Posters Section

01 HUMAN IMMUNOLOGY

01.001 - STUDY OF MAST CELLS IN THE IMMUNE RESPONSE IN PARACOCCIDIOIDOMYCOSIS CUTANEOUS LESIONS

Pagliari, C.[1]; Fernandes, E. R.[2]; Guedes, F.[3]; Pereira, N. V.[4]; Kanashiro, L.[5]; Alves, C.[6]; Sotto, M. N.[7]

[1,2,3]FMUSP - Patologia; [4,5]FMUSP-LIM06 - Patologia; [6,7]FMUSP - Dermatologia

Introduction and Objectives: Recent works have demonstrated that mast cells may have an important role in immunologic reactions and inflammation once they synthesize and secrete some cytokines from Th2 pattern including IL4, IL5 and IL6. In paracoccidioidomycosis (PCM) the compact granuloma are related to a Th1 pattern of cytokines and loose granuloma are related to a Th2 pattern. Our aim was to investigate if mast cells would participate in the local inflammatory immune response against *Paracoccidioides brasiliensis* in skin lesions characterized by a Th2 pattern of cytokines. **Methods and Results:** Forty biopsies from skin lesions (20 with compact granuloma – G1 and 20 with loose granuloma-G2) were analyzed by immunohistochemistry to detect cells expressing IL5 and IL10. The toluidine- blue/HCl staining was used to detect mast cells and the positive cells were quantified and statistically analyzed by the Mann-Whitney test. G2 group presented an increased number of cells expressing both IL5 (20.64±35.94) and IL10 (11.04±6.63) cytokines when compared to G1 (7.46±10.48 and 3.151±3.079, respectively). G2 also presented increased number of mast cells (65.89±26.55) when compared to G1 (42.85±14.01). We also could observe some cells with mast cells morphology expressing IL10. All the differences between G1 and G2 were statistically significant ($p<0.05$). **Conclusions:** Our results suggest the participation of mast cells in the immune response against *P. brasiliensis* in skin lesions and these cells could constitute a source of such cytokines, contributing to a non-effective response against fungal antigens.

01.002 - MODULATION OF CD28 AND CD86 EXPRESSION IN PATIENTS WITH PARACOCCIDIOIDOMYCOSIS IN DIFFERENT PERIODS OF TREATMENT

Bozzi, A.[1]; Reis, B. S.[2]; Prado, F. L. S.[3]; Pedroso, E. P.[4]; Leite, M. F.[5]; Goes, A. M.[6]

[1,2,6]UFMG - Bioquímica e Imunologia; [3,4]UFMG - Faculdade de Medicina; [5]UFMG - Fisiologia e Biofísica

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a systemic human mycosis caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Pb) (Trends Microbiol.10:80-87, 2002). The infection is acquired by inhaling airborne propagules produced by the fungal mycelium form (Am. J. Pathol.156:1811-1820, 2000). The cell-mediated response is the main host defense against *P. brasiliensis* (Infec. Immun. 66:800-806, 1998). The granulomatous inflammation is typical of PCM and is a putative mechanism to overcome the infection (Hum. Immunol. 62:799-808, 2001). In this study, we investigated the cellular immune response of patients with PCM against somatic (PbAg) and extracellular antigens (MEXO) of *P. brasiliensis*, analyzing the production of IL-4 and IFN-γ, as well as cell activation by receptor molecules (CD28 and CD86) and proliferation of PBMC from patients without treatment and with different periods of chemotherapy treatment. **Methodology and Results*:*** Around 30 patients recently diagnosed with active PCM were enrolled in this study and divided in four groups according to with treatment period. The control group comprised healthy individuals volunteers who never had PCM. Human PBMC, isolated by Ficoll–diatriazoate density gradient centrifugation, were use for cellular proliferation, cytokines detection and flow cytometry analyze front MEXO and PbAg antigens stimulation. A significant ($p<0,05$) decrease of cell proliferation and production of IFN-γ are observed when the treatment period was greater than 5 years. The level of IL-4 was high ($p<0,05$) only in the non-treated group. The flow cytometry analysis showed that the Pb antigens modulated the activation of $CD4^+$, $CD8^+$ and B cell populations when we analyses the CD28 and CD86 expressions. **Conclusions:** This study brings results that might explain the frequent recidive observed in this disease. One proposal to maintain the immune reactivity during the treatment period is the adjoining of immunotherapeutical vaccine to chemotherapy treatment to stimulate immune reactivity avoiding the recidive. **Financial support:** CAPES, FAPEMIG and CNPq.

01.003 - INACTIVE AND ACTIVE STAT-1 IS DOWNREGULATED IN PHERIPHERAL BLOOD MONONUCLEAR CELLS OF THE PATIENTS WITH ACTIVE PCM

Romano, C. C.[1]; Mendes-Giannini, M. J. S.[2]; Duarte, A. J. S.[3]; Benard, G.[4]

[1]USP - Dermatologia; [2]UNESP - Departamento de Analises Clinicas, Faculdade de Ciencias Farmaceuticas; [3]FM - USP - Dermatologia e Imunodeficiências; [4]USP - Laboratório de Alergia e Imunologia Clínica e Experimental, Faculdade de Medicina , São Paulo, SP, Brazil

