stress response defects in the RNAi knockouts, we did observe an effect on drug resistance with some of the RNAi knockouts, suggesting further investigation is needed. Cumulatively, *A. fumigatus* RNAi appears to play an active role in defense against foreign RNA, control of resistance to antifungals, and a previously unappreciated function in regulation of conidial ribosomal biogenesis genes.

PII-10

"Should I stay or should I go?" - Strategies of Candida glabrata to survive, persist, and escape from macrophages

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The opportunistic fungal pathogen *Candida glabrata* can cause severe systemic infections in humans. During infection, the fungus faces different types of immune cells, including macrophages, which are crucial for fungal clearance and initiation of antifungal immune responses. Macrophages can efficiently internalize *C. glabrata* cells, however, the fungus has evolved strategies to survive and even proliferate inside the phagosome. The most prevalent

Candida species, *C. albicans*, rapidly forms filaments inside macrophages and escapes. In contrast, *C. glabrata* replicates in the yeast morphology and can persist inside phagocytes for several days. In the first days following phagocytosis, this does not elicit pro-inflammatory responses, programmed cell death, or substantial host cell damage.

We observed that *C. glabrata* can escape after two to three days, when macrophages containing high numbers of replicating cells burst. Fungal replication in the yeast form *per se* does not mediate this delayed exit, as replicating yeast-locked *C. albicans* (*efg1*Δ/*cph1*Δ) escape much faster than *C. glabrata* cells. Host transcriptomic analyses after phagocytosis revealed that the early macrophage response is largely independent of the fungal species, suggesting that the delay is fungal-driven. We identified several *C. glabrata* protein kinases with potentially central roles in macrophage lysis and delayed exit. We now focus on kinases involved in autophagy, which may be connected to formation of *petites*, a respiration-deficient phenotype known to be associated with resistance to antifungals and, importantly, phagocytic killing.

Overall, our findings suggest that *C. glabrata* actively delays its exit from the macrophage, potentially to evade other players of the immune response. Our aim is to now further characterize the underlying mechanisms, as the intracellular phase of *C. glabrata* may serve as persistence stage and a starting point for re-infection of the host.

PII-12

NFDI4Microbiota supporting microbiome research providing data access, services, training and workflows

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Introduction

NFDI4Microbiota aims to support the microbiome research community by providing access to data, analysis services, data/metadata standards, and training. It belongs to the National Research Data Infrastructure (NFDI), which aims to develop comprehensive research data management. Different consortia ensure a broad coverage from cultural sciences, and engineering to life sciences and natural science. NFDI4Microbiota intends to facilitate digital transformation in the microbiological community (bacteriology, virology, mycology, and parasitology).

Goals

NFDI4Microbiota aims to support the German microbiome research network through training and community-building activities, and by creating a cloud-based system that will make the storage, integration, and analysis of microbial data and (microbial) omics data, consistent, reproducible, and accessible. Thereby, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable, and Re-usable) principles and Open Science.

Results

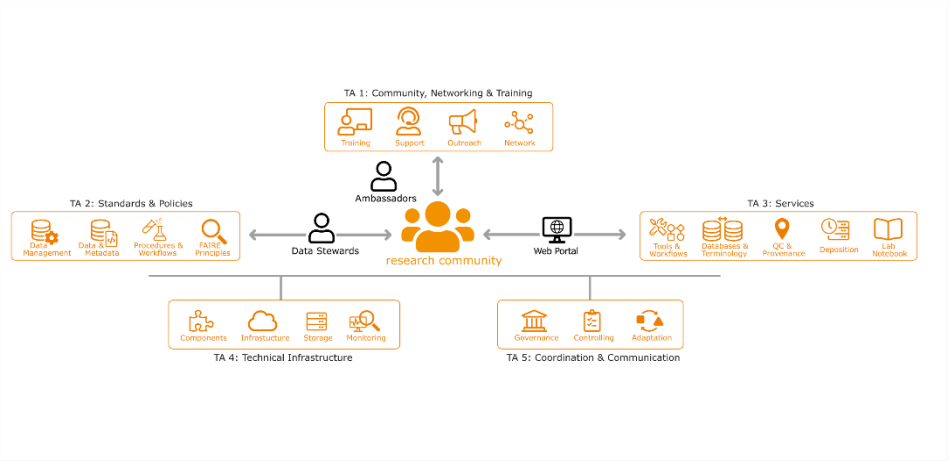
To enable FAIR data management, the NFDI4Microbiota consortium develops and provides computational infrastructure and analytical workflows to store, access, process, and interpret various microbiome-related data types. NFDI4Microbiota works on developing and

implementing software and standardized workflows for users to analyze their data. Further, NFDI4Microbiota offers training, spanning from metagenomics, over courses about programming in R, to research data management and ELN (electronic lab notebooks). To interact with young scientists, the consortium launched an ambassador program, thereby helping to identify the needs of their local community. All relevant information and specific services are available via the web portal.

Summary

NFDI4Microbiota has established community services providing access to data, analysis services, data/metadata standards, and training thereby promoting FAIR principles and Open Science in the microbiology community

Figure 1



PII-14

Free ISG15 dampens neutrophil hyperactivation by *C. albicans*

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Vulvovaginal candidiasis (VVC) is one of the most common fungal infections affecting women in their reproductive years. The interplay between the immune system and the virulence mechanisms of *C. albicans* drives the pathogenesis of VVC, leading to a hyperinflammatory response, neutrophil recruitment and activation as hallmarks of the infection. Type I interferon signalling and stimulation of interferon-stimulated genes (ISGs) has been identified as a common signature of early vaginal epithelial cell responses to infection with *Candida* species. This response improves epithelial resistance to *Candida* infections. While a myriad of ISGs are regulated by interferon signalling, *ISG15* warrants further exploration given its role as both an intracellular and extracellular mediator controlling certain viral and bacterial infections.

We investigated the localization of ISG15 during *C. albicans* infection of vaginal epithelial cells. We found that ISG15 expression increases upon *C. albicans* infection and also accumulates extracellularly. We used recombinant ISG15 to study its effects on neutrophil function, which was assessed by ELISA and ROS assays and live cell microscopy. The presence of ISG15 appeared to dampen ROS production, IL-8 release, and improved lifespan of neutrophils. Yet *C. albicans* clearance was not negatively impacted.

Collectively, our data suggests that exogenous ISG15 dampens hyperinflammatory responses of neutrophils. ISG15 may therefore be the mediator that dampens neutrophil activation following activation of protective type I IFN responses in vaginal epithelial cells. Thus, ISG15 may play a role in dampening the inflammatory responses driving immunopathology in VVC, which warrants its investigation in patient cohorts.

PII-16

(Arginine-induced) Filamentation of *Candida albicans* as an escape mechanism to exit from human macrophages

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Macrophages play a key role in the innate immune response to infections with the opportunistic fungal pathogen *Candida albicans*. Following phagocytosis by the host cell, *C. albicans* can survive intracellularly and even escape within hours.

A crucial step for this escape is rapid initiation of filamentation inside the phagosome since subsequently, hyphae can exert physical forces and drive damage to the phagosome membrane. Furthermore, filamentation leads to the production of the cytolytic peptide toxin candidalysin. After phagosomal escape, the toxin contributes to both host cell damage and activation of the NLRP3 inflammasome, leading to caspase-1-dependent release of IL-1β. However, the candidalysin-dependent activation of the NLRP3 inflammasome is mostly independent of pyroptosis. Taken together, this undermines the importance of understanding the mechanism behind intraphagosomal filamentation.

This key process is likely triggered by the amino acid arginine. Under phagosome-mimetic conditions such as nutrient scarcity and a low pH, arginine as well as the metabolically related amino acids ornithine and proline induce filamentation in both arginine-prototrophic and -auxotrophic strains. During infection with human primary monocyte-derived macrophages, an arginine auxotrophic strain exhibits delayed filamentation but wildtype-like escape rates and levels of damage. These data support the hypothesis that fungal arginine biosynthesis contributes to the induction of intraphagosomal filamentation, however, host-derived arginine seems to be the primary trigger.

In summary, our data show that host-cell escape of *C. albicans* requires a combination of hyphal extension and candidalysin production. In ongoing work, we are investigating potential sources of phagosomal arginine and its central role in macrophage polarization with potential effects on *C. albicans* escape.

