03 IMMUNOLOGICAL DIAGNOSIS

03.001 - PREVALENCE ANALYSIS OF SYSTEMIC AND OPPORTUNISTIC MYCOSES DIAGNOSED IN ADOLFO LUTZ INSTITUTE

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Introduction: Systemic and opportunistic mycoses are invasive diseases that can be fatal if not correctly diagnosed and treated. The former is characterized by infections acquired through inhalation, primary lesions and pulmonary manifestations caused by dimorphic fungi as Paracoccidioides brasiliensis and Histoplasma capsulatum. On the other hand, the opportunistic mycoses are caused by saprophytic fungi and have shown enhanced incidence in parallel with the increased widespread use of antibiotics and immunosuppressors drugs, long-lasting paternal medication and diseases that provoke immunodeficiencies. Methods and Results: We evaluated the prevalence of paracoccidioidomycosis (PCM) and histoplasmosis (HP) among the systemic mycoses and of aspergillosis, an opportunistic mycosis. All of them were immunodiagnosed at the Laboratorio de Imunodiagnostico das Micoses from Adolfo Lutz Institute of São Paulo. Sera samples from 6,041 patients with clinical suspicion of PCM, HP or ASP were analyzed by double immunodiffusion technique in a period from April 2001 to May 2004. Among the clinical suspicious, 67.3% corresponded to PCM, 19.4% to HP and 13.3% to ASP. 86.6% of the patients that presented confirmed PCM serology were male between 40 to 60 years old with only 13.4% female patients, 78% of the HP patients were male with 30 to 50 years old against only 22% female patients. 82% of the individuals with confirmed AP serology were male against only 18% female patients. Conclusion: Among the systemic mycoses, PCM presents the highest incidence in Brazil, mainly in some regions of São Paulo state as Campinas, Jundiaí, São José do Rio Preto and Ribeirão Preto. Beside those regions, it is noteworthy the number of positive cases in Mato Grosso and Mato Grosso do Sul states. Opportunistic mycoses, as histoplasmosis, are observed frequently associated with patients presenting cellular immunity disturbances, mainly those that developed HIV/AIDS, and in individuals with aspergillosis that present allergic process. Financial support: Instituto Adolfo Lutz (Projeto CTC-IAC, #10797).

03.002 - REACTIVITY OF ANTI-HISTOPLASMA CAPSULATUM SERUM TO FRACTIONED H. CAPSULATUM AND PARACOCCIDIIDOIDS BRESILIENSIS CELL FREE ANTIGENS

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Introduction and Objectives: Histoplasma capsulatum is the causative agent of histoplasmosis, a systemic infection of significant consequences especially among immunocompromised patients. The H. capsulatum antigens are known to cross-react with antibodies to Paracoccidioides brasiliensis. The objective of the present investigation was to analyse the reactivity of anti-H. capsulatum serum to fractioned cell antigen (CPA) prepared from H. capsulatum (IMT/HC) and Paracoccidioides brasiliensis (P. brasiliensis) cell free antigen (CFA) from H. capsulatum (IMT/HC) and P. brasiliensis (Ph 18) were submitted to gel filtration chromatography in Sephadex G75-120 column and the fractions were analyzed by dot-blotting using rabbit anti-H. capsulatum serum. Additionally CPA’s samples were analysed by Western-blotting using anti-H. capsulatum serum adsorbed and not adsorbed with P. brasiliensis. Chromatography fractions analysis demonstrated positive reaction between void volume to approximately 58 kDa molecular mass fractions with anti-H. capsulatum serum in both CPA’s samples. Also Western blotting analysis showed that most of CPA high molecular mass were recognized with anti-H. capsulatum serum and after the adsorbing process didn’t present reaction to P. brasiliensis antigen. Conclusion: With this trial was possible to conclude that high molecular weight fractions from H. capsulatum cell free antigens are more immunogenic and less specific and the anti-serum cross-reaction to P. brasiliensis can be abolished through the adsorption with this yeast cell.

03.003 - PARACOCCIDIIDOIDS BRESILIENSIS - INFECTION IN DOGS SEROPOSITIVE AND SERONEGATIVE TO LEISHMANIOSIS

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Introduction and Objectives: Leishmaniosis in dogs. Additional studies are necessary to elucidate the possible association between PCM and leishmaniosis taking into account that both pathogens are controlled by a cellular immune response. Additional studies are necessary to elucidate the possible association between PCM and leishmaniosis in dogs. Financial support: CAPES, FUNDAÇÃO ARAUCÁRIA, CNPq E FUNDAÇÃO FACULDADES LUIZ MENEGHEL.

