02 EXPERIMENTAL MODELS

02.001 - THE PROTECTIVE IMMUNITY TO *PARACOCCIDIOIDES BRASILIENSIS* ELICITED BY A TH2-INDUCING ANTIGENS FORMULATED IN MPL ADJUVANT

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Introduction and Objectives: The paracoccidioidomycosis (PCM) is characterized by a chronic inflammatory granulomatous reaction. Experimental and clinical evidences indicate that cellular, rather than humoral immunity, is the effective mechanism that controls the pathogenesis and the evolution of PCM. One of the main question regard this infection is related to the selection of antigens that could induce protection in susceptible hosts. In this work, our aim was to evaluate the potential of the use of monophosphoryl lipid A (MPL-SE) as adjuvant mixed with surface antigens from *P. brasiliensis* (sPbAg), in order to develop an effective immune response against the infection with the pathogenic fungus. **Methods and Results:** C57BL/6 and MyD88KO mice were immunized three times by subcutaneous route (three weeks apart) with sPbAg mixed with MPL-SE (according to Corixa Corporation protocol). Yeast cells (Pb18) were shook vigorously (in PBS) for 30s in vortex and the supernatant (sPbAg) was used in the assays. Control mice were injected with MPL-SE with PBS or with sPbAg only. Two weeks after the last boost, the animals were challenged (iv) with viable yeast forms of *P. brasiliensis*. The results showed absence of lesions in lungs, liver and spleen of mice immunized with sPbAg plus MPL-SE. Moreover, the colony-forming units (CFUs) was almost absent in the organs. The protection is supposed to be mediated by IFN-γ, given that this cytokine was detected in homogenates of lungs, liver and spleen. Moreover, the levels of specific antifungal IgG2a antibodies in the sera and the DTH reaction were significantly higher when compared to the animals immunized with MPL-SE, sPbAg, or only infected. The mechanism of protection is dependent of the cell activation through Toll-like receptors. Indeed, when MyD88KO mice were immunized with MPL-SE plus sPbAg or MPL-SE plus PBS the animals were not able to control fungal growth and dissemination. Surprisingly, the inoculation of sPbAg prior to infection resulted in a severe granulomatous lesions and fungal dissemination to the all organs analyzed. Clearly, increased fungal growth after sPbAg inoculation was dependent of IL-4, since high level of this cytokine was found in the homogenates of lungs, spleen and liver of mice injected with sPbAg, but not in the other groups of mice (only infected, injected with MPL-SE, or with MPL-SE plus sPbAg). Moreover, the injection of sPbAg did not affect the course of infection in mice deficient of IL-4, suggesting that the exacerbation of PCM after inoculation of sPbAg is mediated by IL-4. **Conclusion:** Our study indicates that the formulation of Th2-inducing antigens from *P. brasiliensis* with MPL-SE, a naturally derived disaccharide adjuvant of *Salmonella minnesota*, is able to elicit a protective immunity against *P. brasiliensis* infection. The protection of immunized mice is mediated by a Th1-type immune response, whose induction is dependent of Toll-like receptor activation. **Financial support:** CNPq, FAPESP, FAEPA

02.002 - PHAGOCYTOSIS, EXPRESSION OF CO-STIMULATORIES MOLECULES AND CYTOKINES PRODUCTION OF PULMONARY DENDRITIC CELLS AFTER INTRATRACHEAL INFECTION WITH *PARACOCCIDIOIDES BRASILIENSIS*

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Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis, caused by the *Paracoccidioides brasiliensis* (Pb), that it commits the lung preferentially. Dendritic cells are called professional antigen presenters, capable to interact the innate and adaptive immune systems. Seen the importance of these cells in the immune system and knowing that the infection for Pb attacks the lung primarily, we evaluated the main biological functions of these cells, as the phagocytosis, expression of co-stimulatories molecules and cytokines production in susceptible (B10.A) and resistant mice (A/J) to PCM. **Methods**: Pulmonary dendritic cells (CDp) were positively selected by anti-CD11c microbeads from collagenase-digested lung (1). The levels of cytokines secretion were determined by ELISA and co-stimulatories molecules were analyzed by flow cytometer. **Results**: We observed that CDp are capable to interact with the fungus, and these cells phagocytized yeasts of Pb *in vivo*. After this interaction, we observed alterations in the expression of co-stimulatories molecules. In purified CDp from A/J mice, a gradual increase of the CD80, CD86 and CD54 expression was observed. However, a decrease of the expression of the same molecules was observed in CDp obtained from susceptible mice. The expression of the MHC-II molecule was similar in the cells obtained of both lineages of mice. Analyzing the cytokines secretion, we observed an increase in the secretion of IL-10, IL-6 and TNF-α in CDp from B10.A. However, we didn't observed alterations in the production of IL-12. **Discussion**: The results suggest that the infection with Pb alters the expression of the main molecules co-stimulatories and cytokines production in CDp. These changes can affect the activation of T cells, mainly in susceptible animals, that could be one of the mechanisms for which the fungus induces a larger chronicity in those animals. **Reference**: (1) Gonzales-Juarrero, M., and I.M. Orme. *Infect. Immun*. 69:1127, 2001. **Financial support:** FAPESP (nº 03/12816-3) and CNPq.

02.003 - INOS KNOCK-OUT MICE ARE NOT MORE SUSCEPTIBLE TO PULMONARY PARACOCCIDIOIDOMYCOSIS (PCM) INFECTION THAN THEIR NORMAL COUNTERPARTS

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Introduction and Objective: Inducible nitric oxide synthase (iNOS) is one key enzyme generating nitric oxide (NO) from L-arginine. iNOS derived NO is a mediator of unspecific host defense and central in the clearance of microbial and parasitic infections. The aim of this work was to investigate the role of NO in the acute and chronic phases of pulmonary PCM. **Methods:** Wild Type (WT) (n=5-8) and iNOS KO C57BL/6 mice (n=5-8) were i.t. infected with $1x10^6$ yeast cells of *P. brasiliensis* (Pb). Mice were sacrificed 48h, 2 and 10 weeks after infection and the severity of the disease determined by organ CFU counts and pulmonary histopathology at week 10. Cytokines and antibodies levels were determinated at several periods of infection. **Results:** At 48h post infection both mouse strains showed similar fungal loads in the lungs and cytokines levels in this organ. On the other hand, at week 2 of infection KO mice presented decreased fungal loads in the lungs (KO 4.55±0.86 $\log_{10}$; control 5.23±0.29 $\log_{10}$) concomitant with high levels of pulmonary TNF-α (KO 5976.7±1954.3 pg/mL; control 1656.2±445.81 pg/mL) and high amounts of hepatic cytokines (TNF-α, IFN-γ, IL-2 and IL-4, $p<0.05$). Interestingly, compared with WT controls, at week 10 iNOS KO mice presented increased fungal burdens in the lungs (KO 5.99±0.79 $\log_{10}$; control 4.64±0.8 $\log_{10}$). In addition, at this period these mice produced significantly increased levels of IgE, IgG1, IgG2a, IgG2b and IgA antibodies ($p<0.01$) and high levels of IFN-γ, IL-4 and IL-5 in liver supernatants ($p<0.05$). iNOS KO mice presented inflammatory reaction composed by well-organized granulomas containing epithelioid cells and multinuclear giant cells, surrounding aggregated yeast cells, whereas WT lesions were less organized (diffuse) and affect most of pulmonary parenchyma. The more organized lesions of KO mice appear to compensate the higher fungal loads resulting in equivalent mortality rates of both mouse strains. **Conclusions:** iNOS deficiency regulates fungal loads and severity of pulmonary lesions leading to equivalent disease outcomes in WT and iNOS KO mice. **Financial support:** FAPESP

02.004 - COMPARED WITH SUSCEPTIBLE MICE, ALVEOLAR MACROPHAGES FROM RESISTANT MICE TO *P.BRASILIENSIS* INFECTION HAVE LOW FUNGICIDAL ABILITY THAT CAN BE REVERTED BY ANTI-TGF-α TREATMENT