Introduction: We have earlier shown that stimulation with the main *P. brasiliensis* antigen (gp43) of PBMC from patients with PCM lead to decreased IL-12, IL-2 and IFN-γ secretion but high IL-10 levels. Modulation of *in vitro* patients´ responses with IL-12 resulted in increased gp43-induced IFN-γ and the addition of neutralizing a-IL-10 Ab also increased the IFN-γ production. Concurrent addition of a -IL-10 and IL-12 not only increased further the IFN-γ but also restored the proliferative responses to gp43. The IL-12R β2 subunit was poorly expressed by patients' lymphocytes with gp43 compared to controls, but normally or near normally expressed with CMA. These findings suggested an imbalance in IL-12/IL-10 regulation. Some studies have suggested that these cytokines can modulate the expression of the same member of the family of the "signal transducers and activators of transcription" (STATs) and to modulate its biological activities. E.g., IFN-γ secretion can induce STAT1 expression in macrophages and increase its function. Methods and Results: Here, we examined intracellular inactive and active STAT 1 expression in PBMC´ s patients and healthy controls by flow cytometry. The suspension of mononuclear cells was isolated by Ficoll-hypaque and cultivated in 96 wells plates in the presence of medium alone, PMA+ ionomycin or gp43. After 24h, the cells was harvested and incubated with monoclonal antibodies for STAT1. STAT1 expression was analyzed by flow cytometry. The expression of inactive or active STAT 1 in PBMC of 6 patients with active PCM was always lower than that in controls cells (9,2 ± 1,4 in control cells vs 5,5 ± 3,9 for patients cells cultivated with PMA+ ionomycin). In cells of controls stimulated with gp43 the STAT 1 expression was 25 ± 3,7, while in the patients was of 2,7 ± 0,9 under the same stimulation. The expression of active STAT1 also was superior in cells from controls when compared with cells of patients (respectively 8,6 ± 2.1 versus 4,6 ± 0,6 for PMA + ionomycin and 28,4 ± 2,8 versus 5.1 ± 1,8 for gp43). **Conclusion:** Our data corroborates findings in experimental models of infectious diseases that describe reduction in the expression of STAT1 as important factor for reduction of the Th-1 response and reduction of phagocytosis for macrophages. **Financial support:** FAPESP 02/14026-7, 02/07306-3; LIM56 HC.

01.004 - ANALYSIS OF THE EXPRESSION OF CD80 AND CD86 MOLECULES IN CD14+ CELL IN PARACOCCIDIOIDOMYCOSIS

Cacere, C. R.[1]; Romano, C. C.[2]; Mendes-Giannini, M. J. S.[3]; Fontes, C. J.[4]; Kono, A. S. G.[5]; Duarte, A. J. S.[6]; Benard, G.[7]

[1]Instituto de Ciências Biomédicas - Microbiologia; [2]Laboratório de dermatologia e Imunodeficiência da Faculdade de Mecicina da USP - Dermatologia; [3]UNESP - Departamento de Analises Clinicas, Faculdade de Ciencias Farmaceuticas; [4]Universidade Federal do Mato Grosso - Infectologia; [5]Faculdaae de Medicina da Universidade de São Paulo - Divisão de Moléstias Infecciosas e Parasitarias ; [6]FM - USP - Dermatologia e Imunodeficiências; [7]USP - Laboratório de Dermatologia e Imunodeficiências Departamento de Dermatologia

Introduction and Objective: Patients with paracoccidioidomycosis present a variable degree of antigen-specific immunosuppression that relates to the severity of the clinical presentation. In this study we aimed to analyze the expression of the costimulatory molecules on CD14+ lymphocytes. We performed cultures of PBMC isolated from patients with active disease and cured controls in presence of a *Candida albicans* antigen [CMA] and gp43. **Results:** Results obtained up to now suggest that the percentages of CD14+ cells expressing CD80 were higher in the cured controls as compared with the patients, either in presence of gp43, CMA or medium only. In the patients, the numbers of CD14+ cells expressing the molecule upon gp43 or CMA stimuli were not higher than without stimulation. In the cured control group, on the other hand, there was augmentation of the expression after gp43 only as compared to the cells without stimulation. The same analyzes were performed for assessing CD86 expression by CD14+ cells. In the cured control group, more cells either stimulated with CMA and gp43 or nor stimulated expressed this costimulatory molecule than patients´ cells in the same culture conditions. Stimulation with CMA or gp43 did not increase the number of CD86+ cells in comparison to the non-stimulated cells. The same was seen with cells from cured controls. In the experiments performed to date, the mean fluorescence intensity (MFI) for the CD80 molecule, both in cells stimulated with the antigens or not-stimulated, were higher in cured controls compared with patients, consistent with the percentage results. In the patients, the MFI did not augment when the cells were stimulated with gp43 and CMA. The same results were observed with the cured control´ s cells. We observed a tendency for the increase in the MFI of CD86 expression in the antigen-stimulated and non-stimulated CD14+ cells from cured controls compared with patients. The CD86 MFI from control´ s cells, differently from the patients, were higher in stimulated cells than non-stimulated cells. The CD86 MFI in patients´ cells were equal with and without stimulation, as for the percentage analyzes. **Conclusions:**These preliminary data suggest that differently from other chronic infectious diseases, where the CD80-CTLA-4 pathway was ascribed a negative signaling for T lymphocyte functions, in paracoccidioidomycosis patients this pathway seems not to be involved in the down modulation of their anti-*P. brasiliensis* immune responses **Financial support:** FAPESP

01.005 - ANALYSIS OF THE EXPRESSION OF THE CD28 AND CTLA4 MOLECULES BY T CELLS FORM PARACOCCIDIOIDOMYCOSIS PATIENTS

Cacere, C. R.[1]; Romano, C. C.[2]; Mendes-Giannini, M. J. S.[3]; Fontes, C. J.[4]; Kono, A. S. G.[5]; Duarte, A. J. S.[6]; Benard, G.[7]

[1]FMUSP - Laboratório de Dermatologia e Imunodeficiências; [2]Laboratório de dermatologia e Imunodeficiência da Faculdade de Mecicina da USP - Dermatologia; [3]UNESP-Araraquara - Laborapório de Micologia Clínica; [4]Universidade Federal do Mato Grosso - Infectologia; [5]Faculdaae de Medicina da Universidade de São Paulo - Divisão de Moléstias Infecciosas e Parasitarias ; [6]FM - USP - Dermatologia e Imunodeficiências; [7]USP - Laboratório de Dermatologia e Imunodeficiências Departamento de Dermatologia