PII-18

IL-1RA in T Cells: Linking Innate and Adaptive Responses

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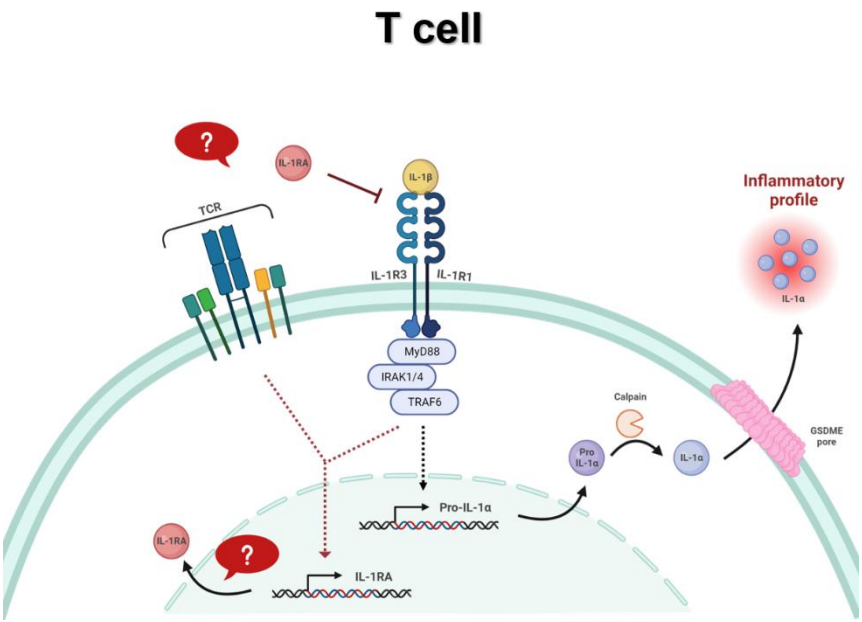
The IL-1 cytokine family, known for its involvement in mediating inflammation, was previously thought to be exclusive to the innate response. However, recent reports revealed that members of this cytokine family are also expressed in T cells (Arbore et al., 2016). In our previous studies, we have shown a marked expression of IL-1α in T helper 17 (Th17) cells, which was correlated with the promotion of phagocytosis of Candida albicans by monocytes (Chao et al., 2023). This finding challenges the previous perception of IL-1 family cytokines restricted to the innate response and further demonstrates an important role of T cell-derived IL-1 cytokines in the control of fungal disease. An interesting member of the IL-1 cytokine family is the Interleukin 1 receptor antagonist (IL-1RA), which principal role is to regulate inflammatory activity by blocking the binding of IL-1α or IL-1β to the interleukin-1 receptor (IL-1R) (Dinarello, 2019). Given the previous report on the expression of members of the IL-1 cytokine family in T cells, we wondered whether IL-1RA could also be expressed by T cells and what role it might play in fungal infections. Therefore, the present study focuses on the characterization of IL-1RA expression in human T cells (Figure 1: Analysis of IL-1RA expression in human T helper cells). Indeed, our group is pleased to report the existence of IL-1RA in conventional T helper cells, demonstrating a correlation of its expression with a specific T helper subset. Moreover, we have identified that the expression of IL-1RA in T cells is enhanced in response to specific T cell activation and cytokine polarization. Understanding the regulatory mechanisms of IL-1RA expression in T cells and its impact on antifungal immunity is promising. For this reason, our future studies aim to further investigate the modulation of IL-1RA expression and its role in fungal infections.

Figure 1



PII-20

Fungal Glucanase That Holds the Two Species of Candida Together

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Candidiasis is one of the most severe mycotic infections with a high mortality rate worldwide. Intriguingly, *C. glabrata* - the second-most frequently isolated *Candida* species, is often co-isolated with *C. albicans* from mixed-species infection sites. When co-cultured in *in vitro* conditions, *C. glabrata* shows ability to induce Yeast-to-Hyphal transition and adhere to hyphae in *C. albicans*. However, the molecular mechanism underlying this inter-species interaction is not studied yet. Our gene-deletion-mutant library screening showed that a *C. glabrata* beta-glucosidase mutant, among a few other, was impaired in hyphal induction in *C. albicans*. Thus, in this study, we are exploring the role(s) of two major *C. glabrata* beta-glucosidases in inter-species interaction. Interestingly, the beta-glucosidase mutant was not only impaired in yeast-to-hyphal transition, but also showed ~80% reduced adherence to pre-induced *C. albicans* hyphae. We further found that, the beta- glucosidase activity likely plays an important role in formation of mono- or dual-species *Candida* biofilm as well as the host epithelial adhesion *in vitro*. We propose, given that, adhesion to *C. albicans* hyphae could be advantageous for *C. glabrata* in accessing deeper tissue, that the beta- glucosidase could be potential target for a novel antifungal strategy.

PII-28

Lung microbiome dysbalance in *Aspergillus fumigatus* infection

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Introduction

Various microorganisms have been detected in healthy lungs by sequencing-based studies. However, the presence of a residential microbiome in the lung and its potential functional role in health and disease remain unclear. Invasive aspergillosis is a fungal lung infection with high mortality rates among immunocompromised patients caused by *Aspergillus fumigatus.* A potential contribution of the lung microbiome to invasive aspergillosis is a matter of debate. Here, we analyze the lung microbiome in healthy lungs of mice and upon disbalance by an *A. fumigatus* infection, and a possible prevention of disbalance by antifungal treatment.

Methods

The murine lung microbiome was studied by isolation of bacteria and 16S rDNA sequencing approaches. A mouse model of invasive aspergillosis was used to investigate the influence of *A. fumigatus* infection and voriconazole treatment on the lug microbiome. Interactions between the isolated lung bacteria and *A. fumigatus* were studied *in vitro.*

Results

Sequencing analysis revealed that the composition of the microbiome changed dramatically under immunosuppression, infection with *A. fumigatus*, and voriconazole treatment.

Interestingly, *Ligilactobacillus murinus* was detected in the lungs under all treatments and increased in abundance in the lungs infected with *A. fumigatus. L. murinus* was also one of the most abundant bacteria that was isolated from the mouse lungs. *In vitro* co-cultivation experiments showed that *A. fumigatus* promotes growth of *L. murinus* indicating a direct influence of the pathogen on resident bacteria.

Conclusion

We showed that both bacterial DNA and living bacteria can be detected in the lungs. *L. murinus* growth is promoted by *A. fumigatus* both *in vivo* and *in vitro* implying that the pathogen can directly affect the lung microbiome. Further studies on the lung microbiome in invasive aspergillosis can lead to the insight about the interdependence of lung microbiome, infection and immune response.

PII-30

Proteome analysis of programmed cell death in *Aspergillus fumigatus* conidia as basis for a "dead or alive" reporter strain

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Conidia produced by the human-pathogenic fungus *Aspergillus fumigatus* are the main cause of invasive aspergillosis in immunocompromised patients. In the lung, alveolar macrophages phagocytose the inhaled conidia and process them intracellularly. However, the fungus interferes with this mechanism to avoid being killed. To study these host-pathogen interactions *in situ*, it is crucial to track dying conidia. Currently, there is only a limited method to visually distinguish between resting and dead conidia, as they show no easily detectable metabolic or morphological differences. By generation of a reporter strain that produces a cell deathassociated protein fused with the green fluorescent protein, it would be possible to distinguish dead from living conidia by fluorescence microscopy. To identify a suitable reporter protein, here, we investigated the induction of the programmed cell death in *A. fumigatus* conidia. We first established an *in vitro* cell death assay by treating conidia with different celldeath-inducing compounds. We found that H_2O_2 in a nutrient-rich medium has a greater ability to kill resting conidia than the antifungal drugs amphotericin B or voriconazole. By proteome analyses of treated resting and swollen conidia we identified eleven proteins which are differentially abundant during regulated cell death. The nature of these proteins gives insight into the programmed cell death in *A. fumigatus* conidia. Moreover, these proteins are currently used for generation of suitable cell death reporter strains and their functionality will be initially analysed *in vitro* and in co-cultivation assays with RAW 264.7 macrophages.

PII-32

Elucidating the contribution of AtrR in *Aspergillus fumigatus* triazole resistance

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Question

Clinically used azole antifungals inhibit a key enzyme in ergosterol biosynthesis, sterol 14-α demethylase (Cyp51), which leads to the accumulation of toxic sterol intermediates and depletion of ergosterol, eventually growth inhibition. In *Aspergillus fumigatus* one of the major transcription factors in sterol regulation is AtrR. In this work we aimed to investigate the effects of differently mutated AtrR on *A. fumigatus* azole resistance.

Methods

To allow transcriptional fine-tuning and study expression level-based effects of different *atrR* variants, tunable promoters were used. Azole susceptibility of different mutant strains was analyzed phenotypically by radial growth assays. Minimum inhibitory concentrations (MICs) were detected using the EUCAST-based broth microdilution method. Expression levels of major AtrR target genes such as *cyp51A* encoding the azole drug target and *cdr1B* coding for an azole efflux pump, were measured in AtrR mutants employing Northern analysis. Simultaneous detection of the subcellular localization of non-mutated and mutated AtrR was achieved by tagging genes with fluorescent proteins.

Results

We uncovered AtrR variants that exert a negative impact on azole resistance. Overexpression of N-terminally truncated AtrR in a wildtype, results in downregulation of *cyp51A* and *cdr1B* and significantly higher azole susceptibility. Notably, the same overexpression does not interfere with growth in the absence of azoles. Microscopic examination of N-terminally truncated AtrR revealed that its subcellular localization is only partially aligned with the wildtype version.

Conclusions

The AtrR N-terminus is essential for correct cellular localization of AtrR and its regulatory action. Expression of N-terminally truncated AtrR perturbs the regulatory function of wildtype AtrR, which results in defective expression of resistance-associated target genes and, as a consequence, increased azole susceptibility.

PII-36

Galleria mellonella* as *in vivo* model for *Debaryomyces hansenii

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Introduction

Debaryomyces hansenii, formerly known as *Candida famata*, is a yeast that is mostly used in food processing industries. However, it has been also described as a rare opportunistic pathogen in immunosuppressed patients. Recent data suggest a higher abundance in Crohn"s disease lesions tending to impair the healing of wounded intestinal tissue. Still, its pathogenic and immunomodulatory functions remain unclear.

Obejctives

To gain further insights into *D. hansenii"s* pathogenic and proinflamatoric attributes we used *Galleria mellonella* infection model.

Materials

G. mellonella larvae were infected with *D. hansenii* strains by direct injection into the hemolymph. Infected larvae were incubated at 37°C for at least 5 days. Survival and health was traced by using the Health Score Index (HSI). Selected larvae were embedded in paraffin and stained with HE, PAS and Grocott. PBS injected larvae served as controls. *C. auris* was used as comparator.

Results

First results indicate that *D. hansenii* strains -in contrast to its comparator *C. auris* - do not affect larvae"s mortality. Even higher infection doses do not lead to larval death. However, dose-dependent and strain-dependent differences in virulence, resulting in lower HSI are observed. By tracing the inflammation process histologically, melanized granuloma-like lesions are found. A granuloma grading system is in establishment to further quantify inflammation between strains.

Conclusions

D. hansenii shows strain-dependent proinflammatory properties in *G. mellonella*, without reducing larval viability. A gut colonization model and variable incubation temperatures will give further insights into possible pathogenic features of this very adaptable yeast.

PII-38

Establishment of an invasive mucormycosis lung-on-chip model for visualizing and analyzing interactions between macrophages and fungal pathogens

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Pulmonary fungal infections encompass fungal asthma and invasive fungal infections. The air-borne spores of *Lichtheimia corymbifera*, *Mucor lusitanicus* and *Rhizopus arrhizus* predominantly cause invasive mucormycosis (IM). Following inhalation, spores settle in the alveoli, causing severe pulmonary infection with a high risk of dissemination in immunocompromised people. Here, IM is reported as breakthrough-infection (BTI) after successful therapy of invasive aspergillosis (IA) with 50 – 90% mortality rate.