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Introduction and Objectives: Previous studies in our laboratory defined susceptible (B10.A) and resistant (A/J) mice to pulmonary *P.brasiliensis* infection. To further understand this experimental model of PCM, the secretory and fungicidal ability of alveolar (AM) macrophages were investigated. **Methods and Results:** Normal B10.A and A/J mice (n=10-15) were submitted to bronchoalveolar lavage (BAL) and cell suspensions ($2x10^5$cells/well) were pre-activated overnight with IFN-γ, IL-12 or the combination of these two cytokines (50,000, 10,000 and 2,000 pg/mL) and *in vitro* challenged with $4x10^3$/mL *P.brasiliensis* (1:50 fungus:AM ratio). 72h after infection fungicidal activity was assessed by CFU counts and levels of nitric oxide (NO) and cytokines were determined in culture supernatants. Data are expressed as means ± SE and analyzed by Student's t test. Our results showed that pre-activation of B10.A AM with 2,000, 10,000 and 50,000 pg/mL of IFN-γ led to reduced number of recovered fungi (r.f.) (258±121; 140±0.2 and 147±2.5 r.f., respectively). With IL-12 and both cytokines equivalent results were obtained. Only the higher IFN-γ concentration was able to activate A/J AM (50,000pg/mL: 105±13 r.f.). At all conditions assayed, B10.A AM secreted higher levels of NO (10,000pg/mL: 26±1.2 and 50,000: 46±0.2mM) than A/J cells (5.2±0.4 and 15±0.2mM). The enhanced fungicidal ability of B10.A AM was associated with secretion of low levels of IL-10 (<100pg/mL) while AM from resistant mice produced high amounts of this cytokine (2,000±18pg/mL). For both mouse strains, the addition of anti-IL-10 mAb (20μg/mL) did not change the fungicidal ability of IFN-γ-activated or non-activated AM. However, in B10.A cocultures IL-10 neutralization led to increased production of NO and TNF-α. Anti-TGF-β treatment (20μg/mL) induced increased fungicidal ability of A/J cells associated with higher levels of NO and TNF-α, and diminished amounts of IL-10. The same treatment did not alter the already high fungicidal ability of B10.A AM but increased the production of the NO, IL-12 and TNF-α. **Conclusions:** AM from susceptible mice are easily activated by IFN-γ and IL-12; present enhanced ability to kill *P.brasiliensis* yeasts and secrete high levels of NO. In contrast, A/J AM are poorly activated by IFN-γ and IL-12, secrete low amounts of NO but their fungicidal ability can be recovered by neutralization of endogenous TGF-β but not IL-10. **Financial support:** FAPESP

02.005 - LEUKOTRIENES IN THE PROTECTION INDUCED BY CFAGS IMMUNIZATION AGAINST *HISTOPLASMA CAPSULATUM*

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Introduction. *Histoplasma capsulatum* is a dimorphic fungus that causes a wide spectrum of disease when mycelial fragments are inhaled. Resistance to *H. capsulatum* is dependent on a cellular immunity mediated by T cells and macrophages and pulmonary histoplasmosis may be leukotriene-modulated. **Objective.** The role of leukotrienes (LTs) in protection induced by cell-free antigens (CFAgs) against *H. capsulatum* was investigated. **Results.** Recently, we standardized the production of a CFAgs of *H. capsulatum* and demonstrated their ability in inducing *in vitro* spleen cells to produce high amounts of IFN-γ and protecting mice against i.v. and i.t. lethal inoculi of *H. capsulatum* (Microbes Infect. v.7, 584, 2005). We also demonstrated the role played by LTs in pulmonary histoplasmosis showing their effect in increased survival, clearance of microorganisms and production of essential cytokines to control the disease (Infect. Immun. 72: 1637-44, 2004). Here, we demonstrated the effects of the LTs synthesis inhibitor MK886, during the immunization with CFAgs on mice infected with *H. capsulatum*. Mice were challenged by i.t. route with a lethal inoculum of *H. capsulatum* yeasts. Most of *H. capsulatum*-infected mice immunized with CFAgs survived (72%) whereas 100% of mice immunized with BSA or CFAgs and treated with MK 886 succumbed to infection until 18 days post infection. Immunization with CFAgs decreased CFU recovery from lungs of infected mice in ~1 $\log_{10}$ ($P < 0.01$) at 14 days post infection in comparison with both groups of infected mice treated with MK 886 (BSA- and CFAgs-immunized). Moreover, CFAgs immunization prevented systemic fungal dissemination, since no CFU were detected in spleen from this group at 7 and 14 days post infection. In contrast, BSA- and CFAgs-immunized mice treated with MK 886 showed more than 2 $\log_{10}$ CFU ($P < 0.01$) per gram of spleen at 14 days post infection. **Conclusions.** These results demonstrate that CFAgs is a potential target for the development of a vaccine against histoplasmosis and that LTs play an intrinsic essential role in the protection induced by CFAgs immunization against *H. capsulatum.* **Financial support:** FAPESP and CNPq.

02.006 - PHAGOCYTOSIS OF *PARACOCCIDIOIDES BRASILIENSIS* BY MACROPHAGES: SIGNIFICATIVE DIFFERENCES IN PHAGOCYTOSIS OF YEASTS AND PSEUDOHYPHAE

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Introduction and Aims: *P. brasiliensis* is a thermodimorfic fungus that grows in a mycelial phase at room temperature and as yeast at 35-37ºC. However, we observed the emergence of a spontaneous mutant of virulent Pb 18 strain that always show up in the pseudohypha form. Considering that macrophages are involved in the host defense against fungal infection and can prevent tissue colonization by *P. brasiliensis* through phagocytosis we evaluated the phagocytosis of yeasts and pseudohyphae in control and infected mice. **Material, methods and results:** Balb/c male mice were i.p. inoculated with yeasts, pseudohyphae of *P. brasiliensis* or PBS. Five days p.i., peritoneal macrophages were collected, cultivated on 13mm round cover slips in RPMI medium (2.10^5 cells/well) for three days and incubated with live pseudohyphae or yeasts (8.10^5 *P. brasiliensis*/well) for six hours. Three samples were fixed and stained with hematoxylin–eosin. To determinate the viability of phagocyted *P. brasliensis*, free cells were removed and macrophages lysed. Cellular suspension was harvested and aliquots of 100 µL were plated in agar plates (BHI + 4% horse serum + 5% *P. brasiliensis* 192 culture filtrate). Colonies per plate were counted after 6-8 days of incubation at 37 °C. We observed larger phagocytic percentage in macrophages challenged with pseudohifas than challenged with yeasts in all control, yeast- or pseudohypha-infected mice (91.2, 86.9 and 85,8 x 70.8, 52.4, 55.1). The phagocytic index of pseudohyphae was also higher than yeasts (2.3, 2.0 and 1.9 x 1.3, 1.0 and 0.9). Of note, control animals presented better phagocytic index than infected. **Conclusions:** suggesting inhibition of phagocytosis by the fungal infection.

02.007 - EFFECTS OF HENE LASER ON EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS LESIONS

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Introduction and Aims. Paracocciodiodomycosis is a systemic mycosis caused by *Paracoccidioides brasiliensis* that induces deep lesions in lungs and skin. Skin lesions are very painful and difficult to be cured. Low level laser therapy has been studied since the 70's and many studies have shown positive effects of skin regeneration in wound herpes and interdigital mycosis besides stimulative effects on collagen, TNF and IFN-γ syntesis. In this study we investigated the effect of laser HeNe on lesions induced by *Paracoccidioides brasiliensis*. **Methods and Results.** Balb/c male mice were infected with 5.10^6 *P. brasiliensis* cells in the footpad and seven days p.i. (pos-infection) were treated with 3 or 5 doses of laser HeNe (»=632.8 nm W= 5Mw. Ø= 4mm). Control mice were inoculated with the same concentration of fungus but they were not treated with laser. After treatment the animals were sacrificed and the footpad containing wounds was collected, fixed, cut and processed to histological analysis or immunohistochemistry to TNF and IFN-gamma. Histopatological analysis shown better progress of healing in treated lesions that shown small granulomas and lesser inflammatory exudate. The footpad in treated animals had normal skin and short lump while in non-treated animals had large edema and crostose ulceration. The immunohistochemistry showed an enhancement in TNF production in treated wounds. The CFUs assay showed that non-treated lesions had more viable fungi. **Conclusions.** We concluded that HeNe laser is capable of inducing TNF production. These results suggest that laser can speed up the lesions resolution and influence local cytokine production, collaborating for fungal clearance and tissue regeneration. **Financial support:** FAPESP

02.008 - EVALUATION OF CELLULAR IMMUNE RESPONSE IN RESISTANT AND SUSCEPTIBLE MICE TO *P. BRASILIENSIS* DURING INFECTION EMPLOYING DIFFERENT ANTIGENS