Introducion and Objective: Patients with paracoccidioidomycosis present a variable degree of antigen-specific immunosuppression that relates to the severity of the clinical presentation. In this study we aimed to analyze the expression of the costimulatory molecules CTLA4 and CD28 on CD3+ lymphocytes. **Methods:** We performed cultures of PBMC isolated from patients with active disease and cured controls in presence of a *Candida albicans* antigen [CMA] and gp43. Gated CD3+ cells were analyzed by flow cytometry. **Results:** Results obtained up to now suggest that, upon CMA and gp43 stimuli, the percentages of CTLA4 expressing CD3+ T cells were higher in patients than controls. However, in unstimulated cells the percantages of CTLA4 cells were comparable. Neither CMA nor gp43 were able to up-regulate the expression of this molecule in patients and controls´ cells. In controls, however, CTLA4 expression was down modulated upon CMA and gp43 stimuli. In parallel we analyzed the mean fluorescence intensity (MFI) of CTLA4 in CD3+ cells of controls and patients. In the same way of the percentage analyzes, antigen-stimulated cells from patients showed a tendency for increased MFI as compared with the antigen-stimulated controls´ cells. The MFI of both patients and controls´ unstimulated cells were not up-regulated by antigen stimulation. Comparison between the percentages of CD28 expression by antigen-stimulated T cells from controls and patients revealed a tendency of decreased expression in the latter. Regarding the unstimulated T cells, there were no differences between patients and controls. CMA and gp43 stimulation did not enhance the MFI of cells from both patients and controls cells. **Conclusion:** The literature suggests that increased expression of CTLA4 by T cells results in negative signaling and feedback for the lymphoproliferative response when this molecule binds to its ligand, the CD80 molecule, on APCs. Our results suggest that such a negative feedback loop is also present in PCM patients, which together with the tendency to decreased expression of CD28, may relate to the decreased antigen-specific immune responses of the patients. However, in PCM patients, the high affinity CTLA4 ligand, CD80, was less expressed by APC than cured controls, thereby suggesting that other molecules participate in the suppression of the immune response of these patients. **Financial support:** Fapesp and LIM-56

01.006 - CD4⁺ AND CD8⁺ T LYMPHOCYTES CYTOKINE PRODUCTION IN HUMAN PARACOCCIDIOIDOMYCOSIS USING DIFFERENT STIMULI: PRELIMINARY RESULTS

Matano, G.[1]; Ferreira, E. C. J.[2]; Brunialti, M. C. K.[3]; Taborda, C. P.[4]; Colombo, A.[5]; Kallas, E. G.[6]; Salomao, R.[7]; Fazioli, R. A.[8]

[1,2,8]Adolfo Lutz Institute - Immunology; [3,6,7]UNIFESP - EPM - Medicina; [4]University of São Paulo - Department of Microbiology; [5]UNIFESP - Infectious Disease, SP, Brasil.

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a deep mycosis caused by the fungus *P. brasiliensis* (Pb). PCM disease can be classified in the chronic and acute forms. Patients, 3 years after the end of the therapy, present a restore cellular immune response against the fungus (defined as treated patients) and are considered excellent model to be used in cellular studies. Our objective was to obtain adequate Pb antigen to evaluate, in the future, the cytokine profile produced by CD4⁺ and CD8⁺ T cells in human PCM. **Methods and Results:** The peripheral blood mononuclear cells (PBMC) of treated patients and healthy controls were stimulated with PMA/Io, LPS and different concentrations of Pb antigens: cell free antigen (CFA), gp43 (immunodominant antigen of Pb) and P10 (fraction of gp43 that stimulated cellular immune response). After stimuli was evaluated CD4⁺ and CD8⁺ T cells subsets for the presence of IFN-γ, IL-2, IL-4, IL-5 and IL-10 production using an intracellular cytokine staining assay. Treated patients and healthy controls PBMC produced high levels of IFN-γ to PMA/Io and IL-10 to LPS stimuli. No cytokine production was observed in treated patients after stimulation with different concentrations of CFA, in contrast, a non-specific production of IFN-γ and IL-4 was observed in healthy controls. Different concentrations of gp43 did not induce cytokine production in treated patients and non-specific response in healthy controls. The P10 fraction induced increased mounts of IFN-γ, low levels of IL-2, IL-5 and IL-10 and no production of IL-4 in treated patients and did not stimulated any cytokine in the healthy controls. **Conclusion:** The non-specific stimuli PMA/Io and LPS induced high levels of IFN-γ and IL-10, respectively, in treated patients and healthy controls, which were considered indicative of a healthy immune system status and a good positive internal control for the assay. Unexpectedly, both CFA and gp43 antigens were not able to induce cytokines production by T cells in treated patients. Althought, CFA induce non-specific cytokine production (IFN-γ and IL-4) in healthy controls, but gp43 did not stimulate. In conclusion, of all antigens thus far tested, P10 showed a better specific stimulus to be used to characterize the cytokine pattern exhibited by CD4⁺ and CD8⁺ T cells in human paracoccidioidomycosis. **Financial support:** Adolfo Lutz Institute, UNIFESP, CAPES

01.007 - PROLIFERATIVE RESPONSE OF LYMPHOCYTES WITH 41 PEPTIDES OF GP 43 IN PATIENTS WITH PARACOCCIDIOIDOMYCOSIS

Sadahiro, A.[1]; Roque, A. C. M.[2]; Iwai, L. K.[3]; Cunha-Neto, E.[4]; Shikanai-Yasuda, M. A.[5]

[1]Universidade Federal do Amazonas - Parasitologia; [2,5]FM - USP - Laboratório de Investigação Médica em Imunologia LIM 48, Hospital das Clínicas, Departamento de Moléstias Infecciosas e Parasitárias; [3,4]Instituto do Coração (INCOR) - Laboratório de Imunologia

