Conferences

PATHOGENESIS OF THE PARACOCCIDIOIDIC GRANULOMA

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A critical review on the granulomatous chronic inflammation induced by *Paracoccidioides brasiliensis*, the agent of paracoccidioidomycosis, will be presented.

The epithelioid granuloma is considered to be the most evolved tissue inflammatory host response against parasites, which consequently implicates complex and intricate molecular mechanisms and cellular modulation.

An effective host granulomatous inflammatory response results in compact, pauci-parasitic granulomata which are seen in the chronic, localized, "benign" , forms of the mycosis in contrast to the loose, exudative, disorganized parasite-rich granulomata seen in the acute, disseminated, "malignant" forms.

During the last years, several national and international groups of research have investigated both host defense mechanisms which act to induce a protective, fungistatic, tissular response to the agent as well as how the parasite evades the host defenses in order to survive and multiply.

The investigative approaches to these mechanisms can be summarized as follows: **i**) *In vitro* experiments with lymphomononuclear cells from treated or "cured" patients as well as from patients with the acute or chronic forms of the disease, or with the paracoccidioidic infection (subclinical asymptomatic form, with positive paracoccidioidin test) and **ii**) *In vivo* testing on human biopsy or on samples from experimentally infected animals.

The *in vitro* experiments have been carried out by evaluating the fungistatic and fungicidal activity, as well as the secretion of cytokines, by circulating leukocytes stimulated with purified fungal antigens with the modulation of pro- and anti-inflammatory mediators.

The *in vivo* studies have tested by immunohistochemistry or PCR **i)** the tissular expression of defensive or modulatory molecules, **ii)** the immunophenotyping of the infiltrating cells and **iii)** the cytokine network acting at and promoting the granulomatous inflammatory response. More recently, experimental models using knockout animals for anti- and pro-inflammatory cytokines genes have contributed significantly to the understanding of several features of the host-parasite relationship in the mycosis.

Based on these results, therapeutic targets have been defined and protocols for immunotherapy and vaccination have been tested aiming at increasing the host defenses against the parasite.

In summary, we will try to look critically at the amount of new information on the physiopathology of the mycosis, in order to interconnect as much as possible the available data as to be able to point out future investigative avenues which will eventually help the patients to survive with no sequelae.

CF 2 - EXPERIMENTAL VACCINES AGAINST COCCIDIOIDOMYCOSIS

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Coccidioides is a fungal pathogen of humans which can cause a life-threatening respiratory disease in immunocompetent individuals. Recurrent epidemics of coccidioidal infections in Southwestern United States has raised the specter of awareness of this soil-borne microbe, particularly among residents of Arizona and Southern California, and has galvanized research efforts to develop a human vaccine against coccidioidomycosis. The focus of our studies of candidate vaccines has been the identification of purified, recombinant antigens which elicit a potent and durable host protective response against *Coccidioides* infection. The strategies used to identify and evaluate vaccine candidates will be discussed, and an update on progress toward development of a vaccine against this endemic pathogen will be presented.

CF 3 - IMMUNITY AND TOLERANCE IN FUNGAL INFECTIONS: MECHANISMS AND PERSPECTIVES

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Protective immunity against fungal pathogens is achieved by the integration of two distinct arms of the immune system, the innate and adaptive responses. The majority of fungi are detected and destroyed within hours by innate defense mechanisms. Most of the host defense mechanisms are inducible upon infection and their activation requires specific recognition of invariant molecular structures shared by large groups of pathogens (also known as PAMPs, pathogen-associated molecular pattern) by a set of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). A number of cell wall components of fungi may act as PAMPs acting through several distinct TLRs. Individual TLR activates specific antifungal programmes on phagocytes and dendritic cells. This implicates that TLRs may govern protection and immunopathology at the level of the innate immune response as well as the quality of the adaptive immune response. In vertebrates, the activation of the adaptive immune response results in the generation of antigen-specific T helper (Th) effector cells that are endowed with the ability to release a distinct panel of cytokines, capable of activating and deactivating signals to effector phagocytes. To limit the pathologic consequences of an excessive inflammatory cell-mediated immune reactions, the immune system also resorts to a number of protective mechanisms, including the generation of regulatory T cells. Thus, innate and adaptive immune responses are intimately linked and controlled by sets of molecules and receptors that act to generate the most effective form of immunity for protection against fungal pathogens.

