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Abstract

S1.1
Basic mechanisms of antifungal immunity
S1.1a
Basic mechanisms of antifungal immunity

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Invasive candidiasis (IC), mainly caused by *C. albicans*, is the most common deep-seated human fungal infection in the western hemisphere. Mortality of patients with IC exceeds 40% despite the administration of antifungal therapy; hence, IC is an unmet medical condition for which better understanding of its immunopathogenesis is essential. The mouse model of IC following intravenous yeast injection that mimics human skin-derived bloodstream candidiasis has been extensively used to study the innate immune response against IC. In this model, the microbiological progression of the infection is organ-specific, with kidney being the main target organ, associated with organ-specific innate immune responses. At the cellular level, neutrophils, monocytes, resident macrophages and $CD11b^+$ dendritic cells, but not lymphocytes or $CD103^+$ dendritic cells, are critical for host defense against IC. Indeed, depletion of neutrophils or mononuclear phagocytes leads to accelerated mortality in the model. Of interest, neutrophils have different effects in the model depending on the phase of the infection; specifically, whereas early neutrophil presence is protective, late neutrophil accumulation mediates tissue injury and immunopathology that results in renal failure and mortality. In recent years, the molecular factors that mediate early protective versus late detrimental neutrophil effects in the model have been emerging and their discovery holds promise for the identification of novel genetic risk factors for IC and targeted therapeutic interventions, respectively. With regard to the protective role of mononuclear phagocytes, the discovery of the prominent role of CCR2-recruited monocytes and CX3CR1-expressing macrophages as well as the unveiling of the orchestrated monocyte/dendritic cells/NK cell axis that drives GM-CSF-mediated activation of neutrophils have shed further light into the pathogenesis of IC. At the molecular level, several studies have demonstrated the orchestrated role of pattern-recognition receptors (e.g., C-type lectins and Toll-like receptors), pro-inflammatory cytokines (e.g., IL-1, IL-6, IFN-g, TNF-a), the inflammasome (e.g., Nlrp3, Nlrp10), the complement cascade (e.g., C5a), and the reactive oxygen species generation machinery (e.g., NADPH oxidase, iNOS) in effective anti-*Candida* innate host defense. Population-genetic approaches have translated several findings to humans with the prospect of devising improved individualized risk stratification and prognostication strategies in patients with IC and better outcomes after IC.

S1.1b
Protection and pathology in pulmonary aspergillosis

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Aspergillus may lead to a spectrum of clinical syndromes in the lung, depending on the host's immune status or pulmonary structure. Invasive pulmonary aspergillosis occurs primarily in critically ill patients patients, such as those with severe immunodeficiency or chronic obstructive pulmonary disease. It is now clear that a three-way interaction between host, fungi, and microbiota dictates the types of host-fungus relationship. Indeed, microbial dysbiosis predisposes to a variety of chronic fungal infections and diseases at local and distant sites. We have explored metagenomics for deciphering the contribution of the microbiota to fungal commensalism and parasitism and metabolomics for capturing the dialogue between the mammalian host and its microbiota. By correlating changes in metabolite profiles with microbiota metagenomic composition, we have defined several functional nodes by which certain bacteria species contribute to or subvert host-fungal symbiosis and mucosal homeostasis in the gut and lung. A microbial tryptophan metabolic pathway activated through the indoleamine 2, 3-dioxygenase 1 (IDO1) enzyme led to the production of the indole-3-aldehyde (IAld). IAld preserved immune physiology at mucosal surfaces while inducing antifungal resistance through the activation of the aryl hydrocarbon receptor (AhR), eventually impacting on mucosal immune homeostasis and host/fungal symbiosis. Thus, the regulatory loop involving AhR and IDO1 may be exploited for the development of multi-pronged host- and microbiota-directed therapeutic approaches in pulmonary aspergillosis.

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S1.1c
Protective immune responses during cryptococcosis

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Cryptococcus neoformans is a fungal pathogen with worldwide distribution and responsible for around 180 000 deaths each year. Exposure to this pathogen is very frequent, but healthy individuals either clear infection or control it as a latent infection, and very rarely develop disease. Instead the overwhelming majority of cases is due to immunosuppression, in particular HIV-mediated immunosuppression.