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Introduction and Objectives: Cell-mediated immunity (CMI) has been ascribed as the most important host defense against *P. brasiliensis* (Pb). Delayed-type hypersensitivity (DTH) response measured by footpad swelling is used to evaluate CMI. Studies examining CMI have been performed using different antigens, yet the lack of a suitable antigen hinders the interpretation of some test results. In the present investigation was standardized the best concentration for two antigens in eliciting DTH responses in a subcutaneous (sc) murine model of PCM, because it is an efficient model to evaluated the CMI. Afterwards was used the best concentration of antigens to evaluate CMI in resistant (A/SN) and susceptible (B10.A) mice after ip infection with Pb. **Methods and Results:** Standardization of Fava Netto antigen (FNAg) and cell free antigen (CFA) concentrations was performed by footpad test at 24h in normal or sc infected B10.A mice at 15 days post infection. FNAg and CFA antigens were obtained from 4 pooled of Pb isolates and the same protein concentration was used to obtain the pool of antigens. The results showed that sc infected B10.A mice injected with FNAg (150, 250 or 350 µg/mL) presented the highest DTH response at 150 µg/mL and CFA (150, 250, 350, 450 or 550 µg/mL) elicited enhanced DTH response at 450 and 550 µg/mL when compared to normal groups. The evaluation of CMI during infection was done at 24h in normal or intraperitoneal (ip) infected A/SN and B10.A mice in different times of infection (1, 2, 4, 8, 12 and 16 weeks) injected with the best concentrations of FNAg and CFA antigens. The results showed that FNAg (150 µg/mL) induced high DTH responses in A/SN mice at 4, 8 and 16 weeks and in B10.A mice only at 2 weeks of ip infection in comparison to controls. CFA (500 µg/mL) always elicited low DTH response in ip infected A/SN mice and a suitable DTH response only at 2 weeks in infected B10.A mice when compared with controls groups. **Conclusion:** The FNAg induced lower DTH response than CFA in sc infected B10.A mice in conditions of standardization. In contrast, the FNAg induced the higher DTH responses in ip infected A/SN and B10.A mice when compared to CFA. Also, ip infected A/SN mice injected with FNAg presented increased DTH responses in comparison to ip infected B10.A mice. The CFA induced low DTH response in ip infected A/SN and B10.A during infection and a suitable difference of DTH response was observed between 2 infected strains of mice. In conclusion the FNAg induced better DTH response than CFA antigen during infection, than was considered a good candidate to be used, in the future, for screening intradermal tests in patients with PCM. **Financial support:** Adolfo Lutz Institute, CAPES

02.009 - INVOLVEMENT OF INTERFERON-γ AND OSTEOPONTIN IN THE GRANULOMATOUS RESPONSE DEVELOPED IN EXPERIMENTAL INFECTION WITH *PARACOCCIDIOIDES BRASILIENSIS*

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Introduction and Objectives: *P. brasiliensis* (Pb) granulomas are characterized by expression of different immune and non-immune cells, cytokines and extracellular matrix components (ECM). IFN-γ has been correlated to the host defence and development of Th1 response in Pb infection. Osteopontin (OPN), a secreted glycoprotein has been described as a cytokine implicated in cell-mediated response and in granulomas. Here, we studied *in situ* expression of IFN-γ and OPN on omentum granulomas developed in a murine model of ip. Pb infection. **Methods and Results:** immunohistochemistry was used to analyze IFN-γ and OPN expression in susceptible (B10.A) and resistant (A/J) mice infected with the highly (Pb18) and slightly (Pb265) virulent Pb strains. IFN-γ expression was detected, mainly on lymphocytes at the periphery of granulomas in both Pb18-infected mouse strains. Semiquantitative analysis (n= 3/group, mean ± SEM) showed an increase in IFN-γ positive (+) cells in B10.A at 120 days postinfection (DPI) (691±50 cells/100 mm^2) compared to 15 DPI (351±83), while in A/J, 4-fold increase of (+) cells was observed at 120 DPI (1793±212) and significantly higher than observed at 15 DPI (404±150). Significant differences were found between infected B10.A and A/J at 120 DPI. In Pb265 infection, the same pattern of IFN-γ localization was found, but there was a decreased staining at 120 DPI due to presence of only residual lesions in B10.A (447±99 *vs*. 25±13) and A/J (237±68 *vs*. 36±7). Higher IFN-γ expression was found in Pb18 infection compared to Pb265 infection at 120 DPI. At 15 DPI with Pb18, OPN was strongly (+) in macrophages and multinucleated giant cells localized mainly in the center of the lesions, but weak expression was seen in the ECM. On the other hand, high OPN (+) ECM was detected at 120 DPI, as well as an increased expression in macrophages and giant cells circumscribing Pb yeasts, mainly localized in the center of granulomas foci in both mouse strains and around necrotic areas in A/J. Semiquantitative data showed a significantly higher degree of OPN (+) cells in B10.A (5.42±0.08) than in A/J (2.42±0.08) at 15 DPI with Pb18, and a significant increase of (+) cells of A/J at 120 DPI (5.00±0.66). Intensity of OPN (+) cells was also evaluated and was significantly lower at 15 DPI (3.25±0.52) than at 120 DPI in A/J (8.58±0.51). On 120 DPI, higher degree of OPN (+) ECM was detected in B10.A (1.08±0.22) and A/J (1.58±0.17), in comparison to 15th DPI. Significant higher intensity of OPN (+) ECM was shown at 120th than at 15th DPI in B10.A (1.00±0.25 vs. 0.25±0) and in A/J (1.75±0.25). Lower staining degree and intensity of OPN (+) cells were observed in Pb265 than in Pb18-infected mice. **Conclusions:** expression of IFN-γ on lymphocytes and of OPN on macrophages and multinucleated giant cells, as well as on ECM in the granulomas, particularly detected in resistant mice, may have an important participation in the tissue response developed during Pb infection. **Financial support:** CNPq and CAPES

02.010 - EFFECT OF CHLOROQUINE ON THE MURINE EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS: MACROPHAGE ACTIVATION, CYTOKINES PRODUCTION AND TRANSFERRIN RECEPTOR EXPRESSION

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Introduction and Objectives: Cellular iron metabolism is of critical importance to the growth of several intracellular pathogens, including *P. brasiliensis* (Pb). Chloroquine has been shown to raise endocytic and lysosomal pH of eukaryotic cells thereby interfering with normal iron metabolism in a variety of cell types. The objectives of this work were to study the effect of chloroquine on the evolution of experimental paracoccidioidomycosis by evaluating the organs viable fungi recovery, macrophage activation, cytokines production and transferrin receptor expression. **Methods and Results:** BALB/c male mice infected by i.v. route, with 10^6 yeasts of Pb 18 and daily treated with Chloroquine (40 and 80 mg/Kg) were sacrificed at 2, 4 and 8 weeks after infection, and evaluated by fungi recovery from lung, liver and spleen. Moreover, peritoneal macrophages were evaluated by H_2O_2 and NO production, TNF-α, IL-6 and IL-10 levels by ELISA and transferrin receptor expression by flow citometry. In all periods Chlor 40 and 80 treated groups showed significant reduction of viable fungi recovery from lung, liver and spleen (more than 50%). At 2nd week the endogenous and PMA stimulated H_2O_2 production was higher in Chlor 40 group (mean±sem 4,35±0,3nmol; 5,54±0,12, respectively) and Chlor 80 group (4,84±0,17; 4,8±0,11) when compared with only infected group (3±0,16; 4,65±0,2). At 4th week the endogenous H_2O_2 levels were higher in Chlor 80 (5,2±0,9) when compared with only infected group (3,54±0,3). At 2nd week, in Chlor 40 or 80 groups, endogenous NO levels were lower (7,93±3 mmol; 1,48±0,02) when compared with only infected group (31,8±0,7). NO levels released by LPS stimulated cells were similar in all groups. At 4th week, endogenous NO levels of infected and Chlor 40 groups decreased in relation to the first period (16,2±1,6; 1,9±0,2 respectively). At 8th week cells of all groups released similar endogenous NO levels in relation to the anterior period. However, LPS stimulated NO levels decreased in Chlor 40 and Chlor 80 groups at 4th and 8th weeks.The transferrin receptor expression was higher in Chlor 40 and Chlor 80 groups in all periods compared to the infected ones. The endogenous and LPS stimulated TNF-α levels of infected groups were low at the 2nd week (42±6 pg/mL, 113,4±2), with increase at 4th and 8th weeks (265±7, 470±11; 303±1,9, 543±23). However, Chlor 40 and Chlor 80 TNF-α levels were always lower than infected groups. Chlor 40 and Chlor 80 IL-6 levels at 2th (97,5±3; 70,9±3), 4th (195±10; 132±9) and 8th weeks (264±95; 176±38) were always lower than infected ones (1613±13; 1920±223; 1227±36 respectively). IL-10 levels were similar in all groups and periods. **Conclusions:** The results showed an important Chloroquine immunomodulatory role on the murine experimental paracoccidioidomycosis suggesting new insights for the mycosis therapy. **Financial support:** FAPESP.