As macrophages are the first line of defence, we strive to understand the interplay between the fungal pathogens and alveolar macrophages. We aim to establish a lung-on-chip model with *L. corymbifera* as the chief pathogen for visualizing infection progression, response of alveolar macrophages, and post-phagocytosis intracellular fate of spores. Moreover, a co-incubation infection model will be setup to investigate the BT infection characteristics of *L. corymbifera* alongside *A. fumigatus*.

Traditional disease modeling relies on mouse models, which are limited in developing advanced diagnostic and therapeutic approaches for IM. The lung-on-chip model, mimicking human alveolar tissue, enables enhanced research in this field. In the chip, lung epithelial and vascular endothelial cells are seeded onto two sides of a porous membrane in a microfluidic environment along with pre-labelled immune cells and fungal spores to simulate infection. The progression of immune reaction is monitored through confocal laser scanning microscopy and flow cytometry to analyze cellular and fungal interaction.

Later, a commensal bacterium will be added to the co-infection model to investigate tri-partite microbial interactions. We also aim to develop a multicellular murine biochip as a preceding step before animal models.

These biochips provide a platform to evaluate host and pathogen interactions and diagnostic markers for developing and testing efficient antifungal therapies against IM.

PII-40

Characterisation of the molecular switch determining phagosome maturation

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Introduction

To degrade or not to degrade is a fundamental question for cells whilst dealing with phagocytosed cargo, especially when facing pathogenic intruders. Previously, we discovered that when *Aspergillus fumigatus* spores are ingested by A549 alveolar lung epithelial cells, phagosomes can either enter the degradative or non-degradative pathway. This duality is orchestrated by the interplay of the fungal protein HscA and the host protein p11. The former anchors p11 on phagosomes, thereby excluding the recruitment of the phagosome maturation mediator Rab7 and ultimately preventing phagolysosomal degradation (Jia et al., 2023). In contrast, when HscA is absent on the conidial surface, p11 dissociates from phagosomes, leading to phagosome maturation into phagolysosomes. Hence, human p11 serves as a pivotal switch in determining the fate of phagosomes.

Objective

Given that p11 excludes the recruitment of Rab7 on phagosomes, to better understand the role of p11 in preventing phagosome maturation, we comprehensively analyze the proteomes of p11+ and Rab7+ phagosomes. This approach aims to define distinct phagosome markers involved and shed light on the molecular mechanisms governing phagosome fate.

Material & Methods

We isolate phagosomes from A549 cells infected with *A. fumigatus* conidia followed by purification into p11+ and Rab7+ subpopulations via FACS. Subsequently, the proteomes are analyzed using LC-MS/MS.

Results

We have optimized a phagosome isolation protocol for efficient isolation from A549 lung epithelial cells.

Conclusion

This optimized phagosome isolation protocol ensures a sufficient quantity of phagosomes for downstream analyses, facilitating comprehensive investigations into the host-pathogen interactions occurring within phagosomes.

PII-42

Employing defined human Macrophage *in vitro* models to describe key factors in early immune defense against *Aspergillus fumigatus* using time series analyses

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Each day, humans inhale over 10,000 liters of air, which can contain various particles like dust, but also pathogens including viruses and conidia of the saprotrophic mold *Aspergillus fumigatus.* It mainly spreads via asexual sporulation and aerial distribution causing life-threatening diseases in immunocompromised individuals in contrast to healthy individuals, which can maintain lung homeostasis. The mechanisms determining whether this pathogen can be contained or will evade the immune system remains elusive. We aim to characterize early decision points in alveolar macrophages upon challenge with *A. fumigatus* by employing different human macrophage (MΦ) populations. We utilized the recently published Alveolar Macrophages Like (AML) cell model1 in comparison to GM-CSF MΦ in dual sequencing. Hereby, we stimulated the cells with *A. fumigatus* for 6 and 9 h to evaluate the MΦ specific and common molecular response upon infection. Using those transcriptomic patterns as a baseline, we employ functional analyses like qPCR, flow cytometry and ELISA to validate the sequencing and perform pathway analysis of the transcriptomic profiles to screen for MΦ infection specific molecular patterns. Likewise, we used both MΦ models in an infection setup using FLARE conidia2 to investigate phagocytic and killing efficacy of both MΦ types in flow cytometry over a specified time series. Eventually, we use microscopic assays to specify the phagocytic and killing efficacy of both MΦ populations further, which we subsequently compare to primary alveolar MΦs. We evaluate the AML and GM-CSF MΦ models for infection with the fungal pathogen *A. fumigatus* and compare them to primary alveolar macrophages. Through both time series analyses, we will be able to identify key factors of MΦ to successfully prevent *A. fumigatus* lung invasion.

1. Pahari et al. *mBio* vol. 14,4 (2023): e0083423. doi:10.1128/mbio.00834-23
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PII-44

Glycolytic control of neutrophil immune responses to *Candida albicans* infection

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Neutrophils are cells of the innate immune system and play a central role in eliminating bacterial and fungal pathogens. In their function as first-line immune defense against invading microorganisms, neutrophils require a rapid supply of energy and building blocks to cope with the pathogens. To meet this demand, neutrophils rely primarily on aerobic glycolysis, making glucose uptake and metabolism indispensable for rapid and efficient responses. Granulocytes express the hexose transporters GLUT1 and GLUT3 to utilize glucose but the specific functions of GLUT1 and GLUT3 in neutrophils remains unknown. In this project, we show that protective neutrophilic immune responses to *Candida albicans* infections are dependent on environmental glucose uptake and aerobic glycolysis. The expression of GLUT1 in neutrophils was markedly increased, while GLUT3 expression was downregulated, upon stimulation with fungal antigens or live *C. albicans*. Genetic depletion of both GLUT1 and GLUT3 almost prevented glucose uptake and lactate secretion of bone marrow-derived neutrophils, demonstrating that GLUT1 and GLUT3 are the main glucose transporters in neutrophils. Surprisingly, combined ablation of GLUT1 and GLUT3 did not affect homeostatic granulopoiesis, suggesting that neutrophil development does not require external glucose consumption. By contrast, the effector function of neutrophils after microbial challenge, such as cytokine and chemokine secretion, was severely impaired, demonstrating that glucose uptake by GLUT1/3 is essential for anti-fungal immunity. Our findings also shed light on the development of metabolic therapies for the treatment of autoimmune diseases. The anti-inflammatory effects of novel GLUT1/3 inhibitors need to be balanced against their potential suppression of protective immune responses, i.e. immunity to bacterial and fungal infections.

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PII-48

The role of neutrophil-derived extracellular vesicles in escape of *C. albicans* from phagocytosis

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Introduction

Candida albicans is a commensal fungus that can cause potentially lethal invasive infections in immunocompromised patients and is one of the leading causes of hospital-acquired sepsis.

Neutrophils comprise the majority of circulating leukocytes and provide a rapid innate immune response against *C. albicans*. In an *ex vivo* whole blood model, a fraction of *C. albicans* cells evaded phagocytosis and killing.

Goals
We aimed to determine whether neutrophil-derived extracellular vesicles (EVs) play a role in the escape of *C. albicans* from phagocytosis.

Methods
Neutrophils were isolated with the MACSxpress Whole Blood Neutrophil Isolation Kit (Miltenyi). We characterized EVs released by neutrophils after contact with opsonized *C. albicans* (CaEVs) in comparison to EVs spontaneously released by mock-infected cells (sponEVs). EVs were isolated by high-speed centrifugation and characterized by mass spectrometry and nanoparticle tracking. After incubation of EVs and *C. albicans*, we investigated fungal surface remodelling and phagocytosis by fresh neutrophils using flow cytometry.

Results
Extracellular *C. albicans* acquired host cell surface markers (e.g. CD66b) after 1 h of confrontation with neutrophils. This transfer also occurred during incubation of *C. albicans* with isolated EVs, suggesting that EVs contributed to the remodelling of the surface of extracellular fungi. While the size of CaEVs and sponEVs was similar, there were differences in the protein content. SponEVs show less protein cargo than EVs produced by challenged neutrophils. Finally, *C. albicans* pre-incubated with EVs showed slightly decreased phagocytosis by neutrophils.

Conclusion
The transfer of host cell proteins to the surface of *C. albicans* and the reduced phagocytosis of pre-incubated fungi with EVs suggest that EVs released by neutrophils contribute to the escape of *C. albicans* from phagocytosis. These results increase our understanding of host-cell interactions with *C. albicans*.

PII-50

Assessing Diagnostic Capabilities and Treatment Accessibility for Invasive Fungal Infections in the Balkan Region

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Background

Advancements in invasive fungal infection (IFI) diagnosis and treatment continue, but economic disparities hinder tool accessibility. IFI prevalence and characteristics vary globally. This study assesses IFI diagnostic capabilities and treatment availability in the Balkan region.

Methods

Data were collected from Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Kosovo, Moldova, Montenegro, North Macedonia, Serbia, Slovenia, and Romania. The survey, at www.clinicalsurveys.net/uc/IFI_management_capacity, explored participating institutions' characteristics, IFI perceptions, and diagnostic method availability.

Results

Surveying 47 Balkan medical center representatives, 38% deemed IFI incidence low, 34% very low. Candida spp. caused IFIs in all centers, followed by Aspergillus spp. (72%) and Cryptococcus spp. (43%). Diagnostic resources included culture/microscopy (98%), susceptibility testing (93%), antigen detection (81%), and PCR tests (Aspergillus 48%, Candida 34%, Pneumocystis 43%, Mucorales 19%). Triazoles were prevalent (96%), followed by echinocandins (79%, mainly micafungin 77%), and amphotericin B (68%, primarily liposomal 57%). Therapeutic drug monitoring was in 30% of centers, mainly for voriconazole (100%), posaconazole (71%), itraconazole (64%), and flucytosine (43%).

