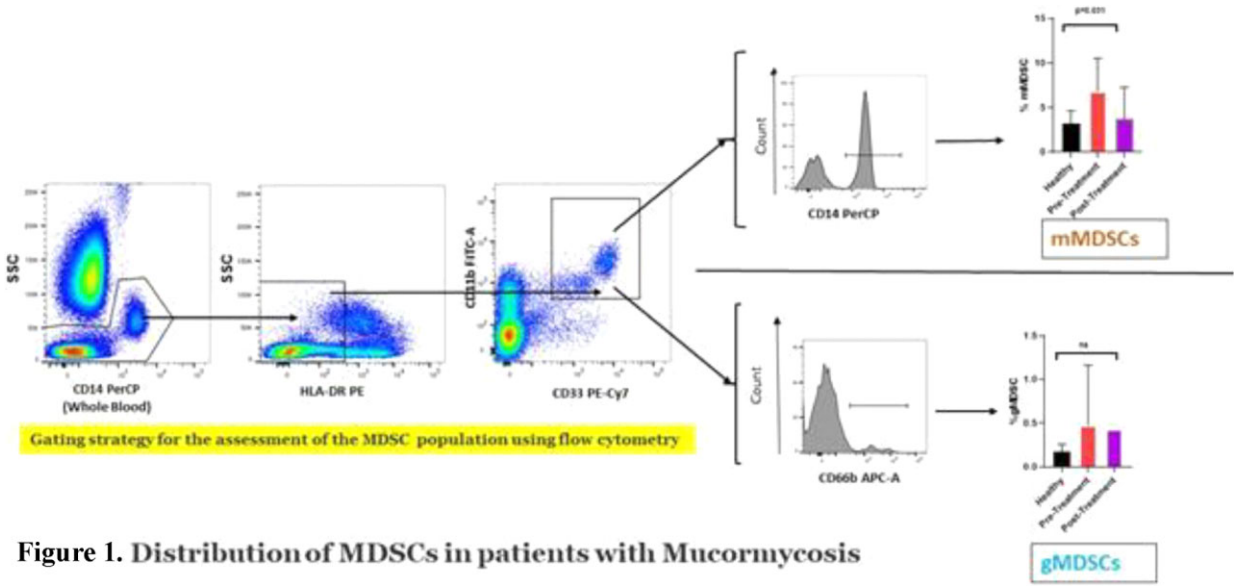

Gating strategy for the assessment of the MDSC population using flow cytometry

Figure 1. Distribution of MDSCs in patients with Mucormycosis

P123

Study of naïve and memory T helper cell response in patients of chronic rhinosinusitis with nasal polyposis (CRSwNP) after *in vitro* exposure to *Aspergillus flavus*

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Objectives: To study the CD45RA (naive) and CD45RO (memory) in CD4 + T cell population after *in vitro* stimulation to *Aspergillus flavus* antigen in CRSwNP patients and healthy controls.

Methods: The study included 30 cases of CRSwNP (before and after six months of treatment) and 30 healthy controls. Postoperatively biopsies (polyp tissues) were subjected to KOH and culture for mycological investigation. Preoperatively, blood sample (4 ml) was collected from cases and controls for peripheral blood mononuclear cells (PBMCs) separation. PBMCs were treated *in vitro* by *Aspergillus flavus* antigen (20 μg/ml) and Phytohemagglutinin and incubated for 18 h at 37°C in CO_2 incubator. Cells were harvested after incubation and stained with different monoclonal antibodies such as CD3, CD4, CD45RA, and CD45RO for flow cytometry analysis. Statistical analysis was done using SPSS software. Data were expressed as mean ± SD and the significance level was considered at probability below 0.05.

Results: The profiles of various CRSwNP patients and healthy controls were studied. The mean age and duration of disease of the patients were recorded as 28.92 years and 9.08 months. A total of 24/30 (80%) cases were found positive for *Aspergillus flavus* from KOH/culture investigation. The percentage positivity of CD3 + CD4 + T cells was significantly increased after *A. flavus* stimulation in patients as compared with healthy controls. Decreased levels of CD45RA + CD4 + T cells were analyzed in patients before and after treatment as compared with healthy controls. The percentage of CD45RO + CD4 + T cells was found to be increased upon *A. flavus* stimulation in patients compared with the healthy control group.

Conclusion: The continuous exposure to fungal spores may induce unusual immune responses to *Aspergillus flavus* spores, triggering an allergic immunological reaction with increased CD4 + T cell responses. Increased levels of CD4 + CD45RO + T cells may transform the pathogenic reaction and highlight the chances of *A. flavus* reactive T cells involvement in initiating inflammation in cases of CRSwNP.

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

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Background. *Cryptococcus neoformans* (Cn), predominantly, and *Cryptococcus gattii* (Cg) cause cryptococcosis, a life-threatening systemic mycosis of global distribution affecting mainly immunocompromised adults.