We discuss the existence of predisposing factors to *C. neoformans* infection, and what constitutes an adequate immune response to this fungal pathogen. Most *C. neoformans* cells are found to closely associate with monocyte and macrophages, effector cells of innate and adaptive immunity, and so we will focus on murine and human monocyte and macrophages interactions. We also examine the strategies that *C. neoformans* uses to achieve survival, latency and growth within a mammalian host. The intracellular lifestyle of *C. neoformans* requires it to survive nutrient starvation and toxicity by host antimicrobial molecules in the phagosome and to counteract the host immunity to ensure its survival. Recent work shows that some of these strategies involve *C. neoformans*-mediated host damage at the molecular and cellular level. There is evidence that the intracellular lifestyle is successful enough that it can provide a protective niche to *C. neoformans*. We will integrate the intracellular lifestyle of *C. neoformans* with the current views in immunometabolism and immunoregulation.

S1.1d
Evolution of Candida albicans colonization and anti-Candida innate and adaptive immunity in patients having undergone surgical resection for Crohn's disease

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Objective: In order to explore a possible role for *C. albicans* in Crohn's disease (CD) a pilot double blind study assessed the effect of 6 months fluconazole (FCZ) treatment *versus* placebo on endoscopic recurrences after surgical resection. Despite the study was not conclusive clinically at 6 month at the tested FCZ dose (200 mg daily) the collected material was used for a longitudinal biological analysis yeast colonization, biomarkers of CD, and /or biomarkers of *C. albicans*-host interplay.

Methods: The study concerned 28 CD patients randomized in two groups,14 with FCZ, 14 with placebo. Patients were examined and sampled for mycological and serological analysis before surgery and 1, 2, 3, 6 months after surgery. Qualitative and quantitative evolution of colonization was assessed in mouth and stool. Serological kinetic analysis concerned levels of *i)* CRP, calprotectin and anti-glycan antibodies biomarkers of CD *ii)* biomarkers of *C. albicans* saprophytic/pathogenic transition: anti-*C. albicans* mannan and anti-*C. albicans* Hwp1 protein *iii)* Innate immunity lectins involved in *C.albicans* sensing: galectin-3 and Mannose Binding Lectin (MBL).

Results: The major finding was that independently of antifungal treatment, surgery was followed by a significant decrease of C. albicans colonization. In parallel, biomarkers of *C. albicans* pathogenic transition decrease to non significant levels. Anti-glycan antibodies levels decreased as well but remain above the cut-off discriminating patients with CD. Galectin 3 exhibited a steady decreases which correlated with the one of calprotectin. MBL levels inversely correlating with anti-*C. albicans* antibodies before surgery remain stable.

Conclusion: With regard to previous evidence that inflammation favors *C. albicans* thriving which, in turn, aggravates inflammation, this is the first evidence that reduction of CD inflammation decreases *C. albicans* colonisation and biomarkers associated with its pathogenic development.

S1.2
Mycoctic keratitis
S1.2a
Update and Epidemiology of mycotic infections of the eye

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Fungi have replaced bacteria as the most common cause of infectious keratitis in the developing world, where $2/3^{rd}$ of the global population reside. It is an ocular emergency and an important cause of visual loss worldwide. An update on the current epidemiological patterns, incidence, organisms profile and treatment option are important for clinicians, laboratory personnel and other scientists to be better equipped in the diagnosis, management and ultimately in the prevention of this disease. It affects young active working population in their prime earning period, thus causing significant loss of man years of productivity. Since it also affects people from the lower socio economic background, the dimension of economic loss becomes even more paramount.

The incidence of Mycotic keratitis varies with the geographical regions and also over time. It is often cited as a public health problem inviting some investigators to term it as a silent epidemic. In India, it is estimated to affect 300,000 people annually. In contrast, in 2011, a combined total of 100 cases per year were reported from 11 centres in the USA. There was a large outbreak of *Fusarium* keratitis associated with use of a contact lens solution a few years back in many countries like USA, Singapore and Hong Kong. After this outbreak, the base line incidence of fungal keratitis in the USA showed an increase even after the outbreak subsided.

More importantly, at least one third of those affected have significant visual morbidity, even after undergoing treatment. Even in a single geographical location, cases of fungal keratitis may be higher than the yearly average at certain times of the year, such as during the harvest or windy seasons, or when there is increased relative humidity (before the rainy season).