Conclusions

Balkan mycology labs are well-equipped, but molecular tools are less accessible in <50% of centers. Triazoles are common, while echinocandins, amphotericin B, flucytosine, and terbinafine are less available. The survey welcomes more insights from the region.

PII-52

Development and validation of a point of care DNA extraction for the detection of Dermatophytes

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Introduction

DNA extraction from skin, nail and hair for dermatomycosis analysis requires lengthy protocols employing multiple processing steps. Typically, existing protocols require overnight incubation and purification of the extracted nucleic acids. A particular difficulty is that these methods show a low DNA yield, especially if specimens contain only very low amounts of biomaterial.

Objective

Our work aimed at simplifying and shortening the DNA extraction process without compromising the sensitivity of subsequent detection assays. That would allow for dermatophyte analysis to be shifted from central laboratory settings towards small laboratories even in dermatologist´s offices, thereby shortening the time to results.

Material & Methods

We developed a fast protocol combining short powerful enzymatic lysis with inhibitor-removal technology that minimizes the loss of nucleic acids released from the biomaterial. The reverse purification methodology removes impurities from nucleic acid extracts using paramagnetic beads. Finally, the extraction protocol was validated with skin, hair, and nail samples using the EUROArray Dermatomycosis PCR-array platform of EUROIMMUN (a Revvity company).

Results

The protocol we developed comprises of a few simple steps: adding the sample to a mixture of lysis buffer, proteinase, and magnetic beads. The total DNA extraction time was shortened

to 20 minutes. Furthermore, the protocol is simple enough to be performed by semi-professional lab personnel. Our study results show that the performance of the fast lysis & reverse purification protocol is equivalent to a silica membrane-based reference DNA extraction kit.

Conclusion

The processing of samples and performance of the analysis at the site of sampling without compromising the diagnostic performance enables a much shorter return of results to the patient, which allows an earlier onset of the anti-fungal therapy.

PII-54

Design and establishment of mitochondrial genes as diagnostic markers for mucormycetes, aspergilli, *Aspergillus* section *Terrei* specific qPCR-assay

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Introduction

Invasive fungal infections, like mucormycosis, are increasingly threatening, as highlighted by recent outbreaks among SARS-COVID-19 survivors. Global incidences are rising due to a growing population of vulnerable patients. However, a diagnostic gap persists in detecting and differentiating between *Mucorales* spp., *Aspergillus* spp., and the *Aspergillus terreus* complex. Early diagnosis and targeted therapy are imperative for improving patient outcomes. Previous studies have shown promising results in the development of novel qPCR assays utilizing mitochondrial genes.

Aim

The aim of this study is to advance the early-stage probe-based pan-Mucorales duplex qPCR assay into a novel pan-Mucorales, pan-*Aspergillus*, and *Aspergillus terreus* section quadruplex diagnostic qPCR.

Material and Methods

Key characteristics of the qPCR assay were determined, including efficiency, sensitivity, cross-reactivity, and specificity. Species-specific efficiency and analytical sensitivity of all four targets—*Aspergillus terreus*, pan-*Aspergillus*, pan-Mucorales, and the human marker—were determined using selected species. A total of 253 DNA samples obtained from fungal pure cultures were evaluated, including: (a) 101 DNA samples of *Aspergillus* spp., (b) 17 samples of *A. terreus* section as positive controls for *Aspergillus* spp., (c) 96 Mucoralean species as positive controls for mucormycetes, (d) 35 other fungal pathogens, and (e) 4 samples of animal species as negative controls.

Results

Cross-reactivity was nearly eliminated for non-*Aspergillus* spp. (N=131) and animal species (N=4), with slight interference observed with *C. glabrata*. The specificity for pure cultures was 81.19% (N=101) for the pan-*Aspergillus* marker and 82.35% for the *A. terreus* complex marker.

Conclusions

The performance of the quadruplex pan-Mucorales, pan-*Aspergillus*, and *A. terreus* section-specific qPCR assay shows promising results and warrants further clinical evaluation.

PII-56

Enhanced identification of filamentous fungi using MALDI-TOF: A routine preparation workflow plus extended spectra library

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Introduction

Fungi appear more frequently in microbiological testing. Identification of filamentous fungi using macroscopic and microscopic methods is time consuming and needs well trained personal. However, MALDI TOF identification of filamentous fungi was still very time intensive before the introduction of a new sample preparation method (MyT) which enables the identification within a few minutes.

Objectives

We present the new, MyT method and compare the identification rates using the two latest Bruker MALDI Biotyper libraries.

Material & Methods

706 clinical MALDI spectra measured with the MBT Filamentous Fungi Module including a specialized spectra acquisition method were used to test the MBT Filamentous Fungi Library (MBT Fil. Fungi Lib.) 2022 compared to the new library 2023. The spectra were mainly generated using the new MyT method. Only samples which could not be identified with it were prepared with the extraction from liquid culture. Formic acid is pipetted onto a MBT Biotarget 96. A wooden toothpick is dipped in formic acid and "front mycelium" is harvested from a 24-48 h incubated Sabouraud agar plate. The spot is dried at room temperature and overlayed with HCCA matrix and dried at 35 ± 0.7 °C with a MBT FAST Shuttle. Targets are measured with MALDI Biotyper sirius® instrument. The resulted spectra were tested with the two libraries.

Results

The identification rates of clinical spectra analysis using the two libraries showed that with the new MBT Fil. Fungi Lib. 2023 eight more isolates could be identified with high confident log(scores). 1.6% of 706 spectra could not be identified with the MBT Fil. Fungi Lib. 2022 but with the 2023 version. The MyT method reduced the hands-on time to a few minutes compared to the extraction from liquid cultivation which takes much longer.

Conclusion

The combination of the new MBT Fil. Fungi Lib. with the MyT method leads to reduced hands-on-time and to increased identification rates of filamentous fungi.

PII-58

Occurrence of environmental *Mucorales* with clinical relevance in Tyrol

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Fungi belonging to the *Mucorales* can cause severe Mucormycosis in patients with immunosuppression or other primary conditions like diabetes or trauma. Generally, Mucorales are found in diverse ecological niches like soil, decomposing material, water, air and dust from indoor environments. Still, whether the occurrence and growth of clinically relevant species in these habitats forms a major exposure and infection risk is not clear.

This work investigates the species distribution of *Mucorales* in agriculturally utilized soils, air samples and indoor dust in Tyrol as potential sources of infectious agents and aims to correlate species occurrence to clinical prevalence and unusually high rates of *Lichtheimia* spp. among Mucorales infections.

Soil samples from agricultural land sites in Tyrol were collected in different seasons and examined with culture-based techniques. Furthermore, dust and air samples were included. Species ID was done by PCR and Sanger Sequencing of the ITS1 region and obtained sequences were blasted against the ISHAM ITS database.

As expected, *Mucorales* were found in all soils independent of the season of sampling. *Mucor circinelloides* was the most frequently isolated species and *Mucor spp.* in general were more abundant than other *Mucorales*. The diversity of *Mucorales* at the sampling sites differed to great extent. In general, the species richness was greatest in autumn, showing a seasonal pattern with an increase throughout the growing season. Dust and air samples are currently evaluated for species ID.

Mucorales are omnipresent in tyrolean agricultural soils but the species distribution depends on the type of soil, the sampling site, the season and the type of crop planted. Against our expectation, *Lichtheimia* spp. were not found so far. This, and other limitations of culture based identification methods, leads us to continue further investigations utilizing qPCR methods to fully detect the mucoralean diversity in our samples.

PII-60

Distribution of *Aspergillus terreus* in soil in Tyrol, Austria

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Aspergillus terreus, is a filamentous fungus known for its role as an environmental decomposer and opportunistic pathogen. Although infections caused by *A. terreus* are rather rare, there are specific medical centers, such as Innsbruck, where they are more prevalent. In Tyrol, cases of infection and natural occurrence appear to be more common in the eastern (lowland) regions than in the western (upland) regions, suggesting a possible pattern. The objective of this study was to verify the elevated prevalence of *A. terreus* in lowland soils in comparison to upland soils and to identify the factors that contribute to its higher occurrence, while confirming its reservoir in soil. Approximately 40 lowland and upland sites were selected for soil sampling. These samples were tested for the presence of *A. terreus* using plate culture methods. In addition, the soil samples were analyzed to assess various soil properties, including pH, soil moisture and contents of organic matter, total carbon and total nitrogen. Site parameters such as altitude and weather conditions were also taken into account in the analysis. The soil immersion tube method was used to assess the soil as a reservoir for *A. terreus*. In this study of 300 soil samples, *A. terreus* was found to be more abundant in soils of the Tyrolean lowland (about 18%) compared to those of the Tyrolean upland (about 8%). Soil samples from the lowland had a significantly higher moisture content (about 28%) than those from the upland (about 26%) and a lower pH (6.4) than those from the upland (6.6). There was also a significant difference in the amount of soil organic matter present, with the upland samples containing 9.0% and the lowland samples containing 7.2%. Although differences were found in the soils of the Tyrolean upland and lowland, further research is needed to determine whether these factors also affect the distribution of *A. terreus* throughout Tyrol. One way to do this is through a planned genotyping study.