Introduction and Objectives: Synthetic peptides from gp 43, an immunodominant antigen of *Paracoccidioides brasiliensis*, were tested in proliferation assay of mononuclear cells from patients treated with different clinical forms of paracoccidioidomycosis. The aim of this study was to investigate the pattern of T cell proliferative response and to understand the mechanisms involved in paracoccidioidomycosis host-parasite interaction. **Methods and Results**: 41 synthetic peptides from gp 43 were tested at 0,1 and 1mM on proliferation assays with mononuclear cells from 44 Brazilian patients with acute form (n=12) and chronic form: unifocal (n=6) and multifocal (n=26) presentations of paracoccidiodomycosis. Moreover, 19 healthy individuals were included. To determine threshold reactivity of 41 peptides the ROC curve (Receiver Operating Characteristic) was applied, considering a specificity of ³ 90%. Ten peptides (P1, P7, P13, P17, P19, P24, P27, P35, P37 and P39) were recognized by 77,3% cells patients and by 26,3% cells of healthy individuals. Lymphoproliferative responses at 0,1 and 1mM concentrations showed differences statistically significant ($p < 0,05$) for P1, P13, P31, P33 and P35. Higher reactivity for P1 was detected at 1mM and at 0,1mM for the others. Comparison between healthy individuals and patients with different clinical forms of the paracoccidioidomycosis (acute, unifocal chronic and multifocal chronic) showed differences statistically significant for six peptides (P15, P16, P22, P28, P29 and P30). Higher lymphoproliferative response was detected in patients with acute form, while most of the patients with unifocal chronic form presented lower levels of lymphoproliferation response. **Conclusion**: In the present study, we could demonstrate peptides from gp 43 antigen capable to stimulate T cells from patients with treated paracoccidioidomycosis. Also, it was established the effect dose of some peptides, to compare the patterns of T cell response from patients of different clinical forms of illness in relation to the control group. The study of new immunogenic gp 43 peptides was proposed for therapeutical approach associated with antifungal drugs as new alternatives for the treatment of disseminated forms, particularly in immunosupressed patients. **Financial support:** FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) Processo No. 02/06481-6 and Fundação Faculdade de Medicina.

01.008 - SPECIFIC ANTI-*PARACOCCIDIOIDES BRASILIENSIS* IGG ISOTYPES IN PARACOCCIDIOIDOMYCOSIS PATIENTS AND CONTROLS FROM RURAL AREA

Ribeiro, S. C. P.[1]; Silva-Vergara, M. L.[2]; Rodrigues Jr., V.[3]

[1]Universidade Federal do Triângulo Mineiro - Departamento de Ciências Biológicas; [2]Universidade Federal do Triângulo Mineiro - Infectologia; [3]Univerdade Federal do Triângulo Mineiro - Departamento de Ciências Biológicas

Introduction and Objectives: The paracoccidioidomycosis is the most prevalent systemic mycosis in the Latin America countries. It is caused by the dimorphic fungus *Paracoccidioides brasiliensis* when its propagules is inhaled. The great majority of individuals remain asymptomatic (primary immunity) or develop sub clinical infection. Some patients will develop the infection with a broad range of clinical manifestations. Once Ig isotype production is controlled by Th1/Th2 cytokines, the aim of this study was to evaluate antigen specific IgG3 and IgG4 as an *in vivo* indicator of Th1 and Th2 immune response respectively. **Methods and Results**: Were selected n=183 individuals from rural area, with activity on (control group) and n=28 individuals with the diagnosis of Paracoccidioidomycosis (PCM) from the General Hospital of Universidade Federal do Triângulo Mineiro (HE-UFTM). Serum from those subjects were tested for the presence of anti *P. brasiliensis* total IgG, IgG1, IgG3 and IgG4 antibodies. Among the healthy individuals, 65 presented relevant levels of specific IgG antibodies and together with PCM patients (n=28) were tested for specific immunoglobulin isotypes. The levels of IgG3 and IgG4 were higher in the individuals with PCM that in the control group ($p=0,0005$, $p<0,0001$). PCM patients were grouped according to the clinical forms on chronic disseminated (n=14), chronic localized infection (n=7) and acute infection (n=7). Patients with the chronic disseminated infection presented higher levels of IgG3 ($p=0,007$) than the other clinical forms, otherwise patients with acute infection presented higher levels of IgG4 ($p=0,03$) than the others. **Conclusions**: These results suggest that the imbalance of Th1/Th2 immune response observed specially in more severe shapes of PCM, detected by cell culture is functional *in vivo*, once the predominance of an IgG4 response is observed on more severe infection. **Financial support:** FAPEMIG

01.009 - EFFECT OF IL-10 ON FUNGICIDAL ACTIVITY OF HUMAN NEUTROPHILS ACTIVATED WITH IFN-γ AND CHALLENGED WITH HIGH VIRULENT STRAIN OF *PARACOCCIDIOIDES BRASILIENSIS*

Costa, D. L.[1]; Acorci, M. J.[2]; Bordon, A. P.[3]; Peraçoli, M. T. S.[4]; Soares, A. M. V. C.[5]
[1,2,3,4,5]*IB - Unesp - Botucatu - Microbiologia e Imunologia*