CF 4 - COCCIDIOIDOMYCOSIS. A REVIEW OF THE DISEASE AND RECENT RESEARCH RESULTS

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There are many similarities between coccidioidomycosis and paracoccidioidomycosis. Both are caused by soil-dwelling dimorphic fungi endemic only to the Western Hemisphere. Symptomatic disease in both is more common in men than women. Both are controlled in the human host by a robust cellular immune response. However, they differ in their precise geographical distribution and in their clinical presentations. Coccidioidomycosis is recognized as a major fungal infection in the southwest United States and northern Mexico. However, there are pockets of endemicity in Central and South America, including northeast Brazil and north-central Argentina. Among those infected, 60% are completely asymptomatic and the other 40% have a disease that most frequently resembles a community-acquired bacterial pneumonia that resolves without therapy. However, approximately 5% have persistent illness, either as a chronic pulmonary infection or as disseminated beyond the thoracic cavity. Sites of dissemination are most commonly the skin, joints, soft tissues and meninges. Risks for dissemination include underlying immunosuppression, such as seen in patients with HIV infection and transplantation recipients. Certain racial groups also appear to be at risk and older age predisposes to symptomatic disease. My laboratory has been interested in the human cellular immune response to coccidioidomycosis for several years. Previously, we have shown that the lack of lymphocyte response among donors with active coccidioidomycosis is correlated with severity of disease. We have been able to overcome this anergy in vitro by incubating cells with autologous mature dendritic cells loaded with coccidioidal antigen. We have also been able to describe immunological events within human coccidioidal granulomata and have demonstrated clusters of lymphocytes that contain principally B cells and CD4+ T cells. These clusters contain relatively more interleukin-10 (IL-10) than interferon-gamma (IFN-γ) and may serve as sites of immune down-regulation. More recently, we have described specific elements that appear important in this response. Specifically, mRNA for IL-12 receptor β2 (IL12Rβ2) and activated intranuclear STAT-4 are upregulated in response to the coccidioidal antigen T27K in cells from immune donors but not from non-immune donors. On the other hand, IL12Rβ1 is increased in both immune and non-immune donors. In addition, the monomer IL-12p40 is increased in cells from immune donors after incubation with coccidioidal antigen but the heterodimer IL-12p70 is not detectable. We now also have evidence that the innate immune system, including the mannose receptor (MR) and Toll-Like Receptor-2 (TLR-2) play a role in recognition of coccidioidal antigens by human peripheral blood mononuclear cells from immune donors. We have demonstrated that the release of IFN-γ and IL-2 are both diminished in cells from immune donors co-incubated with coccidioidal antigen and the inhibitor of MR, mannan. In addition, the release of the cytokine TNF-α is decreased by immune cells incubated with coccidioidal antigen co-incubated with antibody directed against TLR-2 but not TLR-4. These insights should be useful in better understanding the human immune response to coccidioidomycosis and may hold the key to future therapies.

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CF 5 - IMMUNOBIOLOGY OF HISTOPLASMOSIS

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Histoplasma capsulatum is a dimorphic fungus that is endemic to the Americas. Infection is acquired by accidental inhalation of microconidia and mycelial fragments from the soil. Once inhaled, the saprobic form converts to the yeast phase which causes the clinicopathological manifestations of the disease. In mice and in humans, tumor necrosis

factor-a is critically important for host control of infection. Thus, mice given monoclonal antibody to this cytokine fail to control both primary and secondary infection. Humans treated with inhibitors of tumor necrosis factor-a manifest aggressive forms of histoplasmosis. We have explored the mechanisms by which this cytokine modulates the protective immune response. The absence of tumor necrosis factor-a is associated with impaired production of nitric oxide, a gas which is essential for survival of naïve mice infected with this fungus. In addition, neutralization of tumor necrosis factor-a in mice alters the protective function of T cells, induces a population of CD4+CD25+ T cells that dampen protective immunity. We also have shown that a deficiency of this cytokine is accompanied a decrease in the apoptosis of T cells. This decrement is associated with a marked increase in fungal burden. The significance of apoptosis as a mediator of host resistance has been affirmed by demonstrating that caspase inhibitors worsen the severity of infection in mice. These findings demonstrate the complexities of the influence of tumor necrosis factor-a on the protective immune response to *H. capsulatum.*