PII-62

Genetic diversity of indoor isolates of the *Fusarium oxysporum* and *Fusarium solani* species complexes

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Fusarium is a large and diverse fungal genus, with currently over 400 species described. In humans, they can cause life-threatening infections in immunocompromised patients but also those of the cornea in otherwise healthy individuals. In Germany, the majority of these infections is caused by species of the *F. solani* (FSSC) and *F. oxysporum* species complexes (FOSC). Though certain species of both SCs dominate in clinical settings, little is known about their specific ecology. Interestingly, some of those appear to be mainly present in indoor habitats. Further, a dominance of specific sequence types indicating potentially more pathogenic or indoor-adapted species lineages has been described, as well as the presence of mobile, potentially virulence-associated "accessory chromosomes" (ACs) in two human FOSC isolates. To address these issues, we investigated the following: (I) Does the FOSC and FSSC indoor species diversity differ from clinical samples? (II) Do specific sequence types dominate? (III) What known ACs are present? Therefore, we performed multi locus sequence typing for species identification and strain typing with clinical *Fusarium* isolates present at the NRZMyk, and indoor isolates sampled in cooperation with construction biologists. A PCR-based screen was used for AC detection. We found an abundance of three FSSC species in both, clinical and indoor samples, while two other species were only commonly found in indoor samples. For the FOSC, the species diversity from indoor and clinical samples was very similar with a clear dominance of *F. veterinarium*. This species also showed a dominance of a specific sequence type, while other FOSC species had greater diversity. Furthermore, we found AC sequences present in most FOSC species, yet more frequently in two clinically relevant

species. In sum, this work detailed an abundance of clinically relevant *Fusarium* species in indoor environments, proposing them as a likely reservoir for infections.

PII-64

The antiseptic N-Chlorotaurine demonstrates its efficacy as inhaled therapeutic in a murine model of *Aspergillus fumigatus* pneumonia

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Introduction

N-chlorotaurine (NCT) is an excellently tolerated antiseptic substance with broad activity spectrum against pathogens. The well-documented topical applicability recommends inhaled NCT as supportive therapy for fungal infections of the lower airways and was already studied for mucormycetes.

Objectives

We aimed: (1) to evaluate the NCT effectiveness in mouse models of *Aspergillus fumigatus* pneumonia with different immunosuppressive regimens; (2) to compare different NCT concentrations in their capacity to improve the outcome.

Materials and Methods

Mice were immunosuppressed with either cyclophosphamide or cortisone acetate, followed by intranasal inoculation with *A. fumigatus.* Inhalations with 0.1% - 2.0% NCT solution or, as control, sodium chloride three times daily for 10 min started one hour after inoculation and ended after 15 days.

Results

In a subgroup of mice euthanized on day 2, fungi and inflammatory signs were detected in the lungs. In the placebo group, 8/9 or 9/9 mice observed for 15 days died from the infection during this time, while 0/9 to 1/9 died in groups treated with 0.5%, 1.0% and 2.0% NCT ($p < 0.01$ for each concentration versus saline). There was no difference between the two regimens of immunosuppression. With 0.1% NCT, 4/9 mice died ($p = 0.03$ versus the higher NCT-concentrations; $p = 0.0035$ versus control). The fungal load came to 5.28 (4.46; 5.70; median, quartiles) CFU/ml lung homogenate in the control group and to 1.3 (median; maximum 2.45) in the 1% NCT group in mice immunosuppressed with cyclophosphamide ($p = 0.0004$). Values were similar in cortisone groups ($p = 0.0023$). Secondary parameters showed respective significant differences between test and control groups.

Conclusion

Early treatment with inhaled NCT demonstrated highly significant efficacy in improving the outcome of *Aspergillus* pneumonia. A concentration of 1% NCT appears to be optimal, which fits to case experiences with inhalations in humans.

PII-66

Entwicklung eines trägerbasierten Testsystems für die Desinfektionsmitteltestung mit Dermatophyten

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Zoophile Dermatophyten sind als Zoonoseerreger von enormer medizinischer Bedeutung. Neben der antimykotischen Patientenbehandlung ist die effiziente Abtötung von Infektionsmaterial in der unmittelbaren Umgebung notwendig. Dafür müssen hoch wirksame Desinfektionsmittel zur Verfügung stehen. Für Hefen und Schimmelpilze liegen Daten zur Desinfektionsmittelwirksamkeit vor, aber nicht für Dermatophyten.

Daraus ergab sich die Zielstellung ein geeignetes trägerbasiertes Testsystem zu entwickeln, mit dem die Fungizidie von Desinfektionsmittelwirkstoffen auf natürlichem Infektionssubstrat gewachsenen Dermatophyten zeit- und dosisabhängig untersucht werden kann.

Dafür wurde Schafwollvlies auf einem einseitigen Klebestreifen fixiert bzw. Lammwollsohle für Schuhe in Streifen geschnitten. Nach dem Autoklavieren des Trägermaterials erfolgte die Beimpfung mit einem bei Raumtemperatur flüssigen Kulturmediums (halbfest bei Temperaturen über 28°C), dass Dermatophyten (aus Stammlösung mit KBE/ml) enthielt. Nach Inkubation und Kontrolle des Dermatophytenwachstums wurden die infizierten Träger in sterilen 24-Kavitäten-Zellkulturplatten mit unterschiedlichen Konzentrationen von Peressigsäure (Modellwirkstoff, Verdünnung in sterilem Leitungswasser) zeitabhängig bei Raumtemperatur inkubiert. Nach dem zeitversetzten Auswaschen der Prüfsubstanz mit PBS erfolgte die Zugabe von 1 ml je Kavität Kulturmedium. Nach Inkubation bei 28°C über mehrere Tage erfolgt die Kontrolle auf Dermatophytenwachstum.

Unter Anwendung von Peressigsäure als Modellwirkstoff konnte festgestellt werden, dass eine fungizide Wirkung wie folgt vorlag: nach 5 Minuten Einwirkzeit bei 0,4% Peressigsäure, nach 15 Minuten ab 0,2% Peressigsäure und nach 30 Minuten ab 0,1% Peressigsäure. Alle untersuchten Dermatophytenarten wuchsen auf dem Trägermaterial an den Haaren. Somit kann mit dem entwickelten Testsystem praxisnahes Infektionsmaterial gezielt zeit- und dosisabhängig auf fungizide Wirkstoffe untersucht werden.

PII-68

Growth medium and temperature as critical parameters in Microplate-Laser-Nephelometry (MLN) measurements of itraconazole inhibitory concentrations for dermatomycetes compared to EUCAST E.Def9.3.2. or E.Def11.0 conditions

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Introduction

Increased azole tolerance was observed for *Trichophyton indotineae* isolates[1, 2] of the *T. mentagrophytes/ interdigitale* complex, but also for new subtypes of the mouse favus pathogen *Trichophyton quinckeanum*[3]. Measurements of inhibitory concentrations were performed according to the EUCAST E.Def9.3.2 protocol[4] developed for molds or E.Def11.0 adapted for *Trichophyton* species.[5] The use of the Microplate-Laser-Nephelometry (MLN) method[2, 3] allows automated measurements to be carries out throughout the entire growth period.

Objectives

MLN methods were adapted to the EUCAST E.Def9.3.2 or E.Def11.0 methods and mainly showed differences in growth temperatures. The following media were compared with each other: RPMI1640, RPMI1640 with additives and Sabouraud Glucose (SG) broth.

Results

Medium and temperature significantly influenced the growth parameters of the different dermatomycetes. The minimal medium RPMI1640 distinctly prolonged the growth time until the growth maximum was reached. This minimized effects between sensitive and tolerant isolates and generally resulted in higher values of itraconazole inhibitory concentrations. Higher temperatures increased the growth rate and allowed better discrimination between sensitive and tolerant isolates. Addition of a protein source enhanced the growth rate of isolates in RPMI1640, which was accompanied by lower values of itraconazole inhibitory concentrations. The highest discrimination of isolates was detected in SG broth in combination with high temperatures.

Conclusion

It was shown that reduction of the growth rate by using minimal media or low cultivation temperatures helps dermatomycetes to adapt to itraconazole.

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PII-70

Demographic and clinical data for individuals with invasive Candida infection receiving rezafungin during an early access programme in Germany and Italy

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Background

We report demographic and clinical data for patients with invasive candidiasis receiving once-weekly intravenous (IV) rezafungin therapy during an ongoing early access programme in

Germany and Italy. Patients were required to meet ≥1 of the following criteria: ineligible for currently available therapies; eligible for discharge but needing daily IV infusions; requiring outpatient antifungal treatment; receiving critical care (with fluid overload); diagnosed with azole-resistant and echinocandin-susceptible infection; experiencing infusion site reactions (fewer infusions preferable); requiring long-term treatment.

Case(s) description

Seven patients were provisionally enrolled in the programme. Five were male and mean age was 68 (range: 46–78) years. Key risk factors for candidiasis were major surgery, central catheter use, total parenteral nutrition, acute renal failure and diabetes mellitus. Diagnoses included foreign body infections (spondylodiscitis involving non-removable hardware, prosthetic valve endocarditis, and vascular graft infection), candidemia and intra-abdominal candidiasis. Rezafungin treatment was selected to facilitate hospital discharge and outpatient antifungal administration. Four patients were successfully treated with rezafungin in the outpatient setting and therapy was ongoing at the time of reporting. *Candida* species detected at diagnosis comprised *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. albicans.* Length of rezafungin treatment to date ranged between 29–141 days.

Discussion

The rezafungin early access programme mainly included patients with chronic non-albicans Candida infections. These data provide insights regarding rezafungin use in clinical practice. Rezafungin treatment was successfully administered in the outpatient setting and enabled antifungal therapy to be given to individuals who may otherwise have remained in hospital or attended daily for IV infusions.

PII-72

In vitro Susceptibility Testing of Rare Moulds "RaMo Study" – An ECMM-EC/EFISG/ECMM/ISHAM initiative

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Introduction

In recent years, there has been a rise in fungal infections attributed to rare moulds, posing a notable clinical challenge due to their inherent resistance and diminished efficacy of conventional therapies. There is limited data available on their susceptibility profile which adds to the challenges, resulting in the absence of epidemiological cutoff values (ECOFF) and clinical breakpoints.

Objectives

In an ongoing international study, a substantial number of molecularly identified rare moulds (n=450) are undergoing evaluation for their susceptibility to various conventional and novel antifungals. Invasive moulds not displaying *Aspergillus, Mucorales, Scedosporium* and *Fusarium* species were enrolled.

Methods

The susceptibility patterns are analyzed by Etest and broth microdilution methods using Clinical and Laboratory Standards Institute (CLSI) as well as European Committee on

Antimicrobial Susceptibility Testing (EUCAST) guidelines. A freely selected ECOFF of >1mg/L was set for further analyses.

