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**Histoplasma capsulatum modulates the immune response exerted by mastcellenestinal stem cells**

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**Abstract**

Background: Intestinal mast cells (MCs) have a key role not only for tissue regeneration but also for the treatment of inflammatory diseases. Several studies have demonstrated the therapeutic potential of MSCs for the treatment of noninfectious inflammatory diseases; however, they are not able to play a dual role in infection diseases. Histoplasma capsulatum is a systemic mycosis caused by Histoplasma spp., which occurs mainly in immunocompromised individuals; this mycosis can present a severe clinical picture with dissemination to various organs and is associated with an exacerbated inflammatory response and with azotemia and proteinuria if bone marrow is affected. So far, the effect of a possible interaction of Histoplasma with stem cells present in the bone marrow is unknown.

Objectives: To examine, in vitro, the immunomodulatory effects of MSCs in response to Histoplasma capsulatum infection.

Methods: MSCs were obtained from bone marrow of C57BL/6j male mice, after isolation and purification, they were induced to modulated boneons and characterized by flow cytometry. Later, the basal expression of red blood cell receptor (TLR)2, TLR4, and Dictin-1 were determined using flow cytometry. MSCs were incubated with H. capsulatum yeast (isoates CB1990) in a multiplicity of infection (MOI) of 5 and incubated for 24 h. In addition, some of the co-cultures were previously treated with anti-inflammatory blocking antibodies for TLR2 and TLR4 or with a blocking peptide specific for Dictin-1 (CLEC7A). Furthermore, phagocytes, microvascular, and cell proliferation assays were done, and the expression of the genes encoding the cytokines IL-1β, IL-6, IL-10, IL-17, TNF-α, and IFN-γ were assayed, as well as those for arginase-1 and iNOS were assayed.

Results: We observed that H. capsulatum has the capability to adhere and internalize within these MSCs; nonetheless, this process did not affect the survival of the fungus. The interaction of H. capsulatum with MSCs induced a slightly bigger but significantly increased expression of IL-12 and a decrease in the expression of IL-10, IL-17, TNF-α, TGF-β, and iNOS, and as such, the immune milieu Ag-1 and iNOS. Interestingly, blockage of these receptors did not affect phagocytosis, but increased IL-10, IL-17, and TNF-α expression and reduced the expression of IFN-γ. Noteworthy, H. capsulatum-induced apoptosis and ablated the proliferation of these cells; furthermore, this fungus significantly reduced the expression of genes related to adipogenic differentiation and increased the expression of genes related to the osteogenic differentiation process.

Conclusions: The above results indicate that MSCs do not exert a variable antifungal effect against H. capsulatum, on the contrary, this fungal pathogen not only modulates the expression of inflammatory mediators in MSCs, by a mechanism dependent on TLR2, TLR4, and Dictin-1, but also affects their viability and their ability to differentiate into different types of specialized cells. These events could, in principle, affect both hematopoiesis and the immune response in the infected host, and in addition, these stem cells may provide a niche for this fungus, allowing it to persist and evade host immunity.

**P124**

**Cryptococcus neoformans-and Cryptococcus gattii-specific antibodies vary among children and adults with cryptococcosis and healthy from Colombia**

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**Abstract**

Background: Cryptococcus neoformans (C. neoformans) and Cryptococcus gattii (C. gattii) are fungi, spores, and the life-threatening systemic mycosis of global distribution affecting mainly immunocompromised adults.

Objectives: To study and analyze the difference in the expression of IgG antibodies against C. neoformans and C. gattii antigens in sera from patients with cryptococcosis. Our aim was to examine the systemic response and the immune response in Colombia; we also aimed to identify the role of TLRs in the incidence of the disease in the country.

Methods: Sera from children and adult patients with cryptococcosis (n = 109) and sera from healthy children and adults from Colombia (n = 115) were studied. Using ELISA, total and Co- and Cg-specific levels of immunoglobulin IgG, IgA, and IgM were determined in sera. Total IgG, IgA, and IgM levels were higher in HIV- compared with HIV+ patients with cryptococcosis. Specific IgG, IgA, and IgM levels were higher in HIV+ patients with cryptococcosis. These results suggest that the severity of the disease is related to the level of anti-C neoformans antibodies and that the severity of the disease is related to the level of anti-C neoformans antibodies. IgG antibodies were detected in all samples, a positive correlation between total and specific IgG, IgA, and IgM levels was found.