Causative organisms may differ by geographic location and the risk factors. Filamentous fungi like Fusarium sps and Aspergillus flavus are the most common fungal organism in tropical countries. In these countries the majority of patients suffer an injury with vegetative matter making trauma the most important risk factor in developing fungal keratitis. In addition, there is a large group of hyaline and dematoceious fungi that are not identifiable by conventional methods that is responsible for mycotic keratitis as well. In more temperate climate Candida sps predominate and the majority of these patients are immunocompromised.

Interestingly, within India itself the distribution of the different fungal species is different with Aspergillus *flavus* being more common in the northern parts of India and Fusarium keratitis more in southern states. The precise reason for this variation is unknown and has to be investigated further. This observation assumes importance in the context that newer studies show that Aspergillus and Fusarium differ with regard to their susceptibility pattern to different antifungal drugs.

Mycotic keratitis is generally associated with a poor clinical outcome. Currently drugs like natamycin and voriconazole are used in the management. At least a third of the patients may require a corneal transplant and that too with suboptimal visual outcomes. Regular surveys at different geographic locations help us to understand the magnitude of the condition so as to plan effective management strategies. Awareness of this condition and effective public health intervention strategies to prevent this catastrophic problem is the need of the hour.

P089

Virulence evaluation of Aspergillus fumigatus Clinical Isolates in Galleria mellonella model.

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Objective: *Aspergillus spp*. are responsible for causing opportunistic diseases in immunocompromised patients, including Pulmonary Invasive Aspergillosis (PIA), a disease with a high mortality rate characterized by invasion of the lung tissue, inflammation and possible dissemination to other organs. In this study, we analyzed virulence of *Aspergillus fumigatus* clinical isolates from São Paulo Hospital (HSP, UNIFESP) evaluating the percentage of survival and health index post-infection in *Galleria mellonella* model.

Methods: The virulence of 12 strains (2 standard strains and 10 HSP clinical isolates) was evaluated using the experimental model of infection in *Galleria mellonella*, generating a survival and morbidity curve. For infection, 10 μL of a conidia inoculum adjusted to 10^8 CFU/mL was inoculated in the left hemocele of the larvae (2.0–2.5 cm in length). The larvae (n = 16) were incubated at 37°C and observed daily up to 7 days for survivor count and morbidity assessment by health index post-infection. Post-infection, *Galleria mellonella* larvae were monitored for the following attributes: activity, cocoon formation, melanization, and survival.

Results: A significant statistical difference between the survival of larvae infected with *A. fumigatus* and the control group (PBS) ($P < 0.0001$). Seven strains (ATCC 16913, 1220, 1238, 1304, 1341, 1343, 1359, and 1392) showed a high virulent profile with 100% of mortality after 2 or 3 days' post-infection. The clinical isolates 1213, 1228 and 1232 presented a mild virulent profile. Larvae infected with 1213 succumbed on the sixth day, while the ones infected with 1228 or 1232 presented percentage of survival of 6.25% and 17.65%, respectively (Figure 1A). The survival curve results may be correlated with the morbidity data, wherein *A. fumigatus* strains with high virulent profile also promoted higher morbidity. The same was observed for 1213, 1228 and 1232 strains (Figure 1B).

Conclusion: In conclusion, this study demonstrates the differences in the virulence of clinical isolates of *Aspergillus fumigatus* determined by the analysis of the percentage of survival and health index post-infection. We could divide those strains into two profiles according to their virulence, a high-virulence profile (ATCC 16913, 1220, 1238, 1304, 1341, 1343, 1359 and 1392) and a mild-virulence profile (1213, 1228 and 1232).

Picture 1: https://www.eventure-online.com/parthen-uploads/89/8ISH/add_1_419424_ae2ce47d-73f6-4511-94d0-7dfa30560aa8.jpg

P090

Molecular surveillance of healthcare-associated Candida infections in a rehabilitation center for patients with severe acquired brain injuries

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Objective: Healthcare-associated infections (HAIs) affect an estimated 4.1 million patients in the EU annually and cause considerable increases in illness, mortality, and costs. In European intensive care units (ICUs), about 8.4% of the bloodstream infections (BSIs) are caused by pathogenic *Candida* species acquired by patients during their stay in the hospital. The most part of these HAIs is linked to the use of invasive devices (e.g. vascular and urinary catheters) by underlining the key role of hospital environments in the cross-transmission of microbial pathogens.