Results

We determined the susceptibilities of 150 rare moulds to amphotericin B with MICs of 0.03 to >16 by broth microdilution and 0.004 to >32 mg/L by Etest. So far, about 40% of the isolates are potentially non-wild type against amphotericin B in broth microdilution and as well as in Etest.

Conclusion

As mentioned, the increasing challenge posed by rare moulds underscores the importance of gathering extensive MIC data. The knowledge of innate resistance in rare fungi is of high importance for treatment.

PII-74

Candida albicans translocation through the intestinal barrier is promoted by fungal zinc acquisition and limited by host NFκB-mediated barrier protection

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The opportunistic fungal pathogen Candida albicans thrives on human mucosal surfaces as a harmless commensal, but frequently causes infections under certain predisposing conditions. Translocation across the intestinal barrier into the bloodstream by intestine-colonizing C. albicans cells serves as the main source of disseminated candidiasis. However, the host and microbial mechanisms behind this process remain unclear. In this study we identified fungal and host factors specifically involved in infection of intestinal epithelial cells (IECs) using dual-RNA sequencing. Our data suggest that host-cell damage mediated by the peptide toxin candidalysin-encoding gene ECE1 facilitates fungal zinc acquisition. This in turn is crucial for the full virulence potential of C. albicans during infection. IECs in turn exhibit a filamentation- and damage-specific response to C. albicans infection, including NFκB, MAPK, and TNF signaling. NFκB activation by IECs limits candidalysin-mediated host-cell damage and mediates maintenance of the intestinal barrier and cell-cell junctions to further restrict fungal translocation. This is the first study to show that candidalysin-mediated damage is necessary for C. albicans nutrient acquisition during infection and to explain how IECs counteract damage and limit fungal translocation via NFκB-mediated maintenance of the intestinal barrier.

PII-76

Characterization and molecular regulation of human tissue resident T cells and their progenitorsFungiNet project number: C7

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Memory T cells are crucial for long-term immune protection, providing rapid and effective responses to previously encountered pathogens. Resident memory T cells (T_{RM}) in peripheral tissues, such as the skin, play a vital role in local immunity. Naive T cells, expressing markers like CD45RA, CD62L, and CCR7, typically serve as progenitors for memory T cells. Compared to memory T cells, naive T cells exhibit a highly diverse TCR repertoire, enabling them to recognize a wide array of antigens. The high replicative history of naive T cells indicates fewer cell divisions. Additionally, naive T cells demonstrate less effector functionality than their memory counterparts.

The formation of memory T cells begins with the activation of naive T cells, which then differentiate into central memory T cells (T_{CM}) that circulate through lymphoid tissues, and effector memory T cells (T_{EM}) that patrol both lymphoid and non-lymphoid tissues. T_{RM} cells establish long-term residency in peripheral tissues, providing immediate localized immune responses. However, the formation and maintenance of T_{RM} cells in tissue niches remains unclear. Previously, CD45RA+expressing naive-like T cells have been identified in peripheral tissues, suggesting their potential role as T_{RM} cell precursors. Moreover, $CD45RA^+CD69^+$ naive-like T cells have been found in peripheral tissues such as the lung and jejunum, indicating their potential role as resident naïve T cells. Therefore, CD45RA+ expressing naïve-like T cells can be resident and might potentially play a role for peripheral skin T_{RM} cells generation and maintenance.

However, whether $CD45RA^+$ T cells in peripheral tissues are really naïve and a population of precursor cells remains to be investigated since the $CD45RA^+$ cell marker could serve different purposes than stemness. Our research demonstrates that $CD45RA^+$ expressing naive-like T cells do not exhibit the same characteristics as genuine naive T cells found in the blood. Through a series of transcriptomic, phenotypic and functional analyses, we observed that these $CD45RA^+$ expressing naive-like T cells do write instead what they do rather than what they do not do.

Although $CD45RA^+$ naive-like T cells do not act as memory progenitors in the skin, other peripheral progenitor T cells, unrelated to CD45RA expression, may exist. Notably, central memory T cells ($CCR7^+CD62L^+$) have been shown to be effective precursors for human skin-resident T cells in other studies.

In conclusion, peripheral CD45RA-expressing naive-like T cells do not serve as memory progenitors for the generation and maintenance of tissue-resident memory T cells. Further research is needed to identify alternative progenitor T cells in peripheral tissues and to understand the true role of $CD45RA^+$ expressing cells in the skin.

PII-78

Unravelling the impact of interferon-immunotherapy on epithelial resistance to Candida albicans translocation

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Invasive candidiasis is one of the most common opportunistic fungal infections threatening immunocompromised patients. Candidiasis is caused by Candida species, with Candida albicans being the most common pathogen isolated. Under normal conditions, this yeast is a commensal that resides in the gastrointestinal tract of most individuals. However, use of broad-spectrum antibiotics fosters C. albicans overgrowth, and a dysfunctional intestinal epithelial barrier allows translocation from the gut into the bloodstream. When the innate immune defenses are compromised the fungi can reach the bloodstream and cause disseminated infection. Immunotherapy has already been posited as an approach to augment host defense of immunocompromised patients. Despite the immunological interferon gamma (IFN-γ), type II IFNs, communication that augments antifungal effects of myeloid cells, it remains largely unclear how an acute increase in IFN-γ levels influence infection at epithelial barriers. Particularly, since chronic IFN-γ release has been associated with interferonopathy and compromised epithelial barrier function. Conversely, type I IFNs (IFN-I) have been associated with increased epithelial resistance to C. albicans infection. Using an in vitro intestinal epithelial model to study C. albicans translocation, we will evaluate the association between fungal translocation and breakdown of epithelial barrier integrity, upon treatment with IFN-I/II. This will be specifically investigated using microbiological translocation assays, monitoring of tissue damage, confocal microscopy of tight junctions and biophysical assays for assessment of epithelial integrity. Collectively, the project will shed light on the potential detrimental or beneficial effects of interferons on C. albicans colonization, infection, and translocation.

Talks and Poster

PI-05 | S10-02

FungiNet INF: Integrated database for experimental data

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In the CRC/Transregio FungiNet, project INF provided and maintained the infrastructure for the standardized acquisition and central management of -omics data. INF implemented and applied pipelines to support standardized analysis of these data including genomics, transcriptomics and proteomics, as well as for network modeling. INF created metadata templates for efficient structured description of high-throughput experiments, including RNA-sequencing metagenomics and imaging data. Most recent efforts by INF involved developing and applying further software pipelines for (i) support of the analysis of high-throughput data, (ii) storage of these data together with an updated structured experimental description towards

an online meta-data format, and (iii) extension of the service for exploratory genome-scale metabolic modeling.

PI-23 | S10-03

Nested defence strategies of Pseudomonas aeruginosa, an important co-infectant with Aspergillus fumigatus

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Pseudomonas aeruginosa, a versatile Gram-negative bacterium, is a significant human pathogen, capable of causing various infections. In cystic fibrosis, the most common inherited lung disease worldwide, P. aeruginosa and Aspergillus fumigatus are prevalent bacterial and fungal pathogens, respectively, both contributing to severe lung diseases. Their interaction ranges from mutual suppression to potential cooperation, with P. aeruginosa possibly aiding the establishment and growth of A. fumigatus [1]. Thus, developing strategies to counter P. aeruginosa is crucial for combating A. fumigatus infections.

One notable aspect of P. aeruginosa's adaptability is its survival within macrophages, enabled by nested defence strategies where both host and pathogen have evolved to counter each other. For example, P. aeruginosa employs a mechanism for itaconic acid degradation [2], representing a rare form of counter-counter-counter defence. We constructed a minimal model to explore the interplay between P. aeruginosa and its macrophage host. Using a quasi-sequential approach to dynamic optimization [3], we examined the temporal dynamics of counter-defence and counter-counter-counter defene, along with optimal resource allocation from the pathogen's perspective across different infection scenarios. This approach has deepen our understanding of P. aeruginosa infection processes and has generated hypotheses for novel therapeutic strategies. Notably, we found that elaborate regulation is unnecessary for itaconate degradation, and that the putative presence of such a regulation could indicate the existence of even higher degrees of the nested defence strategy. For future studies, it is worth investigating the working hypothesis that also the pathogenic fungus Candida albicans employs an enzyme inactivating itaconate.

References

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FungiNet project B1

PI-25 | S14-04

Surface proteins of *Lichtheimia corymbifera* as novel virulence determinants and as potential interaction partners with the host immune system (FungiNet A6)

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Lichtheimia corymbifera is a ubiquitous, soil-inhabiting, saprobic fungus, belonging to the order Mucorales (Mucoromycotina, ex Zygomycota). In predisposed patients, some Mucorales species, including *L. corymbifera*, can cause life-threatening infections (mucormycosis). In contrast to more common mycoses such as *Candida* or *Aspergillus*-infections, very little is known about the pathogenesis of mucormycoses. The aim of the project is to assess whether fungal attributes contribute to virulence and how the fungal spores interact with host cells. To address these questions, we carried out a proteomic analysis of the spore surface (surfome) and the secreted proteins (secretome). Thereby, we identified the hydrophobic surface binding protein HsbA that is significantly more abundant in a virulent isolate of *L. corymbifera* compared to an attenuated strain. We demonstrate that HsbA interacts with macrophages and contributes to adhesion and invasion of other host cells. In a newly established leukocyte model, a total of 588 and 763 secreted proteins were identified in the < 40 kDa protein fraction of the supernatant of leukocytes infected with *L. corymbifera* spores after 24 and 72 hours, respectively. HsbA and its four isotypes were present in all samples. In parallel, we elucidated the interaction of *L. corymbifera* with macrophages. We found that phagocytosed spores impair the acidification of phagosomes via a novel mechanism. Further, Toll-like receptor 2 (TLR2) was identified as key player in the interaction of *L. corymbifera* with the host. Our findings were confirmed by the use of TLR inhibitors and by the establishment of a TLR2 knockdown protocol in RAW264.7 cells. The identification of the surface protein HsbA and the interaction of spores with the cellular immune defense represent a fundamental basis for understanding the pathogenesis of *L. corymbifera* infections and is important for the development of novel therapeutic strategies.