Conclusions: For cryptococcosis patients from Colombia, serum immunoglobulin levels after different HIV status, as reported previously. However, the study shows for the first time variations in immunoglobulin production among adults and children with cryptococcal disease and between Cg and Cg protein antigens. The observation of differential antibody reactivity with cryptococcal proteins encouraged further studies of the humoral immunity to host defense against cryptococcosis.
Results: We observed that H. capitata has the capability to adhere and internalize within these HSs; however, this process did not alter the survival of the fungus. The interaction of H. capitata with HSs induced a significantly increased expression of TLR2 and Dectin-1 but not TLR4. In addition, this fungal interaction significantly induced an augmented expression of IL-4, IL-10, IL-15, IFN-γ, TGF-β, as well as the innate immune genes Arg-1 and NOS. Interestingly, blockade of these receptors significantly deceased the phagocytic process as well as the expression of all inflammatory mediators evaluated, especially when blocking TLR4 and Dectin-1. Of note, H. capitata induced apoptosis but not the proliferation of these cells.

Conclusions: These results indicate that HSs are capable of phagocytosing H. capitata but do not affect its survival; moreover, this fungal pathogen could induce changes in the expression of pattern-recognition receptors (PRRs), especially TLR2 and Dectin-1, and could subsequently activate the HSs leading to the expression of inflammatory mediators as well as affecting the viability of these stem cells. Altogether, these findings indicate that H. capitata could affect the hematopoietic process as reflected in an increase in diverse leukocytes, erythrocytes, and platelets as observed in patients with severe and disseminated disease, especially in those with dissemination to bone marrow.

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Cytokine gene Polymorphism in superficial Malassezia-associated skin diseases
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Poster session I, September 21, 2022, 12:30 PM – 1:10 PM
Objectives: To isolate and characterize Malassezia species from patients of Pyorrhea Veneralis (PV), Atopic Dermatitis (AD), Seborrhoeic Dermatitis (SD), and healthy controls.

To study single nucleotide polymorphisms in IL-10 and IFN-γ genes of the host and its relation with susceptibility to Malassezia infection.

Methods: It was a prospective observational study done in University College of Medical Sciences and GTB Hospital, Delhi. Sample size comprised of 36 cases of AD, 36 cases of PV, 36 cases were used for fungal culture on Sabouraud Dextrose Agar (SDA) and Modified Dixon Agar (MDA) and isolates were identified as per conventional phenotypic methods. Genetic DNA was extracted from blood samples and Cytokine genotyping was carried out by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) with sequence-specific primers. These SNPs (IL-10 1082G/A; IL-18 760C/G and IFN-γ +874C/T) in two cytokine genes were assessed in all the patients and healthy controls. Chi-Squared Test or Fishers’Exact Test and Bonferroni’s correction were used for statistical analysis.

Results: Malassezia was isolated in 94.4%, 61.1% and 52.6% in PV, AD, SD, and healthy controls respectively.

Malassezia glouvera was the most commonly isolated species from both patient and healthy control.

Malassezia sympodialis was the second most commonly followed by M. flocculosa and M. restricta. Association between specific cytokine gene polymorphisms and clinical outcome was found to be significant in PV, AD, and SD group.

IFN-γ (+874 A) allele was significantly associated with PV and AD respectively.

Conclusion: The identification of Malassezia to a species level is of a great importance to determine which species are implicated in certain skin diseases.

The use of phenotypic methods for identification of Malassezia species is a reliable, easily executed method, that is also inexpensive. Molecular methods are necessary to decribe the turnaround time, especially for slow-growing Malassezia species.

Cytokine gene polymorphisms studies in IL10 and IFN-γ genes demonstrated susceptibility of host to Malassezia infections.

Comparison with the existing cytokine levels will help in understanding the evolution of Malassezia infections in susceptible host.

Population genetics studies requires inclusion of a larger number of subjects to evaluate the probability or the frequency of occurrence of the polymorphism.

Conclusion: A sequenced test to detect Aspergillus fumigatus IFN-γ could complement the Aspergillus fumigatus IFN-γ test in detecting CPA. CPA is a neglected disease with a high morbidity and mortality. Routine follow-up protocol for the averted patients in tuberculosis center by a sezoneetorical test for a minimum period of 5 years may be necessary for early detection of cases in PTB high prevalence countries.