02.011 - THE ROLE OF LEUKOTRIENES IN PULMONARY PARACOCCIDIOIDOMYCOSIS (PCM) AND IN THE FUNGICIDAL AND SECRETORY ABILITY OF PERITONEAL MACROPHAGES INFECTED BY *PARACOCCIDIOIDES BRASILIENSIS*

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Introduction: Paracoccidioidomycosis (PCM) is a chronic sistemic infectious disease caused by *Paracoccidioides brasiliensis* (Pb). Leukotrienes (LT) are important cellular activators and chemotactic factors but little is known about its function in host defenses against infectious agents. **Objectives:** The purpose of the present work was to investigate the role of LT in murine pulmonary PCM of resistant (A/J) and susceptible (B10.A) mice and in the fungicidal and secretory ability of their macrophages after Pb infection. **Results:** First, our results showed that in vivo and in vitro Pb infection induces LT synthesis. Compared with A/Sn mice, levels of pulmonary LT was higher in B10.A animals and increases in the course of infection. To evaluate the importance of LT in PCM, an inhibitor of LT synthesis (MK-0591) and an antagonist of LT receptor (montelukast) were studied after *in vitro* and *in vivo* Pb infection. In vitro, MK-0591 significantly reduced the recovery of Pb yeasts from IFN-γ activated macrophages although *in vivo* MK-0591 treatment did not alter the severity of pulmonary PCM. At 48 h of infection, montelukast treatment of B10.A mice induced impaired fungal loads, diminished influx of PMN leukocytes and increased number of monocytes in the lungs of Pb infected mice. The nitric oxide secretion by lungs of montelukast-treated and untreated B10.A mice was equivalent and proportional to the fungal inoculum. Furthermore, in susceptible mice montelukast treatment led to increased pulmonary IL-10 levels concomitant with diminished amounts of IL-12, TNF-α, and GM-CSF. In contrast, LT inhibition did not alter the fungal loads of B10.A and A/Sn mice at week 8 after infection. **Conclusion:** our results showed that LT are important mediators of the acute inflammatory reaction induced by Pb infection affecting fungal recovery, cellular influx and cytokines synthesis by Pb susceptible mice. In addition, LT affects Pb-macrophages interactions clearly demonstrating that LT are important modulators of murine PCM. **Financial support:** Fapesp, CNPq

02.012 - CYTOKINES PRODUCTION IN LUNGS AND ADRENAL GLANDS OF HIGH AND LOW ANTIBODY PRODUCERS MICE INFECTED WITH *PARACOCCIDIOIDES BRASILIENSIS.*

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Introduction and Objectives: Mice genetically selected for high (H) and low (L) antibody production (Selection IV-A) were used as an experimental model of paracoccidioidomycosis. In previous work of our laboratory, it was observed that the male H_{IV-A} mice were more susceptible to the paracoccidioidomycosis due to adrenal gland damage. The study of cytokines secretion pattern in the compartiments frequently reached by the fungi, such as the lung and adrenal, is necessary for a better understanding of the immunological mechanisms involved in *P. brasiliensis* infection. **Methods and Results:** Male animals H_{IV-A} and L_{IV-A} (Selection IV-A) were infected with *P. brasiliensis*, strain 18, by endovenous route, and sacrificed 2, 4, 6, 8 and 10 weeks after infection. In each period, the lungs and adrenals were removed for determination of the infection degree through the viable fungal recovery and determination of Th1 (IFN-γ, TNF-α) and Th2 cytokines profile (IL-4, IL-10) by capture ELISA. H_{IV-A} animals showed a higher infection degree in analyzed organs. Viable fungal recovery in lungs was higher after 4 and 8 weeks, and there was a lower fungal recovery in the adrenal gland of L_{IV-A} animals after the 2nd week, and fungal total elimination after the 8th week. With regards to the Th2 cytokines determination, there was an inhibition in IL-4 and IL-10 production in the organs from infected animals when compared to control, what varied according to the organ and period analyzed. Interesting data were obtained with the Th1 cytokines determination: IFN-γ production increased in both organs, mainly in the adrenal gland of L_{IV-A} strain after 8 and 10 weeks, when these animals showed a fungal total elimination. It was observed a significant difference between H_{IV-A} and L_{IV-A} strains concerning TNF-α production in the organs and all period of time, the latter strain producing a higher level of this cytokine, mainly in adrenal. **Conclusion:**These results indicate the high susceptibility of H_{IV-A} animals to *P. brasiliensis* infection, mainly the adrenal involvement. The higher production of Th1 profile cytokines by L_{IV-A} strain may be associated with these animals resistance to the fungi, showed in previous works.

02.013 - CELL WALL PROTEIN ANALYSIS AND VIRULENCE OF *Paracoccidioides brasiliensis* SPONTANEOUS MUTANT

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Introduction and Aims: *P. brasiliensis* is a dimorphic fungus that grows in a mycelial phase at room temperature and in a yeast form at 35-37ºC. In our laboratory, it was isolated a spontaneous mutant of the virulent Pb18 strain that always grows up in the pseudohypha form. As literature has shown that Pb18 strain is capable to induce illness in experimental models the objective of this work was to characterize this atypical isolate in terms of cell wall proteins and dynamics of the infection. **Methods and Results:** Two isolates of *P. brasiliensis* were used for this study: yeast cells of virulent Pb18 strain and its spontaneous mutant. To analyze the cell wall proteins, CFA (cell-free antigens) obtained from both isolates were submmited to eletrophoretic separation in 12% discontinuous SDS-PAGE and visualized by silver staining. Moreover, to compare the dynamics of the infection, BALB/c male mice were infected intraperitonially with $5x10^6$ pseudohyphae or yeast forms of the fungus and sacrificed in 1, 3, 5, 7, 14 and 28 days of infection. At sacrifice, mice were weighed and thymuses, livers and spleens were collected, weighted, fixed in 10% buffered formalin for 24 hours, routinely processed, and embedded in parafin. Sections (4mm) were stained with hematoxylin and eosin. In order to represent the weight, the organ index was calculated as: organ weight (in grams)/body weight (in grams) x 100. The results presented a marked difference among the yeast and pseudohyphae form. The SDS-PAGE shows qualitatively that only wild strain expressed proteins with molecular weight about of 39, 50, 70, 90 kDa. Otherwise, a quantitative comparison indicated that wild strain expressed the higher concentration of gp 43. In the pseudohyphae infection thymic atrophy was not observed and spleen and liver hypertrophy as noted only in the yeast infection. Moreover, the histopathological analysis of thymus showed a "starry-sky" pattern, loss of corticomedullary delimitation and presence of juxtacapsular inflammatory infiltrate only in the yeast-infected animals. **Conclusions:** These results suggest that the protein composition and form could be crucial in determining the *P. brasiliensis* virulence. **Financial support:** FAPESP (#01/10551-7; #03/01891-4)

02.014 - COMPARATIVE EVALUATION OF LYMPHOCYTE SUBPOPULATIONS FROM MICE INFECTED WITH PSEUDOHYPHAE AND YEAST FORMS OF *PARACOCCIDIOIDES BRASILIENSIS*

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Introduction and Aims: *P. brasiliensis* infection causes a deep mycosis that runs in parallel with alterations in the immune response. By studying the effects of *P. brasiliensis* infection in the thymus our research group observed atrophy and loss of architecture. In addition, in our laboratory, it was isolated a spontaneous mutant of the virulent Pb18 strain that always grows up in the pseudohyphae form and is responsible for a mild illness. In this work, we evaluated alterations in thymic and splenic subpopulations of T cells from pseudohyphae and yeast infected mice during acute phase of experimental infection. **Methods and Results:** BALB/c male mice were infected intraperitonially with $5x10^6$ pseudohyphae or yeast forms of the fungus. After 1, 3, 5, 7, 14 and 28 days of infection, thymic and splenic T lymphocytes were phenotypically evaluated by flow cytometry. Yeast infected mice presented impairment in thymic $CD4^+CD8^+$ [72,9% (control) to 55,6% (1st day) and 39,8% (3rd day)] associated to an increase in $CD4^-CD8^-$ population [4,7% (control) to 9,8% (1st day) and 8,7% (3rd day)], CD4+ [14,2% (control) to 34,7% (3rd day)] and CD8+ [8,3% (control) to 16,9% (3rd day) and 17,1% (7th day)]. Besides, CD8+ splenic T cells of these mice showed a slight reduction [13,9% (control) to 9,5% (14th day)]. On the other hand, pseudohyphae infected mice did not present any alterations in thymic and splenic T subpopulations. **Conclusions:** Our results show that *P. brasiliensis* infection induces severe alterations in thymic and splenic subpopulations of T lymphocytes only when the isolate Pb18 is used. These changes in cellular dynamics, most probably, will affect the immune response to the fungus. **Financial support:** FAPESP (#01/10551-7; #03/01891-4)