Introduction and objectives: Phagocytic cells play a critical role against *Paracoccidioides brasiliensis*, and there are several papers showing the effects of activator and suppressive cytokines on macrophages and monocytes functions, when these cells are challenged with the fungus, but there are few ones performed with neutrophils. Works in our laboratory, showed that IFN-γ activates human neutrophils for fungicidal activity against *P. brasiliensis*, due to a higher release of H_2O_2 by these cells. Hence, the objectives of this work were to evaluate if IL-10, a suppressor cytokine, inhibits this acquired fungicidal activity, and if this process is related to a lower release of H_2O_2. **Methods and results:** Peripheral blood neutrophils obtained from 20 healthy donors were incubated for 18 h in the presence or absence of IFN-γ (50, 100, 250, 500 and 1000 U/mL), IL-10 alone (12,5, 25 and 50 ng/mL) or IFN-gð (50 U/mL) plus IL-10 (12,5, 25 and 50 ng/mL), and then challenged with high virulent strain of *P. brasiliensis* (Pb18), by 4 h. After, the cells were evaluated for their fungicidal activity, by counting of colony forming units after plating, and for H_2O_2 release. When incubated with IFN-γ (50 U/mL), the cells showed increased fungicidal activity (mean ± sem 22,16% ± 5,28), and higher H_2O_2 release (0,62 nM ± 0,19), compared to nontreated ones (5% ± 3,25 and 0,15 ± 0,05 respectively). IL-10 alone had no influence on fungicidal activity and H_2O_2 release, but it inhibited IFN-γ induced fungicidal activity (26,8% ± 2,00 for IFN-γ alone compared to 13,98% ± 1,25 for IFN-γ plus IL-10), and H_2O_2 release (0,39 nM + 0,14 for IFN-γ alone compared to 0,20nM + 0,03 for IFN-γ plus IL-10). **Conclusion:** IFN-γ increases the neutrophil fungicidal activity, due to a higher release of H_2O_2, and IL-10 inhibits this IFN-γ induced activity, due to a reduction in the metabolite release. This work shows the importance of early neutrophils exposure to activator or suppressor cytokines, since these cells are the first ones recruited to the infection sites. **Financial support:** Fapesp

01.010 - *PARACOCCIDIOIDES BRASILIENSIS* INHIBITS CYTOKINES AND H_2O_2 PRODUCTION BY HUMAN NEUTROPHILS

Acorci, M. J.[1]; Bordon, A. P.[2]; Dias-Melicio, L. A.[3]; Calvi, S. A.[4]; Costa, D. L.[5]; Peraçoli, M. T. S.[6]; Soares, A. M. V. C.[7]
[1,2,3,4,5,6,7]*IB - Unesp - Botucatu - Microbiologia e Imunologia*

Introduction and Objectives: Polymorphonuclear neutrophils (PMNs) are the predominant cells present in the early phases of murine infection with *P. brasiliensis*. In humans, the early histopathologic characteristics of paracoccidioidomycosis are not fully understood, but there are strong evidences that PMNs are involved in the primary host response to fungus, since studies point out the presence of these cells in infected tissues. Despite of these observations, the mechanisms involved in the response of human PMN to fungus challenge are poorly studied. The objective of this work was to better understand the response of human PMNs to *P. brasiliensis* by evaluating cytokines and H_2O_2 release after challenge of these cells with this fungus *in vitro*. **Methods and Results:** Peripheral blood neutrophils ($1X10^6$/ml) obtained from 10 healthy donors were incubated with tissue culture medium alone, LPS (20ug/mL) or high (Pb18) virulent strain of *P. brasiliensis* ($4X10^4$ yeasts/mL). After 12 h of coculture it was evaluated H_2O_2 production and the percentage of cells containing the intracytoplasmatic cytokines TNF-α, IL-6, IL-8, IL-10 and IL-12, by flow-citometry. After 18 h of coculture the cytokines released in the cocultures supernatants were evaluated by Elisa. PMN alone released low levels of H_2O_2 (mean±sem 2,0±0,3 nmoles) that were increased after LPS activation (3,0±0,6). However after fungus challenge the levels of H_2O_2 were yet lower than those detected for PMN (1,2±0,2). The evaluation of intracytoplasmatic cytokines showed that PMN alone produce all the cytokines tested (mean±sem 35,6%±13,7; 84,8%±4,8; 58,6%±22,9; 73,1%±7,3 and 79,6%±11,8 for TNF, IL-6, IL-8, IL-10 and IL-12 respectively). These levels tended to increase after LPS activation (53,4%±20,9; 84,8%±8,8; 53,8%±21,0; 79,1%±11,8 and 88,1%±3,7 for TNF, IL-6, IL-8, IL-10 and IL-12 respectively). The Elisa assays showed that PMN released TNF (545,5±126,9 pg/mL), IL-6 (298,7±148,9) and IL-8 (298,7±47,0). These levels were increased after LPS activation (871,8±248,1; 404,5±161,0 and 404,5±50,9 for TNF, IL-6 and IL-8 respectively). However after fungus incubation the levels of these cytokines were lesser than those released by neutrophils (TNF = 294,3±126,1; IL-6 = 160,9±93,0; IL-8 = 149,9±26,2). Significative levels of IL-10 and IL-12 were not detected in supernatants from cultures submitted to all stimulus (< 5 pg/ml). **Conclusion:** High virulent strain of *Paracoccidioides brasiliensis* inhibits cytokines and H_2O_2 production by human neutrophils. The results open perspectives for studies with the objective to better understand the real role of PMN in paracoccidioidomycosis defense mechanisms. **Financial support:** Supported by FAPESP

01.011 - LACK OF HIGH VIRULENT *PARACOCCIDIOIDES BRASILIENSIS* KILLING BY HUMAN MONOCYTES IS INDUCED BY PROSTAGLANDINS WHICH INHIBIT H_2O_2 AND TNF-ALPHA LEVELS

Bordon, A. P.[1]; Acorci, M. J.[2]; Dias-Melicio, L. A.[3]; Calvi, S. A.[4]; Peraçoli, M. T. S.[5]; Soares, A. M. V. C.[6]
[1,2,3,4,5,6]*Instituto de Biociências-UNESP-Botucatu - Depto Microbiologia e Imunologia*