The aim of this study was to describe the genetic pattern, and population structure, of different pathogenic *Candida* species recovered from blood samples, central venous catheters (CVCs), hands of healthcare workers (HCWs) and hospital environments (air, medical equipment and various surfaces) in one of the most important hospitals of Southern Italy in the treatment of patients with severe acquired brain injuries.

Methods: Twenty-six blood and 28 CVCs *Candida* isolates were examined in this study. Two hundred-eight additional isolates, 161 collected from ward environments and 47 from HCWs, were also included.

All isolates were initially identified using CHROMagar medium and Vitek2 yeast identification system and, subsequently, subjected to molecular identification using species-specific PCRs and/or ITS-sequencing.

The multilocus sequence typing (MLST) scheme used for *C. albicans*, *C. glabrata* and *C. tropicalis* genotyping was based on sequence analysis of 6–7 housekeeping genes as previously described whereas for *C. parapsilosis* (66 total isolates examined), genotyping was performed using four highly-polymorphic microsatellite markers.

Results: In this study, 23 *C. tropicalis*, 20 *C. parapsilosis*, 7 *C. albicans* and 4 *C. glabrata* isolates were isolated from blood cultures and CVCs of hospitalized patients.

C. parapsilosis was the most common species recovered from environmental and HCWs samples (179 isolates) followed by *C. albicans* (14) *C. glabrata* (10) and *C. tropicalis* (5).

Genotyping data revealed 18 different MLST-genotypes (or diploid sequence types; DSTs) for *C. albicans*, 3 for *C. glabrata* and 8 for *C. tropicalis*. Except for *C. glabrata*, 14 *C. albicans* and 6 *C. tropicalis* DSTs were completely new and were submitted in the respective public MLST databases. For *C. tropicalis*, we found that 4 of the new DSTs (747, 748, 749 and 759) composed a single well-defined Italian clonal cluster (CC33) with the DST747 as the putative founding genotype. For *C. parapsilosis*, a total of 28 microsatellites genotypes were detected among 66 isolates genotyped.

The availability of genetic and epidemiological data supported the evidence of recurrent outbreaks of *Candida* infection in our hospital.

Conclusion: This study shows that, in our hospital, candidaemia is predominantly caused by non-albicans *Candida* species colonizing also various hospital surfaces including air, medical devices and HCWs, and therefore the implementation of surveillance programmes is imperative to prevent the spread of HAIs. The study also shows the increase of *C. tropicalis* BSIs and highlights that the population structure of this yeast is far from being fully elucidated as its complexity increases as different categories of patients and geographic areas are examined.

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P091

Miltefosine shows fungicidal effect on Cryptococcus spp. by apoptosis mechanism.

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Objective: *Cryptococcus neoformans* and *Cryptococcus gattii* are the etiological agents of cryptococcosis, a disease with a high incidence among severely immunocompromised patients. Amphotericin B is the standard antifungal to treat the neuronal form of cryptococcosis, but its use has been limited. Thus, the search for new therapeutic options is necessary for the treatment of this mycosis. Previous studies had shown miltefosine (MFS) as an alternative antifungal drug for several pathogenic fungi including *Cryptococcus* spp., however, its mechanism of action on this fungus is poorly understood. The aim of this study was to characterize the fungicidal effects of MFS on *Cryptococcus*.

Methods: The broth microdilution assay was performed to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of MFS on *C. neoformans* (H99 and CAP59*)* and *C. gattii* (ATCC 56990). Subsequent assays were performed to evaluate the activity in biofilms, post-antifungal effect (PAFE) of MFS, yeast viability, plasma membrane permeability, drug interaction with exogenous ergosterol and yeast morphological alterations. Yeasts of *C. neoformans* H99 untreated or treated with fungicidal concentrations of MFS (4× and 8× MIC) were analyzed by flow cytometry or fluorescence microscopy using propidium iodide, Rhodamine123, DCFH-DA, TUNEL, and Hoechst 33342 as fluorescent markers.