02.015 - ACTIVATION OF TH1/TH2 RESPONSE BY B-1 LYMPHOCYTES IN THE EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

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Introdution: The glycoprotein gp43 is the major antigenic protein secreted by *Paracoccidioides brasiliensis,* the ethiological agent of Paracoccidioidomycosis (PCM). In addition, gp43 is the main PCM diagnostic antigen, being recognized by all sera from infected patients. The outcoming of the disease will depend in several factors, especially on the cellular immunity response of the host. The antigen presenting cells (APCs) are one of the responsables for T cells activation, proliferation and cytokines production, which determinate whether Th1 or Th2 subsets will develop preferentially. There are at least three B cell subsets, B-1a, B-1b and B-2, distinguished by diferents phenotype characteristics. B-1 lymphocyte is considered an APC because of its capacity to capture antigenic particules by a IgM receptor and, in the end, present them with a class II major histocompatibility complex (MHC) molecule. **Objectives:** We evalueted the predominant pattern of response (Th1 or Th2) and the activation of "naive"T lymphocytes *in vivo* by B-1 cells in the presence or absence of gp43. **Methods:** Adherent cells from peritoneal cavity of mice, were cultured for 7 days to obtain B-1 lymphocytes. The levels of cytokines secretion were quantified by capture-ELISA and co-stimulatories molecules were analyzed by flow cytometer. **Results:** It was demonstrated a significantly increase of $TCD4^+$ proliferation when this cells were cultured in the presence of B-1 cells and gp43, suggesting that the antigen was presented by B-1 cells to $TCD4^+$ lymphocytes previoslly sensibilized by gp43, besides the down regulation of co-stimulatories molecules expression. Indeed, induced a secretion of cytokines, such as, IL-4 and IL-10, which is associated with a Th_2 response**.** Therefore, we observed that B-1 cells previoslly incubated with gp43 were able to activated "naive"T lymphocytes *in vivo.* **Discussion:** These results suggest that B-1 lymphocytes would be performing as APCs, and seems to induce a more prominent Th2 type of T cells response, which is associated with the PCM dissemination and might contribute to reduce the effectiveness of the immune response. **Financial support:** FAPESP (Process nº 04/01182-6)

02.016 - THE ROLE OF TLR-4 AND TLR-2 IN MURINE PARACOCCIDIOIDOMYCOSIS (PCM)

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Introduction: The host mechanisms of innate immunity against *P. brasiliensis* (Pb) infection are poorly understood. Resident macrophages are the first cells that interact with Pb conidia or yeasts and, depending on their local activation, they can exert an efficient or neglectful fungicidal activity. However, the molecular mechanisms that govern Pb-macrophages interactions are not well understood. The Toll-Like Receptors (TLRs) recognize molecular patterns present in several pathogens and influence the synthesis of mediators and phagocytes activation that will participate in parasite elimination. Although mannose and C3b receptors were described as mediators of macrophages-Pb interactions, to our knowledge the influence of TLRs in this phenomenon was not investigated. **Objectives:** The aim of this work was to study the role of TLR-4 and TLR-2 in Pb interaction with macrophages. We performed studies with peritoneal macrophages that have non-functional TLR-4 or TLR-2 molecules (C3H/HeJ and C57BL/6 TLR-2 knockout strains, respectively) and macrophages from mice that have these functional receptors (C3H/HePas and C57BL/6 strains, respectively). **Methods:** Mice (n=3-5) were submitted to peritoneal lavage and cell suspensions ($2x10^5$cells/well) were pre-activated overnight with IFN-γ (20,000 pg/mL) and *in vitro* challenged with $4x10^3$/mL Pb yeasts (1:50 fungus:macrophage ratio). 72h after infection fungicidal activity was assessed by CFU counts and nitric oxide (NO) levels measured by Griess reagent. **Results:** Macrophages expressing functional TLRs produce higher levels of NO than those from TLRs deficient groups. Thus, macrophages from TLR-4 normal mice (C3H/HePas) produce 143.4 ±1.31 µM NO while the TLR-4 deficient group (C3H/HeJ) produce 128.2 ±1.75µM ($p<0,05$); normal TLR-2 macrophages (C57BL/6) produce 188.10 ±2.81 µM NO but TLR-2 deficient mice (TLR-2 KO) secrete 180.8 ±2.95 µM ($p<0,05$). Compared with TLRs deficient macrophages, higher number ($p<0,05$) of viable yeast cells was recovered from normal TLRs macrophages (780 ±41 viable Pb from TLR-4 normal vs 625 ±18 from TLR-4 deficient group; 4,791 ±198 yeasts from TLR-2 normal vs 3,760 ±15 fromTLR-2 deficient group). **Conclusion:** Our data demonstrated that TLR-4 and TLR-2 receptors are involved in Pb-macrophage interactions and TLRs-4 appears to have a more prominent role than TLR-2. TLRs-Pb interactions were sufficient to induce increased NO secretion by IFN-γ primed macrophages. The higher number of yeast cells recovered from TLRs normal macrophages was probably due to enhanced fungal ingestion after Pb-TLR interaction, but the increased levels of NO were not sufficient to control fungal growth. In this aspect Pb yeasts can use TLRs to gain access into murine macrophages and use this interaction as a virulence mechanism. **Financial support:** FAPESP and CNPq .

02.017 - PULMONARY PARACOCCIDIOIDOMYCOSIS (PCM) IN IL-10 KNOCK-OUT (KO) MICE

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Introduction and Objective: Studies in experimental PCM have demonstrated that immunological resistance is linked to a preferential T helper-1 (Th1) immune response whereas susceptibility is associated with absence or low levels of IFN-γ. Furthermore, control of fungal growth is mainly due to nitric oxide production by macrophages after their activation by pro-inflammatory cytokines. IL-10 and TGF-b usually function as macrophages deactivating cytokines. Gene knockout mice (KO) of IFN-γ, IL-12 and TNF-α a receptor develop a more severe PCM although IL-4 can be protective or a disease exacerbating cytokine depending on the genetic background of the host. Patients with the severe and mild forms of the disease as well as susceptible and resistant mice to PCM secrete IL-10 throughout the course of the disease but its precocious or enhanced synthesis appear to be associated with the most severe aspects of the infection. To better understand the in vivo role of IL-10, pulmonary PCM was comparatively studied in IL-10 KO mice and their normal counterparts of the C57BL/6 strain. **Methods:** Wild type (WT) (n=5-8) and IL-10 KO C57Bl/6 male mice (n=5-8) were i.t. infected with $1x10^6$ yeast cells of *P. brasiliensis* (Pb). Mice were sacrificed 8 weeks after infection and the severity of disease determined by organ CFU counts and lungs histopathology. Pulmonary cytokines and levels of specific isotypes were evaluated by ELISA in lung homogenates or serum samples, respectively. **Results:** At week 8 after infection, IL-10 KO mice presented decreased fungal loads in the lungs (KO 3.43 ± 0.18 $\log_{10}$; control 5.15 ± 0.15 $\log_{10}$) and no fungal cells were detected in the livers and spleens. Lung histology revealed the presence of numerous granulomatous lesions in control mice whereas KO animals presented almost no fungal cells and lesions indicating an evident tendency to cure. The much less severe disease of IL-10 KO mice was accompanied by low levels of IgG2a (KO 2.24 ± 1.10 $\log_2$, control 10.69 ± 0.53 $\log_2$), IgG2b (KO 6.57±0.41 $\log_2$, control 10.75±0.68) and IgG3 (KO 1.99±0.97 $\log_2$, control 5.32±0 $\log_2$) and decreased amounts of pulmonary IL-12 (KO 167.25 ± 34 pg/ml; control 305.63 ± 41 pg/ml). Furthermore, no differences of GM-CSF, TNF-α, IFN-γ, IL-4 and IL-5 levels were detected in lung homogenates. **Conclusion:** Genetically determined deficiency of IL-10 results in a less severe PCM which appears to evolve to microbiological cure. Interestingly, this picture was not concomitant with a decreased Th2 immunity but was associated with a diminished Th1 immunity. We may suggest that the enhanced fungal clearance observed in IL-10 deficient mice may be due to a more efficient phagocyte activation resulting in less severe pathology and decreased activation of T cell immunity. **Financial support:** Fapesp