Introduction and Objectives: Human monocytes lack fungicidal activity against high virulent strain of *Paracoccidioides brasiliensis*, the ethiological agent of paracoccidioidomycosis, even after IFN-γ activation. However, indomethacin (INDO) or INDO plus IFN-γ treated monocytes exhibit an effective killing against this fungus, suggesting a inhibitory role of prostaglandins in this process. Thus, the purpose of this work was to test if this regulatory effect of prostaglandin was associated with alterations on H_2O_2 production and/or on modulatory cytokines levels, such as TNF-α, IL-10 and IL-6. **Methods and Results:** Peripheral blood monocytes obtained from 10 healthy donors were incubated by 18h in the presence or absence of IFN-γ, INDO or IFN-γ plus INDO, and further challenged with high virulent strain of *P. brasiliensis* (Pb18) by 4h. After, the monocytes cultures were evaluated for H_2O_2 release and fungicidal activity calculated by counting of colony forming units after plating. Moreover, on supernatants of the same cultures it was evaluated the concentrations of TNF-α, IL-10 and IL-6 by ELISA. INDO or INDO plus IFN-γ treated monocytes presented higher fungicidal activity (mean±sem 20%±2.8 or 25%±2.5 respectively) associated with higher levels of H_2O_2 (1.0±0.2 or 0.9±0.2nm) and TNF-α, (826.6±230.3 or 989.8±233pg/ml) when compared to nontreated cells (2%±2.4; 0.6±0.1nm; 642±163.8pg/ml). However, the levels of IL-10 and IL-6 were similar between treated (IL-10: 280±75.1 or 290±62.6pg/ml and IL-6: 2406.5±382.3 or 2683.9+364.5pg/ml) and nontreated cells (250.9±79.8 and 2544.6±433.3pg/ml respectively). **Conclusion:** The results suggest that human monocytes when challenged with high virulent strain of *P. brasiliensis* produce prostaglandins that inhibit the fungicidal activity of these cells by reducing H_2O_2 and TNF-α levels. **Financial support:** FAPESP

01.012 - MONOCYTE-DERIVED MULTINUCLEATED GIANT CELLS INDUCED *IN VITRO* BY INTERFERON-GAMMA AND *PARACOCCIDIOIDES BRASILIENSIS* ANTIGEN DISPLAY FUNGICIDAL ACTIVITY

Nascimento, M. P. P.[1]; Soares, A. M. V. C.[2]; Parise-Fortes, M. R.[3]; Martins, R. A. R.[4]; Nakaira, E. T.[5]; Peraçoli, M. T. S.[6]
[1,2,4,5,6]*IB - UNESP - Botucatu - Microbiologia e Imunologia;* [3]*UNESP - Dermatologia e Radioterapia*

Introduction and Objectives: Multinucleated giant cells (MGC) are a common feature of granulomas that occur in chronic infectious diseases like tuberculosis and paracoccidiodomycosis. This study investigated *in vitro* formation of MGC derived from monocytes of healthy individuals, stimulated with recombinant human interferon-gamma (IFN-γ), *P. brasiliensis* antigens (PbAg), and supernatant of Concanavalin A-stimulated peripheral blood mononuclear cells (conditioned medium) (CM-ConA). Fungicidal activity of monocytes and monocyte-derived MGC challenged with *P. brasiliensis* strain (Pb 18) was also evaluated. **Methods and Results:** Peripheral blood monocytes obtained from healthy individuals were cultured for three days with or without IFN-γ (300 IU/mL), CM-ConA, or PbAg (100 mg/mL) stimulus. Fusion index and MGC formation percentage were determined after cell fixing and May-Grumwald-Giensa staining. Fungicidal activity of monocytes and monocyte-derived MGC were evaluated 4h after monocyte co-culture with Pb18 strains at a 50:1 monocyte-fungus ratio, by BHI-agar plating and determination of viable fungi recovery. The results demonstrated that IFN-γ enhanced MGC generation in a dose-dependent pattern, with fusion index and the percentage of formed MGC significantly higher than with CM-ConA and PbAg stimulation. This effect was abrogated by adding anti-IFN-γ monoclonal antibodies to the cultures. Both monocytes and monocyte-derived MGC induced *in vitro* by IFN-γ were able to kill the fungus, and the results were significantly higher in comparison with control cells cultured without stimulus. PbAg incubation also led to lower viable fungi recovery in monocytes and MGC cultures. **Conclusion:** Together, these data suggest that IFN-γ and PbAg are good stimuli for MGC generation and activation, and inducing fungicidal activity against *P.* b*rasiliensis.* **Financial support:** CAPES and FAPESP (nº 03/13743-0)

01.013 - EFFECT OF TRANSFORMING GROWTH FACTOR BETA ON THE FUNCTIONAL ACTIVITY OF HUMAN MONOCYTES "*IN VITRO*" INFECTED WITH *PARACOCCIDIOIDES BRASILIENSIS*

Martins, R. A. R.[1]; Soares, A. M. V. C.[2]; Nascimento, M. P. P.[3]; Nakaira, E. T.[4]; Peraçoli, M. T. S.[5]

[1]INSTITUTO BIOCIÊNCIAS - UNESP - BOTUCATU - MICROBIOLOGIA E IMUNOLOGIA; [2,5]IB - Unesp - Botucatu - Microbiologia e Imunologia; [3,4]IB-Unesp-Botucatu - Microbiologia e Imunologia