PI-27 | S02-05

Insights into the infection biology of fungal pathogens by LC-MS/MS and MALDI-TOF-based proteomic methods

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The incidence of life-threatening fungal infections has significantly risen over the last decades, in particular due to an increasing number of susceptible patients, e.g. those with severe immunosuppression or intensive medical care. Invasive fungal infections are associated with high mortality rates, because of the difficult diagnosis and the limited arsenal of antifungal drugs available in the clinics. Given these challenges, there is a strong demand for new methodological approaches to gain detailed insights into host-fungal pathogen interactions. The advancement in the field of proteomics has led to powerful and sensitive methods that enables to investigate the detailed changes in protein levels in the fungal pathogen and its host. We therefore set out to establish robust and reproducible protocols for fungal infection biology. We developed LC-MS/MS based methods for the characterization of post-translational modifications (phosphorylation, acetylation, cysteine oxidation), the fungal surface proteome, and changes of the host proteome upon infection with fungi. Identification and quantifications of proteins were performed by Orbitrap mass analyzers, while the spatial distribution of proteins was determined by MALDI-imaging measurements using an UltrafleXtreme device. Exemplary

data will be shown to illustrate the power of recent proteomic technologies to accelerate the growth of knowledge on the infection biology of human-pathogenic fungi. In addition, MALDI-MSI could be established as a tool for spatially resolved analysis of host-pathogen interaction direct within infected tissue.

PI-45 | S04-02

Robust induction of pro-inflammatory leukotriene B4 generation in human macrophages during *A. fumigatus* infection

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Aspergillus (A.) fumigatus, a saprophytic pathogenic fungus, is among the greatest concerns in healthcare regarding emerging antifungal resistance, posing a threat especially to immunocompromised patients. Macrophages are major innate immune cells in inflammation, playing a crucial role in inflammation that accompanies microbial infections. The orchestrated production of lipid mediators by macrophages is crucial to initiate and to resolve inflammation. Lipid mediators comprise the pro-inflammatory leukotrienes (LTs) and prostaglandins (PGs) derived from arachidonic acid via 5-lipoxygenase (LOX) and cyclooxygenase as biosynthetic key enzymes, respectively, as well as the specialized pro-resolving mediators formed by multiple LOXs. Here, we employed targeted metabololipidomics using ultra-performance liquid chromatography-tandem mass spectrometry to analyze lipid mediator profiles in human host-pathogen interactions. We found that *A. fumigatus* germ tubes, but not conidia, strongly induce LTB4 and, to a minor degree, PGE2 formation in human monocyte-derived macrophages via the dectin-1 receptor. Particularly, the dectin-1/SYK/ERK axis leads to significant 5-LOX activation and LTB4 generation, confirmed by the use of dectin-1-capturing antibodies that block *A. fumigatus*-induced LTB4 formation in macrophages. By exploiting *A. fumigatus* mutant strains, we found that galactosaminogalactan and α-1,3-glucan are fungal components triggering this cascade. Furthermore, we investigated the macrophages'' capacity to produce lipid mediators after engulfment of *A. fumigatus* spores and found reduced LTB4 formation, while PGE2 was increased significantly. [PJ1] [KG2] Conclusively, we propose that *A. fumigatus*-derived galactosaminogalactan and α-1,3-glucan act via the dectin-1 receptor on macrophages to evoke robust formation of the pro-inflammatory mediator LTB4 within the anti-fungal host immune response.

PI-47 | S18-04

Regulation of Candida albicans virulence traits by protein kinase

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Microbial signal transduction pathways regulate adaptation to changing environmental conditions and facilitating the success of pathogens to cause infections. Most of these pathways are regulated by protein kinases. The opportunistic fungal pathogen *Candida albicans* exists as a commensal of the intestinal mycobiota and can cause disseminated candidiasis upon certain circumstances. Both commensalism and infection require a complex

network of signalling pathways. The *C. albicans* genome was predicted to encode 108 protein kinases yet nearly 50% remain uncharacterised. We aim to dissect the role of *C. albicans* protein kinases during commensalism and infection.

To investigate the impact of protein kinases on the pathogenicity of *C. albicans*, we screened a library containing individual mutants lacking each of the identified *C. albicans* protein kinase genes for their ability to damage intestinal epithelial cells (IEC). Mutants exhibiting an increased or decreased cytotoxicity were further validated.

Our kinase mutant screen revealed that deletion of around 30% of all kinases resulted in altered cytotoxicity compared to the wild-type (18 increased and 19 decreased damage). As filamentous growth is a major virulence mechanism of *C. albicans*, the morphology of these mutants on IECs was investigated. Surprisingly, we found that mutants lacking *CRK1* or *SCH9* showed reduced filamentation, yet induced increased or WT-like IEC cytotoxicity. To fully determine the role of these protein kinases during epithelial infection, specific virulence attributes such as adhesion, invasion and translocation potential as well as metabolic adaptation were assessed for the corresponding mutants.

Collectively, we found that several protein kinases of *C. albicans* are associated with epithelial cell damage. This includes the regulation of filamentation during infection of IECs. However, distinct kinases were shown to negatively and filamentation-independently impact pathogenicity.

PI-63 | S12-04

FungiNet A1: Establishment of the invasive aspergillosis-on-chip model to study dynamics of neutrophil migration and the antifungal activity of host cell-derived extracellular vesicles

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Aspergillus fumigatus is a fungal pathogen that can cause a variety of diseases in immunocompromised hosts. In invasive aspergillosis, the conidia can reach the lung alveoli, where they encounter host immune cells, such tissue-resident alveolar macrophages or neutrophils (PMNs) migrating from the blood. The microfluidic invasive aspergillosis-on-chip (IAC) model closely mimics the course of infection and allows to study the recruitment of PMNs and the interaction of PMNs and macrophages with *A. fumigatus*. In addition, host cells produce extracellular vesicles (EVs), which are important for cell-cell communication. EVs secreted by PMNs exhibit antifungal activity, however, the mode of action of EVs is still unknown.

Here, we investigated the recruitment of PMNs and their physical interactions with *A. fumigatus* and/or macrophages using the IAC model to better understand the life cycle of EVs, their interaction with *A. fumigatus* and their antifungal mode of action.

Confocal laser scanning microscopy (CLSM) showed that PMNs continuously migrate from the blood into the alveoli regardless of the presence of *A. fumigatus*. Upon infection, the number of PMNs increased fourfold within the first hour. Initially, PMNs moved rapidly and gradually slowed down after phagocytosis of conidia. The hyphae were mainly attacked at their tip. Cell death of PMNs started after about 12 hours and occurred mainly in the vicinity of macrophages that had ingested conidia as well as dead PMNs. The addition of EVs to a fluorescent *A. fumigatus* live-dead reporter strain confirmed the antifungal effect of PMN-derived EVs *in vitro*. Proteomic analysis of EVs revealed the presence of antimicrobial peptides. CLSM enabled the visualization of dye-stained and genetically labelled EVs and revealed their co-localization with fungal hyphae.

This study emphasizes the need for recruitment of PMNs to the site of infection and sheds light on the modes of action inhibiting fungal growth.

PI-75 | S06-05

CAR-engineered T and NK cells targeting *Aspergillus* species

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Limited treatment options and the emergence of resistant strains are leading to high mortality rates of invasive pulmonary aspergillosis (IPA). New therapeutic strategies targeting the fungus and modulating the immune response are desired. We explore the therapeutic potential of lymphocytes, which are important in antifungal immunity.

To target *Aspergillus* spp., we designed two chimeric antigen receptors (CARs): Af-CAR specific for a protein expressed by *A. fumigatus* and Carb-CAR for a carbohydrate in the *Aspergillus* cell wall. We engineered human T and NK92 cells, confirming CAR expression and specificity through flow cytometry, microscopy and IFN-γ secretion assays. In co-cultures with *Aspergillus* spp., we assessed cytokine secretion, degranulation marker (CD107a) expression, innate immune cell activation, and hyphal damage. Finally, we evaluated the therapeutic potential in NSG mouse models of IPA.

We showed that Af-CAR-cells specifically interact with *A. fumigatus* hyphae (*Af*). In co-culture with *Af*, $CD8^+$ Af-CAR T cells secreted Th1 cytokines, while $CD4^+$ Af-CAR T cells secreted Th1 and Th2 cytokines. Conditioned media from Af-CAR T cells enhanced the antifungal activity of

innate immune cells. *In vivo* $CD8^+$ Af-CAR T cells were more effective than $CD4^+$ Af-CAR T cells in reducing fungal burden and improving survival. We moved on to evaluate NK92 cells expressing Af-CAR or Carb-CAR. Af-CAR NK92 cells exhibited a mixed pro- and anti-inflammatory phenotype and showed significant therapeutic potential *in vivo*. When compared to Carb-CAR NK92 cells, Af-CAR NK92 cells had the highest activation against *A. fumigatus*, as indicated by CD107a expression and cytokine secretion. However, only Carb-CAR NK92 cells were activated in co-cultures with *A. terreus* and *A. flavus*.

Our data suggest that CAR-based therapy could be promising for IPA patients. A combination of T and NK cells expressing Af-CAR and Carb-CAR could be beneficial and will be investigated.

PI-77 | S13-03

Strategies to treat vulvovaginal candidiasis: neutralizing candidalysin and augmenting the protective potential of probiotic lactobacilli to reduce *Candida albicans* pathogenicity

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While *Candida albicans* is normally a harmless colonizer of mucosal surfaces, this fungus can cause vulvovaginal candidiasis (VVC) under certain conditions. This infection affects millions of women worldwide and significantly impacts quality of life. During infection, the *C. albicans* peptide toxin candidalysin drives host cell damage and activation of immune responses. Unlike other mucosal *C. albicans* infections, neutrophils that are recruited during VVC do not clear the infection and cause hyperinflammation and symptomatic disease. VVC is difficult to treat since the cause of infection is often unknown, infections are recurrent, and antifungal resistance is increasing. We therefore evaluated different treatment strategies to reduce *C. albicans* pathogenicity. We investigated anti-candidalysin nanobodies for their potential to prevent candidalysin-induced epithelial damage and metabolic supplementation to improve the anti-*C. albicans* activity of probiotic lactobacilli as an indirect strategy.