02.018 - INVOLVEMENT OF MELANIN FROM DIMORPHIC FUNGAL PATHOGEN *PARACOCCIDIOIDES BRASILIENSIS* IN PHAGOCYTOSIS AND SUSCEPTIBILITY TO ANTIMICROBIAL OXIDANTS

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Introduction: Paracoccidioidomycosis, caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Pb), is a chronic granulomatous disease restricted to Latin America. Melanin is synthesized by several important pathogenic fungi, including *P. brasiliensis*. It is deposited on the cell wall, and has been implicated in the pathogenesis of a number of fungal agents. In this study, we determined the role of macrophage receptors in the phagocytosis of melanized yeast cells, nitrite production and antimicrobial oxidants. **Methods**: Pb18 yeast cells were grown in modified McVeigh-Morton medium, supplemented with 1.0 mM L-DOPA. Macrophage-like J774.16 and MH-S cell lines were utilized for phagocytosis assays. Inhibition experiments were run with mannan, *N*-acetyl-D-glucosamine and a monoclonal antibody to CD18 on phagocytosis of melanized and nonmelanized Pb 18-yeast cells. Nitric oxide (NO^-) generated during the phagocytosis of melanized and nonmelanized yeast cells was quantified by Griess' colorimetric method. Susceptibility to NO, oxygen and hypochlorite-derived oxidants was investigated by using three *in vitro* systems and fungal viability was determined as colony forming units (CFU). **Results:** The treatment of peritoneal macrophage-like J774.16 cells with mannan, *N*-acetyl-D-glucosamine and anti-CD18 Mab reduced significantly the phagocytosis of nonmelanized cells but only mannan reduced the phagocytosis of melanized cells. When the three inhibitors were added, both melanized and nonmelanized cells showed a significant reduction in the phagocytic index. Similar results were obtained with alveolar macrophage-like MH-S cells. A significant enhancement of nitrite was obtained in J774.16 cells during the phagocytosis of nonmelanized but not of melanized yeast cells. No differences were detected with MH-S cells. Melanized cells were more resistant than nonmelanized ones to *in vitro* generated nitrogen-, oxygen- and hypochlorite-derived oxidants. **Discussion:** Our data indicate that melanin can interfere in the yeast phagocytosis by macrophages. Inhibition of mannan and ²-glucan receptors virtually abolished the phagocytosis by both peritoneal and alveolar macrophages. Melanin also conferred protection against antimicrobial oxidants that are produced during the host defense response. **Financial support:** Supported by FAPESP

02.019 - GALECTIN-3, A BETA-GALACTOSIDE BINDING LECTIN, INTERFERES WITH CYTOKINE AND ANTIBODY PRODUCTION DURING EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

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Introduction and Objectives: Cellular immune response is the main mechanism of host defense against *Paracoccidioides brasiliensis* infection. Triggering of an adequate cellular immune response, characterized by Th1 type cytokines production and compact granuloma formation, is protective against *P. brasiliensis* infection. Galectin-3 is a member of a family of beta-galactoside binding animal lectins, known for its pro-inflammatory activity. Because galectin-3 is expressed by a variety of immune cells, modulates cell-matrix adhesion, and is a monocyte/macrophage chemoattractant, it is candidate to act as a regulator of immune and inflammatory response. This scenario has motivated us to investigate a possible role for galectin-3 exerted during experimental paracoccidioidomycosis. We have evaluated if the absence of galectin-3 interfered with cytokine production by *Paracoccidioides brasiliensis* infected mice. **Methods and Results:** Wild type (WT) and galectin-3 knockout mice (gal-3KO) were i.p. infected with Pb18 virulent strain from *P. brasiliensis.* We investigated *in vitro* response of splenic cells from WT and gal-3KO infected mice to different stimuli. Gal-3KO infected mice produced higher amounts of IL-12p40 than WT infected mice under different stimulus. Surprisingly, despite a high IL-12 production, levels of IFN-γ produced by splenic cells from gal-3KO mice were similar to levels produced by cells from WT mice under the same stimulus. Under no stimulation, however, IFN-γ production by knockout cells was lower than the production observed by WT cells. This suggests that galectin-3 regulates, directly or indirectly, IFN-γ production by T and NK cells after infection. Isotypic analysis of seric specific antibodies of both WT and gal-3KO mice at 30 days after infection revealed that gal-3KO mice presented significantly higher levels of IgG1 - that characterizes a Th2 pattern of immune response - when compared to WT mice. Similar levels of IgG1 and IgG2b antibodies were detected in serum from WT mice, denoting a mixed pattern of immune response to *P. brasiliensis* infection. Therefore, the absence of galectin-3 may have changed the mixed pattern of immune response observed in the WT mice to a Th2 pattern, observed in gal-3KO mice, after *P. brasiliensis* infection. **Conclusions:** Our results suggest that galectin-3 has a role in *P. brasiliensis* infection, since knockout mice showed an altered cytokine and antibody production. **Financial support:** FAPESP e FAEPA

02.020 - ROLE OF FAS/FASL SIGNALING IN SYSTEMIC PARACOCCIDIOIDOMYCOSIS

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INTRODUCTION AND OBJECTIVES: Paracoccidioidomycosis (PCM), a systemic mycosis endemic at South America, is caused by the fungus *Paracoccidioides brasiliensis* (Pb), which reaches the respiratory system through inhalation of conidia, establishing chronic infection. If host-parasite balance ends up in immunosuppression, the infection gives rise to full-blown disease. Cell-mediated immune response is mandatory for protection against PCM, generating Th1 response. A recent study showed high expression of Fas and FasL in monocytes from patients with PCM. In this context, we addressed the question if Fas insufficiency leads to resistance or susceptibility to PCM. **METHODS:** We used an experimental model of PCM. Six- to eight-week-old C57BL/6 wild type (WT) and lpr mice (with a truncated form of Fas) were I.V. infected with 10^6 yeasts of Pb. **RESULTS:** Up to 120 days post infection (p.i.) 100% of lpr and the WT mice were still alive. We found at 7 and 15 days p.i. similar CFU counts in the spleens, livers and lungs of lpr and WT mice. We noticed that starting from 30 days p.i. fungal load were greater in lpr mice, compared to WT. lpr animals take long to heal, eradicating fungi by day 150 p.i. Histopathology of lung lesions of lpr at day 7 were marked by presence of inflammatory cells, scattered throughout the parenchyma in aggregates resembling granulomas, whilst a diffuse inflammatory pool were found in WT mice. Compact granulomas were observed in both mice at day 15 p.i. DTH response to Pb-exoantigen was increased in WT mice, compared to lpr. The levels of IL-10 were higher in lpr mice until day 30 p.i., while MIP-1a and KC were greater in lpr mice during all the course of infection. Specific humoral response were measured and both mice produced high and similar levels of IgG2a and IgG2b. To assess the pattern of inflammatory infiltrate in the lungs we performed FACS analysis. Percentages of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were similar in both mice, although less number of neutrophils (NØ) were found in lpr mice.**DISCUSSION:** The results allow us to suggest that Fas/FasL pathway is crucial for a protective immune response against PCM. The delayed fungal clearance in lpr mice was unpredicted because a probable diminished apoptosis of leucocytes should circumvent fungal growth. We believe that Fas/FasL pathway is involved in controlling immune response, playing a role in NØ activation and migration. We aim to determine in lpr mice infected with Pb if a Th2 response lingers alongside the Th1 one and verify if low numbers of NØ at early stages accounts to a retarded fungal removal in lpr mice. **Financial support:** CAPES and FAPESP

02.021 - CYTOKINE PRODUCTION IN PARACOCCIDIOIDOMYCOSIS INFECTION IN MICE SELECTED FOR ACUTE INFLAMMATORY REACTION

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Introduction and Objectives: The resistance/susceptibility and cytokine production (Th1 and Th2) in genetically selected mice strain to maximum (AIRmax) and minimum (AIRmin) acute inflammatory reaction in response to *Paracoccidioides brasiliensis* was analyzed. These strains showed different susceptibility degrees to the intracellular parasite multiplication, being useful as an experimental model to this infection. **Methods and Results:** Airmax and AIRmin mice were inoculated (i.p.) with $2x10^6$ yeasts of *Paracoccidioides brasiliensis* (Pb18) and sacrificed after 1, 3, 7 and 14 days of infection in other to cytokine (IFN-γ, IL-12, IL-10 and IL-4) production in lungs and spleen by ELISA assay. The AIRmax strain showed a better infection control than the AIRmin by CFU counts. AIRmax did not show any change in cytokine production in the lungs, however, AIRmin strain showed an inhibition in IL-12 production, after 1 and 3 days of infection and increased IL-10 after 1, 3 and 7 days. AIRmax showed an increased IFN-γ production ($p>0,05$) in the spleen and no changes in the other cytokines. **Conclusion:** We analyzed early infection periods, innate mechanisms such as macrophagic activity could be more involved in the immune response in this experimental model.