Introduction and Objectives: Transforming Growth Factor-beta (TGF-β_1) is a cytokine produced by cells such as macrophages and T cells having both pro- and anti-inflammatory properties depending on their environment and concentration. The aim of this study was to analyze the effect of TGF-β_1 on the hydrogen peroxide (H_2O_2) release, Tumor Necrosis Factor-alpha (TNF-α) production, and fungicidal activity of human monocytes challenged with high-virulent strain of *Paracoccidioides brasiliensis* (Pb18). **Methods and Results:** Peripheral blood monocytes from healthy individuals were preincubated with or without different concentrations (7.8 pg/ml to 500 pg/ml) of TGF-β_1 for 24 h at 37ºC, and then challenged with Pb18 in a ratio of 50:1 monocyte:fungus. The release of H_2O_2 by monocytes in response to Phorbol Myristate Acetate (PMA) was evaluated during and after 4h of monocyte infection with the fungus. TNF-α production by these cells was determined in supernatant cultures by enzyme immunoassay (ELISA), and fungicidal activity of monocytes against Pb18 was assessed by viable fungi recovery from 4h co-culture in Blood Heart Infusion-Agar (BHI-Agar) and counting of colony-forming units after 10 days. The results showed that monocyte incubation with TGF-β_1 concentrations (31.2 pg/ml to 500 pg/ml) suppressed H_2O_2 release in a dose-dependent manner. The Pb18 infection of monocytes pretreated with TGF-β_1 maintained the inhibitory effect on the H_2O_2 production by these cells stimulated with PMA, even in low doses of TGF-β_1, suggesting that Pb18 may also interfere with H_2O_2 production by monocytes. These cells challenged with Pb18 produced significantly higher levels of TNF-α in comparison to monocytes not infected. However this production was inhibited when these cells were previously cultured with high concentrations of TGF-β_1. On the other hand, pretreatment of monocytes with high doses of this cytokine enhanced their fungicidal activity against *P. brasiliensis*. **Conclusion:**Together the results suggest that exogenous TGF-β_1 can exert a dual modulatory effect on monocytes infected with *P.brasiliensis*, when used in high concentrations. The effects are stimulatory on fungicidal activity and inhibitory on H_2O_2 release and TNF-α production.

01.014 - FREQUENCIES OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE IL-10 AND TNF-AÐ CYTOKINES GENES IN PARACOCCIDIOIDOMYCOSIS

Pereira, P. N. P.[1]; Bozzi, A.[2]; Reis, B. S.[3]; Goulart, M. I.[4]; Pereira, M, C. N.[5]; Pedroso, E. P.[6]; Leite, M. F.[7]; Goes, A. M.[8]

[1,5,6]UFMG - Faculty of Medicine; [2,3,4,8]ICB/UFMG - Department of Biochemistry and Immunology; [7]ICB/UFMG - Department of Physiology and Biophysics

Introduction and Objectives: Allelic variants of the cytokine genes seem to contribute to the resistance or susceptibility to several diseases (Europ.Journ.Immuno., 29:371-374, 2002). This study was performed in order to evaluate the -308G/A single nucleotide polymorphism (SNP) in the tumor necrosis factor-α (TNF-α) gene and the -1082G/A SNP in the interleukin 10 (IL-10) gene in paracoccidioidomycosis (PCM). This disease is caused by fungus *Paracoccidioides brasiliensis* and affects millions of individuals in the Latin America, having serious morbidity and mortality (Clin.Microbio.Rev., 6:89-117, 1993). We also analyzed the frequency of cell producing cytokines in response to the secreted antigen (MEXO), correlating to polymorphism. **Methods and Results:** This study analyzed the SNP´s using an allele-specific polymerase chain reaction (ASPCR) and PCR restriction fragment length polymorphism (RFLP) methods. Non-infected subjects and patients infected by *P. brasiliensis*, with overt disease were evaluated. The results showed a different distribution of the genotypes in the –1082 SNP IL-10 locus between patients group (GG=28.6%; GA=51.0%;AA=20.4%;n=49) and controls (GG=6.5%;GA=54.8%;AA=38.7%;n=31) (p=0.030). The genotypes distribution in –308 SNP TNF-α locus was different in patients group (GG=87.8%;GA=7.3%;AA=4.9%;n=41) when compared to controls (GG=70.0%;GA=26.7%;AA=3.3%;n=30), but it was not significant (p=0.084). Subjects were grouped in A+ (AG, AA genotypes) and A- (GG genotypes). A lower frequency of A+ subjects for -1082 IL-10 SNP was found in patients group (71.4%; n=49) compared to controls (93.5%; n=31) (p=0.016). In the -308 TNF-α SNP analysis, the patients group also showed a lower frequency of A+ subjects (12.2%; n=41) compared to controls (30.0%; n=31), but the difference was not statistically significant (p-value=0.063). The frequency of cell producing cytokines in response to MEXO was measured by flow cytometry and correlated to polymorphism. The A+ group had a higher frequency of cells producing IL-10 (mean=3.81% ± 3.80; n=13) when we compared to A- group (mean=1.26% ± 0.74; n=6), but it didn't reached the statistical significance (p=0.065). Similar results were seen in frequency of cells producing TNF-α in A+ group (mean=11.43% ± 23.64; n=5) when compared to A- group (mean=6.52% ± 9.00; n=9) (p=0.463).**Conclusion:** These data suggest that the GG genotype in -1082 IL-10 locus and probably in the -308 TNF-α locus could be correlated to an increased susceptibility to PCM, besides, due to a high standard deviation, a strong relation to frequency of cells producing cytokines could not be done. However, the study of SNP´s may help to understand the disease and could be a valid tool for identification of subjects which need a more appropriate therapy. **Financial support:** CAPES and CNPq

01.015 - MODULATORY EFFECT OF AMPHOTERICIN B ON TUMOR NECROSIS FACTOR-ALPHA PRODUCTION BY HUMAN MONOCYTES INFECTED WITH *PARACOCCIDIOIDES BRASILIENSIS*

Nakaira, E. T.[1]; Soares, A. M. V. C.[2]; Arita, L. S.[3]; Matsumoto, L. E.[4]; Peraçoli, M. T. S.[5]

