02 EXPERIMENTAL MODELS

02.001 - THE PROTECTIVE IMMUNITY TO PARACOCIDIOIDIOSES 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: Six-week-old BALB/c mice were immunized i.n. with sPbAg mixed with MPL-SE (according to Corixa Corporation protocol). Yeast cells (Ph18) were shocked 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 (i.v.) 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 of IFN-γ in liver and spleen of mice immunized with sPbAg plus MPL-SE 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 lesion and fungal dissemination to the all organs analyzed. Cleared 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 elict a protective immunity against P. brasiliensis infection. The protection of immunized mice was mediated by a Th1-type immune response, whose induction is dependent of Toll-like receptor activation. Final conclusions: sPbAg, FAPESP, FAEPFA

02.002 - PHAGOCYTOSIS, EXPRESSION OF CO-STIMULATORS MOLECULES AND CYTOKINES PRODUCTION OF PULMONARY DENDRITIC CELLS AFTER INTRATRACHEAL INFECTION WITH PARACOCIDIOIDIOSES 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. See 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-stimulatory 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-stimulatory molecules were analyzed by flow cytometry. 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-stimulatory molecules. In purified CDP from A/J mice, a gradual increase of the CD80, CD86, and Foxp3 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 in both lines 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-stimulatory and cytokines production in CDP. These changes can affect the activation of T cells, mainly in susceptible animals. It is possible that mechanisms for which the fungus induces a larger chronicity in those animals. Reference: (1) Gonzales-Juarrero, M., and I.M. Orne. Infect. Immun. 69:1127, 2001. Financial support: FAPESF (n° 03/12816-3) and CNPq.
02.005 - LEUKOTRIENES IN THE PROTECTION INDUCED BY CFAGS IMMUNIZATION AGAINST HISTOPLASMA CAPSULATUM Medeiros, A. L.; Sá-Nunes, A. C.; Turato, W. H.; Peres, C. M. C.; Sorgi, C. A.; Panunto-Castelo, A. S.; Silva, C. L. S.; Facchioli, L. H. 1,2
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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 histoplasmiasis 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.p. and i.t. lethal inocula of H. capsulatum (Microbes Infect. v.7, 584, 2005). We also demonstrated the role played by LTs in pulmonary histoplasmiasis 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 (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 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: FAEPES and CNPq.

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Introduction and Aims: P. brasiliensis is a thermo-dimorphic 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 pseudoypial 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 phagocytic yeasts of P. brasiliensis and pseudoypial in control and infected macrophages. Material, methods and results: Balb/c male mice were i.p. inoculated with yeasts, pseudoypial of Pb or PBS. Five days p.i., peritoneal macrophages were collected, cultured on 13mm round cover slips in RPMI medium (2.105 cells/well) for three days and incubated with live pseudoypial or yeasts (8.105 P. brasiliensis/well) for six hours. Three samples were fixed and stained with hematoxilin–eosin. To determine the viability of phagocitized P. brasiliensis, 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 + 0.01% L-glutamine). Colonies per plate were counted after 6-8 days of incubation at 37 ºC. We observed larger phagocytic index in macrophages challenged with pseudoypiala than challenged with yeasts in all control, yeast- or pseudoypiala-infected mice (91.2, 86.9 and 85.8 ± 70.8, 52.4, 35.1). The phagocytic index of pseudoypiala 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.

1Instituto de Biologia - UNICAMP - Microbiologia e Imunologia; 2UNICAMP - Microbiologia e Imunologia; 3UNICAMP - Eletrônica Quântica; 4UNICAMP - Histologia e Embriologia

Introduction and Aims. Paracoccidioidomycosis 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 synthesis. 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 5.107 P. brasiliensis cells in the footpad and seven days p.i. (pos-infection) were treated with 3 or 5 doses of laser HeNe (λ=632.8 µm W= 5mW. Δ= 4mm). Control mice were inoculated with the same concentration of fungi 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 showed better progress of healing in treated lesions that showed small granomas 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: FAEPES

02.008 - EVALUATION OF CELLULAR IMMUNE RESPONSE IN RESISTANT AND SUSCEPTIBLE MICE TO P. BRASILIENSIS DURING INFECTION EMPLOYING DIFFERENT ANTIGENS Ferreira, E. C. J.; Matano, G.; Fasoli, R. A.;
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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 (CFAg) concentrations was performed by footpad test at 24h in normal or sc infected B10.A mice at 15 days post infection. FNAg and CFAg 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 CFAg (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 and 16 weeks) injected with the best concentrations of FNAg and CFAg 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 only mice at 2 weeks of ip infection in comparison to controls. CFAg (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 CFAg 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 CFAg. Also, ip infected A/SN mice injected with FNAg presented increased DTH responses in comparison to ip infected B10.A mice. The CFAg 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 CFAg antigen during infection, then 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.
0.02.09 - INVOLVEMENT OF INTERFERON-γ AND OSTEOPONTIN IN THE GRANULOMATOUS RESPONSE DEVELOPED IN EXPERIMENTAL INFECTION WITH PARACOCCIDIOIDOMYCOSIS