Results: We confirmed the inhibitory and fungicidal activity of MFS on planktonic cells (0.5 - 2.0 μg/ml) and also observed that the dispersion cells from *Cryptococcus* biofilms exhibited similar MFS susceptibility to their planktonic counterpart. In contrast, MFS did not present inhibitory effect on sessile cells from biofilms at concentrations up to 16 μg/ml. Interestingly, MFS showed post-antifungal effect on *Cryptococcus* spp. that lasted for up to 8.15 h suggesting prolonged MFS inhibitory activity even when the drug pressure is no longer present, and this effect is associated with the fungicidal effect. Morphological changes were observed by light and transmission electron microscopies after exposure of the yeasts to MFS, such as a significant reduction of the mucopolysaccharide capsule thickness, plasma membrane irregularity, and presence of swollen mitochondria. In addition, treatment with fungicidal concentrations led to significant extravasation of DNA and proteins through the membrane. Conversely, in the competition assay with ergosterol we observed a reduction in the antifungal activity of MFS. Thus, the increased permeability in the plasma membrane (Figure 1A) may be related with a direct interaction of MFS with the ergosterol in the plasma membrane. Also, our flow cytometry results showed that MFS interferes with the mitochondrial potential (Figure 1B) leading to increased ROS production (Figure 1C), and DNA fragmentation (Figure 1D) and DNA condensation (Figure 1E) as final consequence. Amphotericin B, an antifungal gold standard, was used to compare with MFS.

Conclusion: Together, our results suggest that MFS acts on the membranes of *Cryptococcus* spp. yeasts leading to two important events: increased plasma membrane permeability and a deleterious effect on the mitochondrial activity inducing cell death by apoptosis; both effects corroborating to the MFS fungicidal activity. These findings reinforce the potential use of MFS as an alternative treatment for cryptococcosis.

Picture 1: https://www.eventure-online.com/parthen-uploads/89/8ISH/add_1_419508_b9fad543-8c47-402a-9ff3-6fcf07dff9af.jpg

Caption 1: Effects of MFS on C. neoformans H99 yeasts.

P092

Paracoccidioidomycosis in a university hospital of Buenos aires city

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Objective: Paracoccidioidomycosis (PCM) is a systemic and endemic fungal disease that is restricted to Latin America. In Argentina, the endemic area is located north of the 34° S parallel. Diagnosis is made by visualization of the yeast cells of *Paracoccidioides* spp in tissues, fungal isolation and anti-*Paracoccidioides* antibodies detection.

To report cases of PCM treated in a university hospital in the city of Buenos Aires, Argentina.

Methods: Retrospective study. Source documents were medical records from patients with PCM diagnosed from 2002 to 2017. Demographic, clinical and laboratory data are described.

Results: The total number of patients diagnosed with PCM was 22. All patients were adult men with an average age of 53 years (range 29 to 72 years); 11/22 (50%) were natives and still lived in endemic area at the time of diagnosis. A total of 10/22 (45%) of the patients had been born in this area but had migrated years before to Buenos Aires (between 11 to 45 years). Only one patient was born in the metropolitan Buenos Aires region and had not visited the endemic area of PCM.

In 17/22 (77%) of patients multifocal lessions (lung and other organs) were observed while in 5/22 (23%) unifocal lessions (1 in cerebellum, 1 in adrenal glands, 1 in larynx, 1 in ropes vowels and 1 in lymph node) were found. *Paracoccidiodes* spp. / *Leishmania* spp. co-infection was documented in one patient in 2017.

In 16 of 22 (72%) patients *multi-budding* yeast cells (pilot's wheel) of *Paraccocidiodes* spp. were observed in fresh examination and / or with histopathological techniques of different samples. The fungus was isolated in pure culture only in three patients. In 12 of 22 (54%) patients specific anti-*Paracoccidoides* antibodies were detected using the immunodifussion (ID) test. Positive ID was the only confirmatory test for diagnosis in 5 of these patients.

Nested PCR amplified a GP43 gene fragment in the last six cases from sputum samples, biopsies from adrenal glands, larynx, vocal cords and oral mucosa. In five of these cases multi-budding yeast cells were observed and in one case direct examination was negative.

All patients received itraconazole antifungal treatment (100-400 mg/day). When follow-up was performed, favorable outcome was achieved in 12/18 (66%) cases.

Conclusion: There are few record of PCM cases in the city of Buenos Aires. However, the large number of migrants from Argentina's northern provinces and neighboring countries, where PCM is endemic, should be an alert for its diagnosis.

Clinical suspicion and specific tests inclusion for its diagnosis are mandatory in patients who inhabit the PCM endemic area. It is also important to include the differential diagnosis with pathogens that share ecological and physiopathological similarities with PCM.

Quick and efficient direct examination helps to diagnose most cases of PCM. On the other hand, culture, as reported in the literature, has low sensitivity. In addition, serology is also an important test and, sometimes, the only evidence of this mycosis.