02.022 - THE THE ROLE OF $CD4^+CD25^+$ T CELLS IN THE *PARACOCCIDIOIDES BRASILIENSIS* INFECTION IN MICE

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Introduction and Objective: Paracoccicdioidomycosis (PCM) is a chronic systemic disease with high rates of morbidity and mortality in rural areas of Latin America, that is caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Pb). The disease is characterized by a chronic inflammatory granulomatous reaction and, although the protective mechanism is related to Th1 type response, the balance of the cellular immune response is essential to the control the fungus growth as well as infection-induced immunophatology. There are recent evidences that regulatory T cells ($CD4^+CD25^+$) control excessive immune response and are involved with latency of several pathogens, including the chronic immune response in patients with PCM. In this study, we investigated the role of regulatory T cells ($CD4^+CD25^+$) in the experimental PCM and to comprehend the mechanisms involved in the maintenance of the fungus in the host tissue. **Methods and Results:** To assess the presence and function of regulatory T cells during the PCM, C57BL/6 mice were infected with $1x10^6$ yeast cells of Pb and the presence of CD25+ and GITR+ cells into the granulomas was investigated by immunohistochemistry. Afterward in the intention to inhibit the functional role of natural regulatory T cells, mice were treated ip. with monoclonal antibodies anti-CD25 (PC61), anti-GITR (DTA-1) or Ig-rat as control (all in the concentration of 500mg/ml). After 15, 30, 60 and 90 days of infection, we analyzed the amount of fungus in the organs (CFU) and the granuloma formation. The results showed positive immunorreativity to CD25 and to GITR in the lungs of infected mice. These surface molecules are related with the regulatory T cell phenotype, suggesting the presence of such cells in the lesions. The treatment with anti-GITR, did not interfere in the migration of $CD4^+CD25^+$T cells to the lungs at days 15 after the fungus inoculation. However, it resulted in increased resistance of mice to the Pb-infection, with significant reduced amount of yeast cells recovered from lung, spleen and liver and decreased granulomas formations when compared with the control mice. Reduced CFU mainly in the lungs at days 15, 30 and 60 after infection was also observed in mice treated with anti-CD25. **Conclusion:** These combined data indicated that the regulatory T cells ($CD4^+CD25^+$) in the site of Pb-infection could modulate the immune response against this fungus. Moreover, the treatment with anti-CD25 or anti-GITR results in the control the fungus growth and the severity of the disease. **Financial support:** FAPESP.

02.023 - POST - ANTIFUNGAL EFFECT OF AZOLES AND SULFAMETHOXAZOLE-TRIMETHOPRIM IN EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

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Introduction – The dugs prescribed for treatment of paracoccidioidomycosis are frequently interrupted by patients with poor socioeconomic level or alcoholics. In this study, the efficacy and post-antifungal effect of ketoconazole, fluconazole, itraconazole and sulfamethoxazole-trimethoprim was tested comparatively against experimental infection of female Wistar rats with *Paracoccidioides brasiliensis*. **Methods** – The animals were treated for one to four weeks by gavage with 4 to 16mg/Kg of ketoconazole (K), 4 to 16mg/Kg of fluconazole (F), 2 to 8mg/Kg of itraconazole (I), and 10 to 100mg/Kg weight/day of sulfamethoxazole-trimethoprim (ST). **Results** – Itraconazole and fluconazole were more effective in reducing the fungal burden in respect to control group. The amount of yeast in the lungs was reduced 24, 45, 661, and 4 times, respectively, for K, I, F and ST. In the spleen the reduction of fungal burden obtained with the same drugs was, 2, 2, 5 and 2 times, respectively. The survival 61 day after inoculation of *P. brasiliensis* was 27% for untreated animals and 27%, 67%, 82%, and 17% for the rats treated with K, F, I, and ST, respectively. The post-antifungal effect on the animals that received 6 or 12 doses of the drugs, followed three weeks of interruption of treatment, showed that K, in the lung, and F, in the spleen, provided greater inhibition of the return of fungal multiplication. **Discussion** – It is concluded, for the model and experimental design used, that itraconazole and fluconazole had a stronger action, immediate and post-treatment, on the control of infection with *P. brasiliensis*. **Financial support:** FAEPA Foundation, Hospital das Clínicas da FMRP-USP.

02.024 - INVOLVEMENT OF EXTRACELLULAR MATRIX PROTEINS IN THE COURSE OF EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

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Introduction and Objectives: Previous studies have been shown that laminin, an extracellular matrix protein (ECMp) binds to *Paracoccidioides brasiliensis* yeast cells enhancing both virulence in the hamster testicle model and fungal adhesion to the surface of epithelial Madin-Darby canine kidneys cells. In addition, yeast cells of the Pb265 strain that had been treated with soluble laminin, gave rise to a less severe infection with diminished fungal loads in the lungs and a less intense inflammatory reaction. Recently, we demonstrated the presence of two proteins of 19 and 32-kDa on *P. brasiliensis* surface that interacted with laminin, fibronectin and fibrinogen. The aim of this study was to determine the participation of ECMp (laminin, fibronectin and fibrinogen) on the course of experimental paracoccidioidomycosis. **Methods and Results:** Isogenic 6 weeks old BALB/c male mice were infected intranasally with 4 X 10^6 *P. brasiliensis* conidia previously incubated in the presence of 100 mg/ml of soluble laminin, human fibronectin, bovine fibrinogen (Sigma), bovine serum albumin (BSA) in PBS or PBS alone for 2h at 37ºC. In addition, a monoclonal antibody (MAb 2G4) against a *P. brasiliensis* adhesin of 32-kDa protein was produced, and used to treat the conidial suspension as described above. Also and as a control mouse IgG1 was used at concentrations of 100 mg/ml. Animals were sacrificed at different time intervals 0 (2 hours post-inoculation), 2, 4 days; and 1, 4, 8 and 12 weeks. At each period, 5 mice form each experimental group, as well as non-infected control animals, were sacrificed by the intraperitoneal injection of 1.0 ml of 2.5% sodium penthotal. Different mouse groups were used to determine both the production of cytokines (IL-4, IL-6, TNF-α and IFN-γ) and chitin levels. Reliability of chitin assays was confirmed using a suspension of yeast grown for six days in brain heart infusion (BHI), which showed a linear relationship between the volume of yeast suspension and the chitin content. Chitin content in the lungs was significantly decreased at week 8th in mice infected with conidia previously treated with each one of the ECMp tested when compared with control mice infected with untreated conidia. Contrary to what was expected, when the animals were infected with the MAb 2G4-treated conidia, infectious process became exacerbated, as shown by chitin increase in the lungs. When mice were infected with conidia treated with soluble ECMp or MAb 2G4, a significant increase of IFN-γ levels at day 4th post-infection was observed when compared with animals infected with untreated conidia; TNF-α, IL6 and IL-4 did not show difference with respect to controls. **Conclusions:** These findings point towards an inhibitory effect of ECMp treatment on *P. brasiliensis* conidia infectivity, and also suggest that these proteins could interfere with the interaction of the fungus with ECMp and pulmonary host cells. In addition, it is possible that these ECMp could modulate the immune response (cell activation and cytokines production) in PCM. **Financial support:** Wellcome Trust, Project No. 062247/Z/00Z, and the CIB. The National Doctoral Program of COLCIENCIAS supported A. González.