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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 defense 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 IPb 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 (DPF) (69±50 cells/100 mm²) compared to 15 DPF (351±83), while in A/J, 4-fold increase of (+) cells was observed at 120 DPF (1793±212) and significantly higher than observed at 15 DPF (404±150). Significant differences were found between infected B10.A and A/J mice. On the other hand, IFN-γ expression was found in Pb18 infection compared to Pb265 infection at 120 DPF. At 15 DPF 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 DPF with Pb265. Overall, the same pattern of IFN-γ and OPN expression on Pb18 infection is 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 and OPN expression 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

0.02.10 - EFFECT OF CHLOROQUINE ON THE MURINE EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS: MACROPHAGE ACTIVATION, CYTOKINES PRODUCTION AND TRANSFERREIN 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 the fungicidal and secretory ability of their macrophages after Pb infection. 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 10⁸ yeasts of Pb 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 H2O2 and NO production and TNF-α and IL-10 levels in all groups and periods. Conclusions: These results showed that Chloroquine immunomodulatory role on the murine experimental paracoccidioidomycosis suggesting new insights for the mycosis therapy. Financial support: FAPESP.

0.02.01 - THE ROLE OF LEUKOTRIENES IN PARACOCCIDIOIDOMYCOSIS - IN Vivo and In Vitro

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Introduction: Paracoccidioidomycosis (PCM) is a chronic systemic 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 Pb infection. Objectives of this work were to study the role of LT in murine PCM and compare the responses of resistant (A/J) and susceptible (B10.A) mice. Methods: The role of LT in murine pulmonary PCM of resistant (A/J) and susceptible (B10.A) mice and in the fungidical 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 vivo and in vitro Pb infection. In vitro, MK-0591 significantly reduced the recovery of Pb yeasts from Pb-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.

0.02.02 - CYTOKINES PRODUCTION IN LUNGS AND ADRENAAL GLANDS OF HIGH AND LOW ANTIBODY PRODUCERS MICE INFECTED WITH PARACOCCIDIOIDOMYCOSIS

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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 HIV-A and LIV-A (Selection IV-A) were infected with P. brasiliensis; strain 18B, 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) and Th2 (IL-4, IL-10) cytokines production by ELISA. HIV-A animals showed a higher infection degree in analyzed organs. The study of cytokines secretion pattern in the compartments 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 H18-A and L18-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) and Th2 cytokines profile (IL-4, IL-10) by capture ELISA. H18-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 L18-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. Interestingly data were obtained with the Th1 cytokines determination: IFN-γ production increased in both organs, mainly in the adrenal gland of L18-A strain after 8 and 10 weeks, when these animals showed a fungal total elimination. It was observed a significant difference between H18-A and L18-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 L18-A animals to paracoccidioidomycosis infection, mainly the adrenal involvement. The higher production of Th1 profile cytokines by L18-A strain may be associated with these animals resistance to the fungi, shown in previous works.
**02.013 - CELL WALL PROTEIN ANALYSIS AND VIRULENCE OF Paracoccidioides brasiliensis SPONTANEOUS MUTANT**

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*UNICAMP - Microbiologia e Imunologia*; *UNICAMP - Pediatría*;

**Introduction and Aims:** *P. brasiliensis* is a dimorphic fungus that grows in a mycelial phase at room temperature and in a yeast form at 35-37°C. In our laboratory, it was isolated from mice infected with pseudohyphae and yeast forms of this fungus. As literature has shown that Pb18 strain is capable to induce illness in experimental animals at room temperature and in a yeast form at 35-37ºC. In our laboratory, it was isolated from mice infected with pseudohyphae and yeast forms of this 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 paraffin. 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 pseudohyphal form. The SD-SAGE 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 pseudohyphal 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 pseudohyphal 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 5x10⁶ pseudohyphal or yeast forms of the fungus and sacrificed in 1, 3, 5, 7, 14 and 28 days of infection. To analyze the cell wall proteins, CFA (cell-free antigens) obtained from both isolates were submitted to electrophoretic 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 5x10⁶ pseudohyphal 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 paraffin. 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 pseudohyphal form. The SD-SAGE 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 pseudohyphal 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)