CF 6 - MEDICAL MYCOLOGY MEETS MOLECULAR GENETICS: *HISTOPLASMA* AS A MODEL SYSTEM

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Histoplasma capsulatum is one of the classic dimorphic fungal pathogens which undergo a transition from a mycelial phase that grows in soil to a yeast phase that establishes infection in the lung. Macrophages are the primary host cells for *H. capsulatum*, and the yeasts survive and proliferate within phagolysosomes. To help identify and study factors that are involved in this intracellular parasitism, we have developed a series of molecular genetic tools for analysis of *Histoplasma* gene function: shuttle plasmids, reporter genes, and a two-step gene disruption strategy. These have allowed us to evaluate the expression and role of two yeast phase-specific genes: *CBP1*, which encodes a secreted calcium-binding protein that is essential for virulence; and *AGS1*, which encodes an enzyme responsible for synthesis of a virulence-associated cell wall polysaccharide. More recently, we have designed a telomeric plasmid-based expession vector for double stranded RNA-mediated interference (RNAi). Compared to the process of generating null mutants in *H. capsulatum*, silencing genes by RNAi is a rapid method that can be implemented with less sequence information, fewer cloning steps, and a much shorter period of genetic selection. Therefore, RNAi is well-suited for functionally testing the large number of interesting *Histoplasma* genes that will be identified in future transcriptional profiling and comparative genomics projects.

CF 7 - PARACOCCIDIOIDOMYCOSIS: FROM BASIC RESEARCH TO CLINICAL APPLICATIONS.

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Introduction: From January 2003 to July 2005, a literature search specifically oriented towards experimental studies dealing with paracoccidioidomycosis (PCM) and *Paracoccidioides brasiliensis* revealed 131 articles. This rather large number of top, basically-oriented products indicates that extremely important efforts are being done to better understand the genetic constitution of the fungus and the immunopathogenesis of both infection and disease processes. Although not overstated, such scientific productions underlines the need to improve or restore the patient's health status avoiding the ultimate ill effects of fungal invasion.

Objectives: To extract from chosen articles those sentences and conclusions used by the authors to emphasize the benefits derived from their studies concerning the understanding of the molecular and cellular events taking place during the host-fungus interaction and the possible ways to avoid cell and tissue injury. This scenario would - hopefully - permit to undertake joint studies or novel paths to arrive with basic solutions to the clinical arena**.**

Methods: Literature search, analysis of published data, selection of clue sentences and clinical relevance, visualizing applications.

Results: Twenty-five articles were chosen and their data classified according to main subjects, namely: GENES, IMMUNITY, VACCINES. Various previously unknown genes were characterized and their expression confirmed and, in certain cases, their role in form transition, in heat shock and in oxidative stress responses, as well as in metabolic function, were pinpointed. Additionally, one ribosomal protein was identified. Novel *P. brasiliensis* genes were identified with their expressed proteins considered to represent virulence factor candidates and potential new drug targets. Advances in the understanding of immune responses clarified the already known Th1 immunosuppression exhibited by some patients, which was shown to be associated with down-modulation of the IL-12 pathway and correlated, later on, in a patient with an inherited deficiency in the β1 subunit of the interleukin (IL)–12/IL-23 receptor. On the same token, genetically determined deficiency of IL-4 in experimental animals was shown to exert a protective role in pulmonary PCM. Treatment of PCM patients was shown to promote regulation of IFN-gamma, IL-4 levels and CD28, CD86 expression bringing new insights into the cellular immune responses. In the realm of treatment and vaccination, gp43-derived killing peptides obtained through biotechnological methods proved strongly effective against *P. brasiliensis* yeast cells with the advantage that the patients' mononuclear cells recognized such peptides indicating that they should have been present during infection; as such they are prospective candidates for a vaccine. Several purified and characterized antigens such as F0 and FII have also been considered reliable vaccine candidates; additionally, identification of certain encoded proteins as structural membrane segments represent new alternatives for protection.

Conclusions: Basic research offers a completely different approach to the management of patients based on the understanding of genes, of their function and expression, as well as of the cellular immune mechanisms. We should promptly profit from these advances to curtail the miseries that PCM imposes on our patients.