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a systemic mycosis endemic in Latin American countries. The etiologic agent Paracoccidioides brasiliensis is a thermomorphic fungus that grows as yeast in the host or at 37 °C and as mycelia at 25 °C (Clin Micr Rev 89-117, 1993). Despite several attempts to find the P. brasiliensis habitat, until now, it is unknown although that the fungus lives in soil. Also, the role of other animal species in the fungus ecology remains unclear. Paracoccidioides brasiliensis was isolated from frugivorous bats (Sabouraudia 4:124-125, 1965), penguin [Inst Venez de Invest Cient, Abstract B-2, 1989] and armadillos (Med Mycol; 38(3): 193-199,2000). Epidemiological studies suggest that other species as cows (Antioq Med; 24: 339-358, 1974), sheeps (Sabouraudia; 16:93-101, 1978), monkeys (Vet Pathol. 1977; 14:368-371) and dogs (Med Mycol; 2001; 39: 277-282, 2001) may be infected by P. brasiliensis. The aim of this work was to evaluate the humoral immune response in bovine immunized with Paracoccidioides brasiliensis and realize a seroepidemiologic study of paracoccidioidomycosis in bovine from four microregions of Mato Grosso do Sul, Brazil. Methods and Results: Two bovine were inoculated with suspension of P. brasiliensis in Freund incomplete adjuvant. Samples of blood were collected periodically to evaluate humoral immune response by immunodiffusion and ELISA, using exoantigen and gp43 as antigens, respectively. The production of antibody was detected by immunodiffusion and ELISA, in both animals 14 days after immunization. The seroepidemiologic study was carried out in 400 bovines of Mato Grosso do Sul from 4 regions: Dourados, São Gabriel d’ Oeste, Corumbá and Nova Andradina. The reactivity to gp43 was 17.5% and municipalities of Corumbá (30%) and Nova Andradina (28%) showed higher positivity than São Gabriel d’ Oeste(4%) and Dourados (8%). Conclusion: In this study we concluded that bovine immunized with P. brasiliensis can elicit humoral immune response against gp43, remaining with high titles of antibodies and that this animal species could be an epidemiologic indicator of paracoccidioidomycosis. Financial support: CAPES, FUNDAÇÃO ARAUCÁRIA, CNPq E FUNDAÇÃO FACULDADES LUIZ MENEGHEL.

