Studies on innate immune Mechanisms in Aspergillosis in Mice Model

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Aspergillus fumigatus is the most common mould causing a wide spectrum of allergic (Allergic bronchopulmonary aspergillosis [ABPA] and Aspergilloma) and invasive disorders (Invasive pulmonary aspergillosis [IPA]) in immunocompetent and immunocompromised patients respectively. Patients at risk (frequency of disease) for ABPA also include those suffering from asthma (15-20%) and cystic fibrosis (25%). The crude mortality from IA is around 85% when untreated. Most of the ABPA patients are treated with cortcosteroids and Amphotericin B is the choice of anti-fungal agent for IPA. So in the present scenario we need a novel therapeutic agent for allergic as well as IPA. Various studies have shown that innate immune defense mediated by macrophages and neutrophils is the main line of defense against A fu. Recently it has been observed that human lung surfactant proteins A and D (SP-A and SP-D) and a recombinant fragment of SP-D (rSP-D) play a major role in pulmonary defense against Afu by enhancing the phagocytic activity of alveolar macrophages. In this context the current study aimed at the evaluation of the therapeutic potential of SP-A, SP-D and a recombinant fragment of SP-D (rSP-D) in the murine model of ABPA and IPA.

Native human SP-A and SP-D were purified from the lung lavage obtained from alveolar proteinosis patients and were judged to be pure by using SDS-PAGE, Western blot analysis.

Murine models of ABPA (by intranasal administration 300 m g antigen of Afu) and IPA (by intranasal administration of 1×10^8 Afu spores) were developed to study the effect of SP-A, SP-D and rSP-D

In vitro studies with rSP-D were performed to examine their immunomodulatory properties. Effect of rSP-D on Agglutination, binding, phagocytosis and killing of A.fu conidia have been performed. Histamine release in sensitized basophils and lymphoproliferation in sensitized lymphocytes were also evaluated.

RESULTS:
rSP-D showed increased Agglutination, binding, phagocytosis and killing of A.fu conidia

and decreased histamine release in sensitized basophils and lymphproliferation in sensitized lymphocytes. The untreated ABPA mice showed increased IgG and IgE antibody levels, high peripheral and pulmonary eosinophil count and a shift form TH1 to TH2 type of cytokine profile which is non protective in the case of ABPA. Intranasal administration of physiological concentration of human SP-A, SP-D and rSP-D in the murine model of ABPA decrease in IgG and IgE antibody, low peripheral and pulmonary eosinophil count and a shift form TH2 to TH1 type of cytokine profile have been observed. The IPA mice showed 100% mortality at seven days, high CFU counts and decreased levels of IFN-g and TNF-a . The AmB, SP-D and rSP-D increased the survival rate, lowered the CFU counts in IPA mice. Lung sections of the IPA mice showed dense growth of fungal hyphae which were significantly reduced following treatment with AmB, SP-D and rSP-D, consistent with marked increase in the levels of TNF-a , as compared to untreated IPA mice.

FUTURE PLAN:

1. Evaluation of different doses of rSP-D (5m g, 10m g, 20m g) to find the optimum dose
2. Study of the combined treatment of rSP-D and Amphotericin B (a standard drug for IPA).
3. Estimation of toxicity of rSP-D by various biochemical tests and enzyme indicators for hepato, nephro and cardiac toxicity