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Optimization of MTI assay protocol in Pythium idahoense zoospores incubated together with neutrophils
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Objectives: The pythium-based MTI assay is widely used to assess cell viability. This colorimetric assay measures cell viability through the enzymatic reduction of MTT, a yellow water-soluble tetrazolium dye, to purple-colored formazan crystals. The use of Pythium idahoense is the informative form causing pythiosis. There remains a lack of standardized protocol for Pythium idahoense viability assay. Hence, we optimized the MTI assay to assess the activity of neutrophils on zoospores.

Methods: Neutrophils and zoospores were incubated for 2, 4, and 6 hours at 37°C. Neutrophils were lysed in water (pH 5 ± 1); MTI was added to each well and incubated at 37°C for 2 ± 0.5 h. The MTT solution was added to each well, and the absorbance at 720 nm was measured using a microplate reader. The percentage of viable zoospores was then determined.

Results: The viable zoospores metabolized MTT to soluble purple formazan product. The zoospores germinated into hyphae when incubated for <2 h. Because of the rapid germination of zoospores into hyphae, reliable results would be obtained with an optimum incubation time of 2 h for the assay.

Conclusion: The simplicity and low cost of the assay make it a valuable tool for zoospore viability studies, with a focus on immunology and immunotherapy.

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Post-COVID-19 recurrent fungal urinary tract infections: A case series
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Poster session I, September 21, 2022, 12:30 PM – 1:10 PM
Background: COVID-19 has opened a pandora’s box of opportunistic infections and immune alteration. We hereby present a series of patients with recurrent fungal urinary tract infections triggered by an episode of COVID-19.

Case 1: A 46-year-old male diabetic, known case of chronic kidney disease, had moderate COVID-19 in September 2020 needing renodsecure and insulin, 2 months later, he was treated for a fungal UTI-dweller. In May 2021, he was presented with right-sided pyelonephritis, haematuric and pyrexia. ESIT was done and cultures grew C. tropicalis. Recurrent anti-fungal susceptibility from the bacilomycete resistant and was hence treated with 21 days of micafungin with significant improvement. Despite 3 weeks of directed anti-fungal treatment, he had recurrent episodes of candidiasis with azonnia. Cytospora and selective sampling yielded C. tropicalis from the right pelvicalyceal system, but he was unrevealing for nephroptosis and local antibiotic sensitivities he was started on amikadiol and long-term 5-fucosidase suppressor.

Case 2: A 6-year-old male, diabetic, with uncontrolled sugars, had severe COVID-19 in September 2020 needing oxygen, renodsecure, and insulin, 1-month later, presented with right-sided flank pain and four episodes of purulent passage of Rocky stone in the last year (Fig 2). He had multiple episodes of bacterial UTIs which were treated, with complete resolution. The Rocky stone showed fungal elements and culture grew Aspergillus flavus sensitive to voriconazole, itraconazole, and posaconazole. PUC was grown and multiple penicillin resistant C. tropicalis with minimum 5-fucosidase suppressor. He grew in right-sided pyelonephritis with mild pelviccalyceal system. The patient was treated on 2 weeks of macrolide following a prolonged course of voriconazole and 5-fucosidase with close clinical and therapeutic drug monitoring.

Case 3: A 68-year-old female, diabetic, had moderate COVID-19 in April 2021, and was given renodsecure and insulin. She was admitted 1 month later with UTI (Pyelonephritis with early forming renal abscess) with urine cultures growing CR K. kingii and C. tropicalis for which she was given colistin, amikacin, and voriconazole and underwent bilateral ESIT followed by stent removed after 1 month along with switching to macrolide in view of repeated candidaemia on treatment. She has developed multiple UTIs in the subsequent months with C. tropicalis being isolated twice and bacterial UTIs treated with antibiotics and macrolide. Subsequent CT revealed retroperitoneal fibrosis causing the ureters obstruction, a biopsy was mandated but was essentially started on nephrectomy for UTI-disease.

Discussion: The proposed immune alteration mechanism of COVID-19 of decreased phagocyte function, uncontrolled sugars, and uncontrolled neutrophil dysfunction predisposes to several opportunistic infections including fungal infections. It is intriguing that these patients with refractory funguria never underwent any immunization. The challenges associated with the use of these cases included delayed renal function precluding the use of intravenous contrast for imaging and several anti-fungal drugs; inadequate urinary percutaneous of azonnia especially with delayed renal function and immunosuppressive measures needed to correct this problem.