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

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**Introduction:** The glycoprotein gp43 is the major antigenic protein secreted by *Paracoccidioides brasiliensis*, the ethiological agent of Paracoccidiomycosis (PCM). In addition, gp43 is the main PCM diagnostic antigen, being recognized by all sera from infected patients. The outcome 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 responsible to T cells activation, proliferation and cytokines production, which determine 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 different phenotype characteristics. B-1 lymphocyte is considered an APC because of its capacity to capture antigenic particules by a fagM receptor and, in the end, present them with a class II major histocompatibility complex (MHC) molecule. **Objectives:** We evaluated 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 previously sensitized 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 Th2 response. Therefore, we observed that B-1 cells previously 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 PARACOCCIDIOMYCOSIS (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 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/HePas 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 (2x10⁶ cells/well) were pre-activated overnight with IFN-γ (20,000 pg/mL) and in vitro functional 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 ±98 yeasts from TLR-2 normal vs 3,760 ±15 from TLR-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 Pb 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-β usually function as macrophages deactivating cytokines. Gene knockout mice (KO) of IFN-γ, IL-12 and TNF-α 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 C57BL6 strain. Methods: Wild type (WT) (n=5-8) and IL-10 KO C57BL6/6 mice (n=5-8) were i.t. infected with 1x10^6 yeast cells of the C57BL/6 strain. OUT (KO) MICE

02.019 - GALECTIN-3, A BETA-GLUCOSIDE 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 chemotactic factor, 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 experimental conditions. 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 sera 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 FAEP
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. Results and Methods: AIRmax and AIRmin mice were inoculated (i.p.) with 2x10⁴ yeasts of Paracoccidioides brasiliensis (PB18) and sacrificed after 1, 3, 7 and 14 days of infection in other cytokine (INF-γ, IL-12, IL-10 and IL-4) production in lungs and spleen by ELISA assay. 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 3, 7 and 3 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, immature 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 Objectives: Paracoccidioidomycosis (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 immunopathology. There are recent evidences that regulatory T cells (CD4+CD25+) in the site of Pb-infection could modulate the 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. Results and Methods: To assess the presence and function of regulatory T cells during the PCM, C57BL/6 mice were infected with 1x10⁴ 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 (in all 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 immunoreactivity 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: FAEPDA.

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

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Introduction – The drugs 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 antifungal effect on the animals that received 6 or 12 doses of the drugs, followed three weeks of interruption of treatment, showed that in the lung, and F, in the spleen, provided greater inhibition of the return of fungal multiplication. Conclusion – 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: FAEPDA 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 32kDa on P. brasiliensis surface that interacted with laminin, fibronectin and fibrobrinogen. 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: Iogenic 6 weeks old BALB/c male mice were infected intranasally with 4 x 10⁶ P. brasiliensis conidia previously incubated in the presence of 100 mg/ml of soluble laminin, human fibronectin, bovine fibrobrinogen (Sigma), bovine serum albumin (BSA) in PBS or PBS alone for 2h at 37°C. In addition, a monoclonal antibody (MAb 2G4) against a P. brasiliensis adhesin of 32-kDa protein was produced, and used to treat the conidal 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 pentotal. 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, IL-6 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/00/Z, and the CIB. The National Doctoral Program of COLCIENCIAS supported A. Gonzalez.
02.025 - ADHERENCE OF PARACOCIDIOIDES BRASILIENSIIS 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-neuraminic acid (NANA), all at 0.2M]; mucine, asyalomucine, a monoclonal antibody against a 32-kDa protein (adhesin), as well as all 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, fibronectin and fibronecin) 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 IKVAV) 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/00/Z, and the CIB. The National Doctoral Program of COLCIENCIAS supported A. González.

02.026 - DEPLETION OF NATURAL KILLER CELLS INDUCES A MORE SEVERE PULMONARY PARACOCIDIOIDES BRASILIENSIIS INFECTION IN HUMAN 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 exacerbated 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 and Methods. Athymic and euthymic BALB/c mice (n=8-9) were used and in vivo depletion 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 (log10) in the lungs, liver and spleen. It was also assessed DTH reactions, survival times (n=8-12), bronchoalveolar lavage (BAL) (c=8-12, no specific antibodies (log1)) 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=data). The results 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. Increased levels of IgM (5.8±0.3 X 9.1±0.2), IgG1 (5.3±0.4 X 6.9±0.2) and IgG2b (4.8±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 134/9±0/42) and reduction of IL-4 synthesis (120±34 X zero). In depleted athymic mice decreased levels of IL-5 (803±250 X 320±517) and IL-18 (545±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 splenic 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.