PTX3 was performed by ELSA. Fixed cells and gminated cells were opsonized with different serum factors and co-incubated with C. tropicalis yeast cells and recombinant capsular multivalent carbohydrate: SMG1-24 at 24°C. Culture supernatants were collected, and pro-inflammatory cytokines were measured by sandwich ELSA.

Results: PTX3 did not bind A. fumigatus cell line directly but in the presence of human serum, purified collagen (serum protein S) in SMG1, and complement proteins (C3), opsonisation of candida with these complement proteins or SP-D stimulated pro-inflammatory cytokine expression by MDMM upon interaction (Fig 1a). In contrast, secondary opsonisation of completion proteins or SP-D opsonised with PTX3 significantly reduced pro-inflammatory cytokine and increased anti-inflammatory cytokine expression from MDMM. PTXM1 opsonised PTX-Facted gminated candida significantly reduced pro-inflammatory cytokine and increased anti-inflammatory cytokine expression from MDMM (Fig 1b).

Conclusion: PTX3 is an acute phase protein expressed in response to pro-inflammatory stimuli during infection and is that is increased in bronchoalveolar lavage of patients with aspergillosis. Our recent data with A. fumigatus suggest that PTX3 is an immunological protein that reduces pro-inflammatory response. Although an inflammatory response is necessary to fight against fungal pathogens, the tissue damage associated with enhanced inflammation can be deleterious and facilitates A. fumigatus infection.

P110

Vaccine induced protection by secreted aspartyl proteinase 2 from Candida parapsilosis in Candida tropicalis mediated murine systemic candidiasis: a role of B-cells and antibodies

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: To study the IL-23R (Th17) and CD25 (+) in CD4 + T cell populations in rhino-orbital mucormycosis positive patients and healthy controls.

Methods: The study included 20 cases of mucormycosis and 20 healthy controls. Nasal swab, collected post-surgery was subjected to DNA extraction for the identification of the pathogen. Venous blood sample from each patient for 3 months after treatment was subjected to Flowcytometry. Statistical analysis was done using SPSS software and the P-value ≤ 0.05 considered as significant. All the data are expressed as the mean ± SD.

Results: All the cases were positive for KOM and confirmed for Rhizopus arzeus by culture.

The flow cytometry analysis showed that the percentage of CD4 + IL-23R (+) Th17 cells was significantly high in patients before treatment compared to healthy controls and found to be lower post 3 months of antifungal treatment. The percentage positivity of CD4 + CD25 (+) Th17 cells was decreased in patients (before treatment) as compared to controls and after treatment groups. The percentage positivity of CD4 + CD25 + cells was significantly increased in patients after treatment.

Conclusion: We observed systemic immune imbalance, with elevated CD4 + IL-23R (+) Th17 cells and CD4 + CD25 + regulatory cells. The findings prominently indicate the mechanism of immune dysregulation involving Th17 and Th17 pathways in mucormycosis and provide evidence that restoration of Th17/Treg may be considered a therapeutic option for long-term benefit. Recovery of CD4 + CD25 + T cells after treatment indicated a favorable phenotype outcome.

P121

Myeloid-derived suppressor cells as a potential biomarker and therapeutic target in rhino-orbital mucormycosis patients

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Background: Mucormycosis is a deadly fungal infection that orinates in patients afflicted with COVID-19. All fungal illnesses are caused by dysregulated adaptive immunity, but Myeloid-derived suppressor cells (MDSCs) exhibit a novel di-mension to the chronic inflammatory response.

Objective: We attempted to measure the MDSC immune response in rhino-orbital mucormycosis patients before and after treatment and compared the data with healthy controls.

Methods: A total of 3 ml of blood samples were taken in an EDTA tube from 21 patients with mucormycosis and 20 age-matched healthy controls. A second blood sample was collected to examine the immune system post three months of treatment.

Results: Myeloid cells were superficially cold-stained and as recombinant proteins from C. albicans, C. tropicalis, and C. parapsilosis strains prevalent in India. Groups of wild type BALB/c mice were vaccinated with individual CpG proteins along with alums as adjuvant, followed by systemic infection with a lethal dose of C. tropicalis. The protective potential of each CpG protein was evaluated using survival analysis and estimations of organ fungal burdens. Pathological assessment was performed using H&E and PAS staining. Serum cytokine levels and antigen-specific antibody titers were measured by ELISA.

Cellular responses were analyzed in detail using flow cytometry. Functional evaluation of antibody response was performed using in vitro (both whole blood and neutrophil-neutrophil killing and in vitro (phenotypic screening) studies). B-cell responses were carried out using immunomodulatory approaches.

Results: Mice vaccinated with CpG alone from C. parapsilosis (Sap2-parapsilosis) showed higher increase in survival rate (P < 0.02) and maximum reduction in organ fungal burden (spleen, kidney, lung, brain) (P < 0.05), compared with sham immunized controls. Vaccination with CpG alone from C. albicans did not improve survival in non-toxic candida C. tropicalis infection, despite the protein having ~40% homology across species. Mice vaccinated with CpG-parapsilosis also exhibited significantly higher levels of IFN-γ, IL-17, and IL-4 cytokine levels post infection, which correlated with protection. In addition, CpG-parapsilosis vaccinated induced high levels of IgG1-specific antibodies, and a fraction of antibodies could bind whole fungi which were predominantly of IgG1 isotype. Notably, sera from CpG-parapsilosis vaccinated mice showed increased C. tropicalis induced cell protection and enhanced neutrophil-mediated killing in vitro. Sap2-parapsilosis was further confirmed by detecting antigen-specific antibodies. Presence transfer of Sap2-parapsilosis immune serum significantly reduced fungal burden in naive mice, as compared to mice receiving sham-immunum serum, upon infection. Higher numbers of total CD11b+ MDSCs were observed in Sap2-parapsilosis challenged mice before and during early stages of Sap2-mediated immune response. Ex vivo analysis performed using identified B cell epitopes provided insights about important IgM and IgG epitopes, when designing multivalent or multi-epitope anti Candida vaccines.