ACTIVATION OF THE IMMUNE SYSTEM IN A PULMONARY MODEL OF FUNGAL INFECTION (PARACOCCIDIOIDOMYCOSIS). EFFECT OF FUNGAL AND HOST FACTORS IN THE SEVERITY OF THE DISEASE

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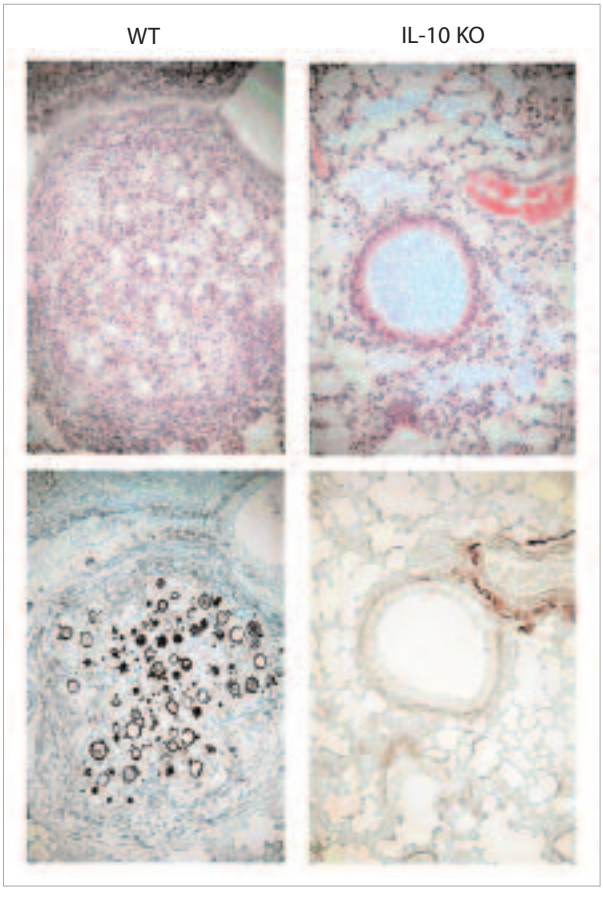

Genetic deficiency of IL-10 (IL-10 KO mice, right micrographs) leads to microbiological cure of P. brasiliensis *pulmonary infection (WT control mice, left micrographs). Upper, H&E; lower, Groccot stained lesions (100x)*

Paracoccidioidomycosis (PCM) is the most prevalent deep mycosis in Latin America and presents a wide spectrum of clinical and immunological manifestations. Our group established a genetically controlled murine model of PCM, where A/Sn mice develop an infection which mimics the benign disease (immune responses which favor cellular immunity) and B10.A animals present the progressive disseminated form of PCM (preferential activation of B cells and impairment of cellular immune responses). This pattern of immunological reactivity led us to postulate that the Th1/Th2 paradigm of immune response could be applied to explain the resistant/susceptible patterns in experimental PCM. Cytokines studies, mainly in the pulmonary model of infection, have confirmed that production of IFN-Y, TNF-a and IL-12 are linked with resistance but more complex immunological mechanisms, not Th1/Th2 mediated, are associated with genetic susceptibility to *P. brasiliensis* infection. We are now proposing further studies in our experimental pulmomary model of PCM. We intend to expand our studies on the influence of some cells and mediators of innate immunity (macrophages, NK cells, nitric oxide, IL-10, leucotrienes, *P. brasiliensis* lipids, TLR-4, chemokines) in the adaptative immunity and severity of pulmonary PCM. We also intend to further explore the role of TDC8 and TCD4 lymphocytes in the immunoprotection against *P. brasiliensis* infection. Several approaches, genetic strains of mice as well as immunomanipulations will be used to reach a better comprehension of the immunoprotective mechanisms operating in pulmonary PCM. After i.t. infection with on million yeast cells, mice will be studied regarding the severity of infection in lungs, liver and spleen, production of specific isotypes, delayed hypersensitivity reactions, levels of pulmonary and hepatic cytokines and organs histopathology. In addition, studies on the cellular composition of bronchoalveolar lavage fluids and lung infiltrating lymphocytes will be performed. When required, the activation of these cells will be evaluated by the expression of adhesion and co-stimulatory molecules besides the characterization of its ability to secrete pro- and anti-inflammatory mediators.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main purpose of this project is to study some mechanisms of innate immunity which interfere with the protective or deleterious adaptative immune responses that further develop in a pulmonary model of fungal infection. Our studies on the function of CD4+ and CD8+ T cells in the immunity developed by susceptible, intermediate and resistant mice after pulmonary infection with *P. brasiliensis* demonstrated that: a) unexpectedly, fungal loads are mainly controlled by CD8α + T cells; b) genetic susceptibility of hosts appears to be associated with deletion or anergy of CD4+ T cells, and finally, d) a balanced type1/type2 immunity is associated with genetic resistance to *P. brasiliensis* infection. Our results on the role of TLRs in paracoccidioidomycosis suggest *P. brasiliensis* yeasts use TLR2 and TLR4 to gain entry into macrophages and infect mammalian hosts. Indeed, *P. brasiliensis* yeasts appear to be recognized by TLR2 and TLR4, resulting in increased phagocyte ability, NO secretion and fungal infection of macrophages. Thus, interaction with TLRs could be considered a pathogenicity mechanism of *P. brasiliensis*. We could also verify that alveolar macrophages of susceptible mice are very reactive to *P.brasiliensis* components and pro-inflammatory mediators are secreted by cells involved in the innate immunity of lungs. The excessive production of NO, however, inhibits the initial development of CD4+ T-cell-immunity by active induction of T cell anergy or deletion. The elevated expression of co stimulatory molecules (MHC class I, CD40, CD80) by APC can directly activate CD8+ T cells without the help of CD4+ T lymphocytes. This pattern of immunity can explain the efficient mechanism of innate immunity resulting, however, in poor T cell mediated immunity. Alveolar macrophages from resistant mice respond to *P.brasiliensis* infection by secreting low amounts of IL-12, but high levels of TGF-β and TNF-α. This explains the inefficient natural immunity of resistant mice. The low levels of NO production, however does not impair T cell immunity. So, resistant animals slowly develop *P. brasiliensis* specific CD4+ and CD8+ T lymphocytes, which control fungal growth and organize lesion morphology. This model does not exclude the previously proposed Th1/Th2 model of *P. brasiliensis* control, but explains why the excessive activation of the immune system can result in enhanced susceptibility to the fungus and severe pathology.

MAIN PUBLICATIONS

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