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a systemic mycosis endemic in Latin American countries. The etiologic agent Paracoccidioides brasiliensis is a thermomorphic fungus that grows as yeast in the host or at 37 °C and as mycelia at 25 °C. The individuals that develop PCM are mainly male agricultural workers. The granulomatous lesions are frequently observed in lungs, lymphnodes, spleen, liver, skin and mucosa. Probably infection occurs by fungus propagule inhalation (J. Med. Vet. Mycology, 23:323-334,1985). The epiocological aspects of PCM remains poorly understood. P. brasiliensis was isolated from frugivorous bats [Sabouraudia 4:124-125, 1965], penguin [Inst Venez de Invest Cient (IVIC), Abstract B-2, 1989] and armadillos (Med Mycol; 38(3): 193-199,2000). Recently the first case of natural PCM in dogs was reported [Medical Mycology 42: 379-383, 2004). Taking into account that endemic areas for PCM can be endemic for other diseases that affect dogs as leishmaniosis. The aim of this study was to evaluate the infection by P. brasiliensis in dogs seropositive and seronegative to leishmaniosis. Methods and Results: Sera from 836 dogs (449 positive and 387 negative to leishmaniosis) were analysed by ELISA and immunodiffusion test using gp43 and crude P. brasiliensis exoantigen respectively. The analysis of the 836 serum samples by ELISA and immunodiffusion test showed a positivity of 67.8% and 7.3%, respectively for P. brasiliensis infection. The dogs positive to leishmaniosis (n=440) showed a higher reactivity to gp43 (79.95%) and to exoantigen (93.5%) by ELISA. Four out 61 dogs seropositive by ID test were examined for clinical signs of paracoccidioidomycosis. One dog showed cough and dyspnea and the other ones showed haematological alterations. Pulmonary discrete radiological alterations were observed in two dogs. Conclusion: The higher reactivity to P. brasiliensis antigens may be due to a cross-reactivity or a co infection of dogs by Leishmania sp and P. brasiliensis. The lower correlation (0.040) observed between reactivity to gp43 and Leishmania antigen reinforce the latter hypothesis. Probably the dogs seropositives by ID were in an initial phase of PCM with very discrete signs of disease, Probably dogs at higher risk of infection by Leishmania also are more exposed to P. brasiliensis infection. Unfortunately the following of a greater number of animals was not possible taking into account that the dogs positive in leishmaniosis test were killed. The results of this study suggest that co infection of dogs by P. brasiliensis and Leishmania are occurring. The association between these diseases in dogs is very interesting taking into account that both pathogens are controlled by a cellular immune response.
Introduction and Objectives: *H. capsulatum* (He) is the ecbologic agent of histoplasmosis (HP), a mycosis that principally affects the lungs. Proven diagnostic modalities include cultures, fungal stains of tissues or body fluid, and tests for antibodies and antigens. The aim of this study was to obtain a mycelial-phase soluble antigen of *He* as well as evaluate the usefulness of this antigenic preparation in standards serological reactions. Methods and Results: Soluble antigens were obtained from the 4P, 40, 200, 212, 268, 299, 340, 361, 406, 584 802 and 2030 He isolates cultivated at 27°C Con Bauround-dextrose agar for 15 and 33 days. After incubation mycelial cells were suspended in aqueus solution of thimerosal (1:5000) at room temperature for 24 h. Antibiotics were concentrated by lyophilization procedure and stored at -20°C. The specificity and sensitivity of the different He soluble antigens were evaluated, using the double immunodiffusion (ID) assay for panel of sera from: HP patients (illness or infection); individuals with clinical suspicion of HP but non-reactive with He reference antigen by ID; paracoccidioidomycosis, aspergillosis and leishmaniasis patients; sera anti-exoantigens of He, P. brasiliensis and A. fumigatus and reference positive control serum anti-Hc (H and M fractions). Moreover, the electrophoretic profile of those antigens were analyzed by SDS-PAGE and the immunoreactivity, by immunoblotting (IB). Through ID, it was verified that the 20-fold concentrated soluble antigens presented reactivity only against serum anti-He and anti- H and M He fractions, being observed the presence of H and M bands, and also against sera from patients with HP infection or illness. The best pattern of reactivity was observed for antigens obtained with 33 days of growth, the antigens of 15 days presented better seroreactivity results. The analysis of the electrophoretic profile by SDS-PAGE disclosed great proteic complexity, presenting antigenic components of apparent molecular mass from 17 to 119 kDa. Through the IB, it was observed intense reactivity of the sera from patients with HP (infection or illness), being observed bands between of 74 to 119 kDa. It is noteworthy that the antigen from sample 200, with 15 or 33 days of culture, presented the best pattern of recognition against the homologous sera. Conclusion: The results suggest the employment of the soluble antigen from sample 200 in the ID assay due to its good capacity to discriminate both sera from patients with HP illness and HP infection, besides its high specificity (100%) against heterologous sera. Financial support: Adolfo Lutz Institute (Projetos # 107/97c # 06/04).
03.009 - PRELIMINARY STUDY IN THE IDENTIFICATION AND CHARACTERIZATION OF NEW SEQUENTIAL MARKERS TO HISTOPLASMOSIS
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Introduction and Objective: Histoplasmosis, a systemic fungal disease caused by *Histoplasma capsulatum*, is one of the most common systemic mycoses in Brazil where epidemiological surveys carried out using the histoplasmin skin test indicate that this mycosis is endemic in all surveyed areas. Definitive diagnosis of histoplasmosis is still reliant on the visualization of the organism and/or isolation of the fungus in culture; these methods are time-consuming and lacking in sensitivity. Serological tests, which consist in useful tools for the detection of either antibodies and/or antigen in clinical fluids specimens (such as serum, urine and liquor), have been developed and they offer a rapid alternative in order to diagnosis of histoplasmosis. The H and M glycoproteins constituents of the culture filtrate of *H. capsulatum* are considered pluripotent antigens that elicit both humoral and T-cell mediated immune responses, and suitable molecules to diagnosis of histoplasmosis. However, the characterization of new antigenic proteins and their heterologous production will allow a broader spectrum of molecules to be used in the diagnosis of histoplasmosis. In an attempt to contribute to the discovery of useful biomarkers for its diagnosis and therapeutic monitoring new potential antigens are studied for our group. An immunoproteomic approach was taken to separate and identify proteins from a crude extract of *H. capsulatum*. Methods and Results: In this study, the SDS-PAGE, Western blot test and the 2D electrophoresis was used to analyze this antigen. A protein profile composed by 7 bands was identified in silver stained SDS-PAGE gels with molecular masses ranging from 117 to 14.7 kDa. When these gels were analyzed by Western blot probing with human serum from mycologically confirmed histoplasmosis, positive reactions occurred with protein profiles ranging in size (120, 110, 60, 57, 44, 32 kDa). There were not reactive bands pattern in the negative control. After immunoproteomic approach (2D electrophoresis) 16 spots were observed in western blot test, 80 kDa (pI 6.2), 70 kDa (isoforms of 6.3, 6.5), 68 kDa (pI 4.8), 66 kDa (pI 7.0), 63 kDa (pI 7.1), 57 kDa (isoforms of 6.1, 6.8), 50 kDa (pI 6.5), 45 kDa (pI 5.0), 43 kDa (isoforms of 5.7, 6.8), 30 kDa (pI 6.9), 25 kDa (isoforms of 6.0, 6.2, 6.4). Conclusion: Further studies using proteomics tools must be done in order to achieve a possible relationship of such proteins as a new serological marker and its application in immunological diagnostic assay. Financial support: CNPq and FAPERJ