Objectives. This study aimed to determine total and specific antibodies against *C. neoformans* and *C. gattii* antigens in sera from patients with cryptococcosis and from healthy individuals from Colombia, which will help to elucidate sero-epidemiological variations in the incidence of the disease in the country.

Methods. Sera from child and adult patients with cryptococcosis ($n = 109$) and sera from healthy children and adults from Colombia ($n = 119$) were studied. Using ELISA, total and Cn- and Cg-specific levels of immunoglobulin (Ig)G, IgA, and IgM were determined in sera.

Results. Total IgG, IgA, and IgM levels were higher in HIV + compared with HIV− patients with cryptococcosis. Specific IgG, IgA, and IgM levels tended to be higher in cryptococcosis patients than in healthy controls and to be higher in adults than in children, with a positive correlation between antibody reactivity and age. All serum immunoglobulins were more reactive against Cn-proteins than Cg-proteins. Including all samples, a positive correlation between total and specific IgG, IgA, and IgM levels was found.

Conclusions: In cryptococcosis patients from Colombia, serum immunoglobulins levels differ depending on HIV status, as reported previously. However, this study shows for the first-time variations in immunoglobulin production among adults and children with cryptococcal disease and between Cn and Cg-protein antigens. The observation of differential antibody reactivity with cryptococcal proteins encourages further studies of the humoral immunity for host defense against cryptococcosis.

P125

Histoplasma capsulatum modulates the immune response exerted by mesenchymal stromal cells

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Background: Mesenchymal stromal cells (MSCs) have become a tool 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 appear to play a dual role in infectious diseases. Histoplasmosis is a systemic mycosis caused by *Histoplasma* spp., which occurs mainly in immunosuppressed individuals; this mycosis can present a severe clinical picture with dissemination to various organs and is associated with an exacerbated inflammatory response and with anemia and pancytopenia 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 *H. capsulatum* infection.

Methods: MSCs were obtained from bone marrow of C57BL/6 male mice; after isolation and purification, they were induced to mesodermal lineages and characterized by flow cytometry. Later, the basal expression of toll-like receptor (TLR)-2, TLR4, and Dectin-1 was determined using flow cytometry. MSCs were infected with H. capsulatum yeasts (isolate CIB 1980) in a multiplicity of infection (MOI) of 5 and incubated for 24 h. In addition, some of the co-cultures were previously treated with specific blocking antibodies for TLR2 and TLR4 or with a blocking peptide specific for Dectin-1 (CLEC7A). Furthermore, phagocytosis, microbicidal, 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 TGF-β as well as of those for arginase-1 and iNOS were assessed.

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 slight but significantly increased expression of TLR2 but not TLR4 nor Dectin-1. In addition, this fungal interaction significantly induced an augmented expression of IL-6 and a decrease in the expression of IL-1β, IL-17, TNF-α, TGF-β, as well as the immune mediators Arg-1 and iNOS. Interestingly, blockade of these receptors did not affect phagocytosis, but increased IL-1β, IL-17, and TNF-α expression and reduced the expression of IL-6. Noteworthy, H. capsulatum induced apoptosis and inhibited the proliferation of these stem 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 notable 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 Dectin-1, but also affects their viability and their ability to differentiate into a different type 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.

P126

Effects of Histoplasma capsulatum infection on activation and proliferation of hematopoietic stem cells

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Background: Hematopoietic stem cells (HSCs) are considered a multipotent population with high proliferative potential, and are widely used in the treatment of leukemias, multiple myeloma, and some lymphomas. In the context of infectious diseases, some microorganisms have been reported to induce changes in the expression of surface markers in HSCs by a direct effect or through the induction of cytokines. Systemic infections are characterized by inducing stress on the bone marrow, which is reflected in an increase or decrease in leukocytes and platelets in peripheral blood, a process known as 'emergency hematopoiesis'. Histoplasmosis is a systemic mycosis caused by *Histoplasma* spp., which occurs mainly in immunosuppressed individuals; this mycosis can present a severe clinical picture with dissemination to various organs, including the bone marrow, and is associated with anemia and pancytopenia. So far, the effect of a possible interaction of *Histoplasma* with HSCs is unknown.

Objectives: To evaluate, *in vitro*, the effects of Histoplasma capsulatum infection on activation and proliferation of HSCs.

Methods: HSCs were obtained from bone marrow of C57BL/6 male mice; after isolation and purification, they were characterized by flow cytometry. Later, the basal expression of toll-like receptor (TLR)-2, TLR4, and Dectin-1 was determined using flow cytometry. HSCs were infected with *H. capsulatum* yeasts in a multiplicity of infection (MOI) of 5 and incubated for 24 h. In addition, some of the co-cultures were previously treated with specific blocking antibodies for TLR2 and TLR4 or with a blocking peptide specific for Dectin-1 (CLEC7A). Furthermore, phagocytosis, microbicidal, 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 TGF-β as well as arginase-1 and iNOS were assessed.