02.025 - ADHERENCE OF *PARACOCCIDIOIDES BRASILIENSIS* CONIDIA TO HUMAN TYPE II ALVEOLAR CELLS: ROLE OF EXTRACELLULAR MATRIX PROTEINS

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Introduction and Objectives: It has been shown that *Paracoccidioides brasiliensis* conidia have the capacity to interact with extracellular matrix proteins (ECMp) (laminin, fibronectin, fibrinogen), also of adhering to human epithelial type II alveolar cells (A549). Recently, two proteins of 19 and 32-kDa detected on the fungal surface were shown to interact with ECMp. The aim of this study was to establish the mechanisms underlying adherence of *P. brasiliensis* conidia to A549 cells. **Methods and Results:** Initially and by flow cytometry (FC) we determined the presence of ECMp on the surface of A549 cells using antibodies against laminin, fibronectin and fibrinogen. We also determined the adherence capacity of *P. brasiliensis* conidia (previously obtained by discontinuous percoll gradients and labeled with FITC) to A549 cells at different times post-infection (0.5, 1, 2 and 3h) and at different ratios of conidia to A549 cells 1:1 and 5:1. All assays were done using FC. Likewise, inhibition adherence assays were done using antibodies against the ECMp or soluble ECMp tested above, or employing different compounds such as specific synthetic peptides (RGD, RGDS, IKVAV, YIGSR and xYIGSR, all at 1mg/ml); sugars [glucose, mannose, galactose, N-acetyl-glucosamine, N-acetyl-galactosamine and N-acetyl-neuroaminic acid (NANA), all at 0.2M]; mucine, asyalomucine, a monoclonal antibody against a 32-kDa protein (adhesin), as well as this purified protein. The results indicated the presence of the three ECMp on the surface of A549 cells, with fibrinogen and laminin predominating. Adherence of *P. brasiliensis* conidia was observed as early as 0.5h with maximal values at 3h post-infection (23.2 ± 3.0 and 50.6 ± 10.1, respectively), when the ratio conidia to A549 cells was 5:1. Inhibition assays showed a significant decrease on fungal adherence to A549 cells when different treatments were used, mainly when the epithelial cells were treated with anti-ECM antibodies and the purified 32-kDa protein or when conidia were treated with soluble MECp, MAb anti-32-kDa protein, NANA, glucose, lactose, amino-sugars and the specific synthetic peptides (mainly peptides containing RGD and IKVAV fragments). **Conclusions:** These results suggest that the presence of ECMp (laminin, fibrinogen and fibronectin) on A549 cell surface facilitates the adherence of conidia to these alveolar cells possibly through the interaction of adhesine-type molecules, such as the 32-kDa protein and the various ECMp. In addition, other mechanisms such as interactions with specific fragments (RGD and IKVAVA) present in the ECMp or a dependant-NANA system may be involved in this interaction. These findings could be helpful in understanding the complex adherence process, an important step in the host-parasite interaction during the early events corresponding to the pathogenesis of paracoccidioidomycosis. **Financial support:** Wellcome Trust, Project No. 062247/Z/00Z, and the CIB. The National Doctoral Program of COLCIENCIAS supported A. González.

02.026 - DEPLETION OF NATURAL KILLER CELLS INDUCES A MORE SEVERE PULMONARY PARACOCCIDIOIDOMYCOSIS IN ATHYMIC AND EUTHYMIC BALB/C MICE

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Introduction. In a previous work we observed that athymic and euthymic BALB/c mice depleted of IFN-γ or IL-12 demonstrated exacerbation of disease and increased dissemination of yeasts to lungs, liver and spleen 15 days after infection whereas depletion of PMN cells did not alter the severity of infection. These results demonstrated that IFN-γ and IL-12 are essential to control pulmonary PCM and indicate a role for NK cells in the protective natural immunity against *P. brasiliensis* infection. **Objectives.** The purpose of the present study was to define the role of NK cells in natural resistance to *P. brasiliensis* infection. **Material & Methods.** Athymic and euthymic BALB/c mice (n=8-9) were used and *in vivo* depleted by i.p. injection of anti-Asialo GM1 polyclonal Ab (200mL at days -6, -3 and + 3 of infection with 1x106 *P. brasiliensis* yeasts by i.t. route). After 15 days, the severity of infection was determined by CFU counts ($\log_{10}$) in the lungs, liver and spleen. It was also assessed DTH reactions, survival times (n=8-12), bronchoalveolar lavage (BAL) cells (n=6), specific antibodies ($\log_2$) (total Ig, IgM, IgA, IgG1, IgG2a, IgG2b, IgG3, IgE) and levels of pulmonary cytokines (IL-2, IL-3, IL-12, IFN-γ, IL-4, IL-5, IL-10, IL-18) (n=5). Data are expressed as means ± SE and analyzed by Student´s *t* test. **Results.** Increased CFU numbers was observed in the lungs of depleted euthymic (5.2±0.1 X 5.6±0.1) and athymic (6.2±0.1 X 6.5±0.1) mice. In NK-cells-depleted athymic mice increased CFU counts were also observed in liver (2.7±0.1 X 3.2±0.1) and spleen (2.5±0.1 X 3.3±0.1) while no increased dissemination was detected in euthymic animals. NK-cells-depleted athymic mice presented increased mortality but no differences were found between depleted and untreated euthymic mice. Augmented levels of IgM (5.8±0.3 X 9.1±0.2), IgG1 (5.3±0 X 6.9±0.2) and IgG2b (4±1.3 X 7.1±0.3) specific isotypes were observed in the sera of depleted athymic mice but not in the euthymic strain. Both strains demonstrated reduced numbers of PMN leukocytes in BAL fluids. Cytokines determinations in pulmonary homogenates showed that NK-cells-depleted euthymic mice presented increased IL-12 (108±15 X 13459±942) and reduction of IL-4 synthesis (120±34 X zero). In depleted athymic mice decreased levels of IL-5 (8032±520 X 3280±157) and IL-18 (5456±1004 X 2183±1001) was concomitant with elevated amounts of IL-10 (37±21 X 186±41). **Conclusion**. This work demonstrated for the first time that NK cells are protective *in vivo* to both athymic and euthymic BALB/c mice exerting their effects in the lungs (both strains) and controlling fungal dissemination to liver and spleen (athymic strain). It was also verified that NK cells control PMN leukocytes influx to the lungs and regulate antibodies and cytokines levels. **Financial support:** FAPESP and CNPq

02.027 - SUPERIOR EFFICACY OF THE LIPOSOMAL FORM OF AMPHOTERICIN B COMPARED WITH THE CONVENTIONAL DRUG PRESENTATION IN EARLY STAGES OF THE PARACOCCIDIOIDOMYCOTIC INFECTION IN MICE

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Introduction and Objectives: Amphotericin B is the golden standard treatment for paracoccidioidomycosis (PCM). The efficacy of the liposomal (L-AmB) or the conventional (c-AmB) drug therapy were compared in mice infected with *Paracoccidioides brasiliensis* (Pb). **Methods and Results:** Therapy was initiated 24 hours prior to the infection with a virulent Pb isolate; 2mg c-AmB and 5mg L-AmB/kg/day were given to susceptible (B10.A) mice by the ip route, three times a week. It has been postulated that L-AmB has to be given in an at least two-fold higher amount than c-AmB; therefore, both dosages may be considered similar. Delayed-type hypersensitivity responses in the 7th day were similar between the groups; at the 15th day, mice submitted to either therapies showed lower responses than untreated subjects (0.2±0.05 for c-AmB and 0.2±0.1 for L-AmB treated mice and 0.4±0.1 for the untreated ones), developing a pattern very similar to the one presented by resistant animals in the murine model of PCM. At both time points, L-AmB-treated mice showed lower fungal loads than untreated or c-AmB-treated mice in spleen (0 for L-AmB, 3.9±0.6 for c-AmB-treated mice and 4.4±0.4 for the untreated ones in the 7th day; respectively, 3.0±0.1, 4.4±0.2, 4.5±0.5 in the 15th day), epiploon (0, 4.4±0.3, 4.4±0.6; 3.0±0.3, 3.9±0.2, 4.8±0.4), liver (0, 4.0±0.3, 4.4±0.2; 0, 4.5±0.1, 4.4±0.6) and lungs (0, 2.3±0.5, 3.9±0.3; 1.6±0.6, 3.5±.1, 4.2±0.3). At both time points, L-AmB-treated mice tended to show lower secretion of NO than untreated or c-AmB-treated mice in spleen (0, 1.6±1.0, 15.2±4.8; 0, 0.02±0.02, 0), epiploon (0.2±0.2, 38.8±12.9, 67.0±23.6; 0, 10.5±4.4, 20.4±10.6), liver (2.7±0.7, 58.0±7.8, 49.6±7.5; 7.7±1.1, 97.5±13.7, 50.1±14.2) and lungs (0.7±0.7, 65.4±31.9, 66.9±15.5; 0, 16.6±5.9, 6.4±3.3). **Conclusion:** Our results show that L-AmB has a superior efficacy in diminishing the fungal load and establishing a resistant pattern of immune response when compared with c-AmB. However, the mechanisms implicated in this superiority are yet to be investigated and may involve immunostimulatory properties of L-AmB. **Financial support:** CNPq (304986/03-8) and FAPESP (02/10905-6 and 04/14718-1)