03.010 - IMMUNOPROTEOMIC IDENTIFICATION OF SPECIFIC ANTIGENS OF THE DIMORPHIC FUNGUS SPOROTROCHIS SCHENKII
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Introduction and Objective: Sporotrichosis is the most prevalent subcutaneous mycosis in Brazil, especially in Rio de Janeiro State, where an outbreak, related to zoonotic transmission from infected cats to human patients has been described. Although the definitive diagnosis of this infection requires the isolation of the etiologic agent in culture, serologic evidence of these fungal infection is important since the isolation of *Sporothrix schenckii* is time-consuming and lacking in sensitivity, mainly in unusual clinical manifestations of the disease. In an attempt to contribute to the discovery of useful biomarkers for its diagnosis and therapeutic monitoring, we embarked on a mapping of *S. schenckii* immunogenic proteins specifically recognized by antibodies produced during the natural course of this infection. Methods and Results: An immunoproteomic approach was taken to separate and identify proteins from an aqueous extract from yeast form. About 154 protein spots were identified in silver stained gel with molecular mass ranging from 110 kDa to 11 kDa. The most prominent protein species were those of 70 kDa (isoforms of 5.0 and 5.5), 60 kDa (pI 4.8), 50 kDa (pI 4.7), 39 kDa (isoforms of 5.3 and 5.4), 35 kDa (pI 4.6), 30 kDa (pI 4.8), 25 kDa (pI 5.0) and 13 kDa (pI 6.8). The protein species of 13 kDa (pI 6.8 and 7.1), 42 kDa (pI 6.4), 50 kDa (pI 4.7), 53kDa (pI 5.1), 70 kDa (pI 5.0 and 5.5) were reactive to sera of infected patients, but not with sera from patients with paracoccidioidomycosis, histoplasmosis or American tegumentary leishmaniasis. Conclusions: The use of proteomics can provide useful information on the antigenic make up of this fungus and the purification and characterization of these specific proteins can improve the differential diagnosis between sporotrichosis and other related diseases.

03.011 - ANTIBODY DETECTION IN SPOROTROCHISIS USING MYCELIAL PHASE SPOROTROCHIS SCHENKII EXOANTIGENS IN AN ELISA TEST
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Introduction and objective: Infections caused by *Sporothrix schenckii* have increased in recent years, especially in immunocompromised patients. *S. schenckii* can cause either limited cutaneous lesions or invasive, disseminated infections. Systemic sporotrichosis may be due to bloodstream dissemination from a cutaneous lesion or conidia inhalation. Risk factors, such as alcoholism, diabetes, use of immunosuppressive drugs and chronic granulomatous disease may predispose to severe infections, including pulmonary and osteoarticular sporotrichosis. The diagnosis of these clinical manifestations is often difficult and the therapeutic follow-up of patients is usually made by clinical findings, without any knowledge about their immunologic responses against the fungus. In order to solve these questions, we have developed an immunoassay for the serodiagnosis of sporotrichosis. Methods and results: An enzyme-linked immunosorbent assay was developed as a method for specific antibody detection in serum specimens of sporotrichosis patients. The assay was made with mycelial phase *Sporothrix schenckii* exoantigens and tested against 90 sera from patients with several clinical forms of sporotrichosis. Cross reactivity was analysed with 72 heterologous sera from patients with paracoccidioidomycosis, cryptococcosis, aspergillosis, histoplasmosis, tuberculosis and American tegumentary leishmaniasis, as well as 76 sera from healthy controls. Sensitivity of 97% and specificity of 89% were observed in this assay. These parameters were higher than previously published data relating the use of ELISA in serodiagnosis of sporotrichosis. Also, we have observed that all cutaneous forms of this disease responded well in the described ELISA, indicating the useful of this assay in the serodiagnosis of sporotrichosis, especially in cutaneous forms that are not promptly diagnosed with immunoprecipitation or agglutination techniques described up to now. Conclusion: These results suggest that the ELISA using mycelial phase *S. schenckii* exoantigens is a very sensitive diagnostic tool that could be applied to the serodiagnosis of sporotrichosis.