We showed that anti-candidalysin nanobodies reduced vaginal epithelial cell (VEC) damage during *C. albicans* infection *in vitro*. The nanobodies reached candidalysin within the invasion pocket of hyphae invading VECs. The presence of nanobodies, dampened proinflammatory cytokine release by infected VECs leading to decreased neutrophil activation. Candidalysin-neutralizing nanobodies can thus reduce epithelial cell damage and inflammation responsible

for VVC symptoms. Further, by using untargeted metabolomics, we identified metabolites that support lactobacilli growth to improve their protective potential against *C. albicans*. This data set also gives us insight into the antagonistic mechanisms of lactobacilli and helps us to identify anti-*Candida* metabolites. In conclusion, we describe direct and indirect treatment strategies to reduce *C. albicans* pathogenicity during VVC by neutralizing candidalysin or metabolically enhancing the protective potential of probiotic lactobacilli.

PI-79 | S13-04

The effect of lung colonisation with *Candida albicans* on *Staphylococcus aureus* infection

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Introduction

The fungus *C. albicans* and the bacterium *S. aureus* are both commensal organisms that can cause severe systemic infections in immunocompromised patients, and are often co-isolated. However, whereas *S. aureus* can cause pneumonia in mechanically ventilated patients and disseminate from the lung, *C. albicans* almost never causes invasive lung infections.

Goals

We aim to investigate the different behaviour of *C. albicans* and *S. aureus* in the lung, and the effect of *C. albicans* on *S. aureus* infection.

Materials & Methods

We established a novel lung colonisation/infection model using Balb/c mice. Mice were administered *C. albicans* intranasally at day 0 and infected intranasally with *S. aureus* on day 1. At 24, 48 and 96 hours post *S. aureus* infection, lung, liver and kidney bacterial/fungal burdens as well as lung and spleen immune responses were analysed.

Results

After intranasal administration, *C. albicans* and *S. aureus* colony numbers declined though the *C. albicans* Δece1 mutant persisted until day 5 . Only *S. aureus* disseminated into the liver and kidney. This mimics the clinical situation where *C. albicans* does not cause invasive infection via the lungs, but *S. aureus* does. Short-term *C. albicans* and *S. aureus* colonisation primarily increased numbers of neutrophils and CD11b+ dendritic cells in the lung, while the presence of *S. aureus* alone further increased ILC3 at day 5. Single-cell RNAsequencing analysis of these infiltrating cells indicated that *C. albicans* modulated macrophage responses during co-infection, and that IL-15 and TGFb were linked to multiple changes in the gene expression.

Summary

We successfully established a mouse model of *C. albicans* and *S. aureus* colonisation/infection in the lungs. The effects of *C. albicans* on *S. aureus* infection will be further investigated, and mutant strains will be used to explore host-pathogen interactions

PII-22 | S10-01

Spatiotemporal modeling reveals cellular contributions to uptake of Aspergillus fumigatus in the human lung

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The human immune system constantly removes daily inhaled microbial invaders, such as the small-sized conidia of the opportunistic fungus *Aspergillus fumigatus.* In immunocompromised patients, they quickly swell and form hyphae within six hours resulting in life-threatening infections like invasive aspergillosis. Therefore, the pulmonary host cells, the alveolar macrophages (AMs) and stationary alveolar epithelial cells (AECs) must act quickly to remove conidia.

As the individual fungal uptake contribution of AMs and AECs remains unclear, we utilized a previously developed hybrid agent-based model (hABM) to simulate virtual infection scenarios of *A. fumigatus* in the lung. The hABM provides a realistic, to-scale representation of one alveolus, consisting of a ¾ sphere, type 1 and type 2 AECs constituting alveolar tissue, pores of Kohn, and AMs. The model includes conidia-induced chemokine secretion by AECs, which is sensed by AMs, directing their migration towards the source of infection to take up the fungus. In this study, we extended the hABM by the conidial swelling process proposing a size-dependent mechanism for uptake by host cells. For this, we calibrated a bottom-up modeling approach to experimental data and incorporated an increasing uptake rate of type 2 AECs for an increasing physical contact area between fungus and host cell into the hABM. Based on the swelling, we also considered a delayed secretion of chemokines, mimicking a size-dependent delayed detection of the conidium. Including this conidial swelling mechanism into our hABM, also for type 1 AECs and AMs, we could run millions of virtual infection simulations screening over respective uptake rates and a delayed chemokine signaling. Based on our simulation results, we predict the majority of infections being cleared by AMs. Also, AECs can contribute to a certain extent, especially in compromised parameter regimes while a high dependence on surface dominant type 1 AECs was evidenced.

PII-24 | S10-04

Modelling *Candida albicans* metabolism, adaptation, regulation and virulence (B2)

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Candida albicans is a human commensal, however, under the right circumstances it can cause infection, sepsis and even death. We describe how bioinformatics can help to elucidate the adaptations of *C.albicans* during infection and interaction with host immune cells.

Application 1

Growth on different media – which pathway differences become apparent? We analyzed *C.albicans* metabolism of TCA cycle and arginine biosynthesis. Software YANA shows resulting pathway adaptation capabilities also important during infection (elementary mode analysis lists all pathways accessible with the given set of enzymes).

Application 2

Studying metabolic enzyme regulation: Using YANAvergence on these results, we calculate metabolic fluxes. which enzymes are regulated. Next, plotting flux values versus gene expression we can examine flux and regulation differences during growth on glucose, succinate and malate mimicking different environments.

Application 3

Investigating the metabolic network regulation: We study next how a sky2 knock-out (regulatory Ser/Thr kinase) influences adaptation capabilities. Using network biology, we identify in detail how sky2 is connected and how this influences phenotype including infection (1).

Application 4

Analyzing the virulence phenotype: We give an example of a metabolic well adapted filament-deficient *Candida albicans* strain (2).

Application 5

What changes Candida from commensal into virulent attack? Well, at least cytokines and the host cell seem to respond to the change. We show results on human neutrophils CEACAM1, CEACAM3, and CEACAM6 receptors in response to *C. albicans* infection (3).

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PII-26 | S14-02

Analysis of the differential effect of reactive oxygen intermediate deficiency during the response against *Candida albicans* and *Aspergillus fumigatus*

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Chronic Granulomatous Disease (CGD) is a genetic immunodeficiency disease caused by mutations in any of the genes encoding components of the NADPH oxidase in phagocytic cells, leading to a defect in their full antimicrobial activity. Preliminary results using *ex vivo* whole-blood samples from patients with CGD showed a massive dysregulation pattern triggered by *Aspergillus fumigatus* that could be not observed for *Candida albicans*. The predominant upregulated expression of genes encoding for cytokines and chemokines pointed towards a monocyte-dependent effect. To systematically dissect the differences in innate immune response between both fungi and to overcome the issue concerning the availability of patients" samples, we aimed at mimicking the CGD mutation effect in primary monocytes using GSK2795039, a specific inhibitor of the NADPH oxidase. GSK inhibited reactive oxygen species production in monocytes upon confrontation with either *A. fumigatus* or *C. albicans*

and the treatment led to significantly increased levels of pro-inflammatory cytokines (e.g. IL-1β) and chemokines (e.g. GRO-β) in response to *A. fumigatus*, but not *C. albicans.* This pathogen-specific immune response was independent of morphology and viability. To determine what drives this dysregulated monocyte activation by *A. fumigatus,* GSK-treated monocytes were incubated with purified components of the *A. fumigatus* cell wall. We found that β-1,3-glucan, α-1,3-glucan, and galactosaminogalactan (GAG) drive dysregulation in inhibitor-treated monocytes. GAG specifically contributed to the increased inflammasome-dependent cytokine secretion. These results were validated using different *A. fumigatus* cell wall mutants.

In conclusion*, A. fumigatus*, one of the major fungal pathogens in CGD, triggers dysregulation of cytokine secretion in GSK-treated monocytes, mainly by the fungal cell wall GAG. Ongoing work aims to identify pathways that are triggered during dysregulated monocyte activation.

PII-46 | S16-01

Sucrose enables *C. albicans* intestinal colonization

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Candida albicans is a frequent colonizer of the human gut. Increased colonization is one risk factor for disseminated infection, often mediated by antibiotic treatment. In contrast, the effects of diet on intestinal *Candida* colonization are less well studied. We observed that the addition of sucrose to the drinking water of C57BL/6 mice facilitated intestinal colonization with *C. albicans*. To identify the responsible mechanisms, we investigated the consequences of sucrose supplementation and different diets (fiber-rich grain-based diet, fiber-poor purified diet with moderate or high carbohydrate content) on *Candida* colonization and microbiome composition. In SPF mice, both purified diets facilitated stable *C. albicans* colonization. Sucrose supplementation elevated the fungal burden in mice fed grain-based chow to the level of the purified diets. The highest fungal density was reached by combining sucrose supplementation of drinking water with a purified diet. In contrast, sucrose supplementation did not further increase fungal load in germ-free mice fed with a purified diet, suggesting that the maximum level of colonization was reached. Microbiome analysis of murine feces showed clear changes in bacterial composition after switching to purified diets and after colonization with *C. albicans*, both characterized by reduced α-diversity. A *C. albicans* mutant, unable to hydrolyze sucrose, displayed a fitness defect in competition with the corresponding wildtype *in vitro* but not in the murine gut, suggesting that sucrose hydrolysis by the host or microbiota might provide monosaccharides, or that alternative nutrients can complement for the inability to use sucrose. In conclusion, sucrose affects microbiome composition and promotes increased *C. albicans* colonization. Furthermore, the type of fiber present in the diet might impact fungal colonization. Therefore, the effect of diet should be considered in patients at risk for disseminated candidiasis.

FungiNet project C5

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