[1,2,3,4,5]IB - UNESP - Botucatu - Microbiologia e Imunologia

Introduction and Objectives: Itraconazole (itra) has been utilized as the choice antifungal drug for the treatment of patients with paracoccidioidomycosis due to its high clinical efficiency. Therapeutic use of amphotericin B (amph B) is limited by its toxicity to patients including fever, chills, anorexia and nausea as well as chronic renal toxicity. These side effects may be resultant from pro-inflammatory cytokines release by inate immune cells induced after amph B stimulation. We investigated whether amphotericin B (amph B) and itraconazole (itra) can modulate tumor necrosis factor alpha (TNF-α) production by human monocytes *in vitro* infected with viable yeast-like form cells of *Paracoccidioides brasiliensis*. **Methods and Results:** Peripheral blood monocytes obtained from healthy individuals were *in vitro* cultured for 18 h at 37ºC with or without suspensions of *P. brasiliensis* viable yeast cells (Pbv) in the ratio of 1:50 fungus-monocytes. Different concentrations of amph B (0.0625 to 2 mg/mL) or itra (0.001 to 0.016 mg/mL) were added to these cultures and TNF-α was determined in the supernatant by enzyme immunoassay (ELISA). The results showed that monocyte stimulation with amph B or itra alone did not induce significant levels of TNF-α in relation to non-stimulated cultures. Addition of low concentrations of amph B such as 0.0625, 0.125 or 0.25 mg/mL to monocyte cultures infected with Pbv led to significantly higher TNF-α levels 314.3 $\pm$ 61.2, 338.5 $\pm$ 82.5 and 323.7 $\pm$ 49.2 mg/mL respectively in comparison to those detected in cultures only stimulated with Pbv (142.4 $\pm$ 23.5 mg/mL). This modulatory effect was not observed when itra was employed to monocyte treatment. **Conclusion:** This study provides evidence that low concentrations of amph B are able to enhance TNF-α production by human monocytes during infection with *P. brasiliensis*. This may explain the side effects observed after treatment of patients with amph B. **Financial support:** FAPESP no. 03/13743-0

01.016 - PRODUCTION OF TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKIN-6 BY HUMAN MONOCYTES STIMULATED *IN VITRO* WITH *PARACOCCIDIOIDES BRASILIENSIS* TREATED WITH AMPHOTERICIN B AND ITRACONAZOLE

Nakaira, E. T.[1]; Sugizaki, M. F.[2]; Soares, A. M. V. C.[3]; Nascimento, M. P. P.[4]; Martins, R. A. R.[5]; Peraçoli, M. T. S.[6]

[1,2,3,4,5,6]IB - UNESP - Botucatu - Microbiologia e Imunologia

Introduction and Objectives: Monocytes from patients with paracoccidioidomycosis are important source of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin–1 (IL-1), and IL-6, that may be associated with the pathogenesis of the mycosis. On the other hand, the disease treatment with antifungal ats led to fungal load diminution, being a promising option for the control of paracoccidioidomycosis. The present work aimed to evaluate TNF-α and IL-6 production by human monocytes, *in vitro* stimulated by yeast-like form cells of *Paracoccidioides brasiliensis*, previously submitted to the treatment with amphotericin B (amph B) or itraconazole (itra). **Methods and Results:**Peripheral blood monocytes obtained from healthy individuals were *in vitro* cultured for 18 h at 37ºC with suspensions of *P. brasiliensis* viable yeast cells (Pbv), yeast cells killed by autoclaving (Pbaut), viable cells treated for 10 min with amph B (Pbamph-10), or with itra (Pbitra-10), yeast cells killed by amph B treatment for 36 h with the minimun fungicidal concentration of amph B (Pbamph-36) or yeast cells treated for 72 h with the minimum inhibitory concentration of itra (Pbitra-72), in the ratios of 1:1 and 1:50 fungus-monocytes. The supernatants obtained from these cultures were employed for TNF-α and IL-6 determination by enzyme immunoassay (ELISA). The results showed that human monocytes *in vitro* cultured with Pbv, or Pbaut release significantly higher levels of TNF-α and IL-6 in comparison with monocytes not stimulated with the fungus (control). Preincubation if Pb with amph B ou itra does not reduce cytokine levels produced by monocytes when the fungus:monocyte ratio is 1:1. The lower production of TNF-α by monocytes stimulated, with Pbv, Pbaut, or Pbitra obtained in the 1:50 fungus:monocyte ratio, suggests that TNF-α release depends on the concentration of fungal cells components. The high levels of IL-6 produced by monocytes stimulated with yeast-like cells of *P. brasiliensis* in the fungus:monocyte ratios of 1:1 and 1:50, suggest that low concentration of cell wall components are enough to induce this cytokine production. **Conclusion**: The results suggest that cell wall components of *P. brasiliensis* may be responsible for the induction of the inflammatory cytokines by monocytes. The high concentration of TNF-α produced by monocytes stimulated with Pbamph both at fungus:monocyte ratio of 1:1 and 1:50, indicate that amph B exerts modulatory effect on TNF-α production by monocytes. **Financial support:** FAPESP no. 02/12462-4 and no. 03/13743-0

01.017 - HUMAN COMPLEMENT SYSTEM ACTIVATION BY *PARACOCCIDIOIDES BRASILIENSIS*, IN VITRO

Toledo, R. G.[1]; Gomes, G. I.[2]; Dias da Silva, W.[3]; Kipnis, T. L.[4]
[1,2,3,4]Universidade Estadual do Norte Fluminense Darcy Ribeiro - Laboratório de Biologia do Reconhecer

Introduction and objectives: The pathogenicity of *Paracoccidioides brasiliensis* is influenced by its cell wall hexoses (Sabouraudia, 20:31-40, 1982). These carbohydrates are strong candidates for activation of the complement system (CS) particularly the lectin pathway. It´s well described that the fungus is capable of activating the alternative pathway, is opsonized by C3 and C4 fragments and augments its ingestion by murine macrophages (Clin. Immunol. Immunopathol., 12:20-30, 1979; J. Med. Vet. Mycol., 30:317-321, 1992; J. Med. Vet. Mycol., 30:481-484, 1992). This study aimed to investigate the activation of the alternative, classical and lectin pathways (AP, CP, LP) activation by the fungus, *in vitro.* **Methods:** *P. brasiliensis* yeast forms at concentrations of 10^5, 10^6 or 10^7 cells/mL were incubated with normal human serum (NHS). The total hemolytic activity was assayed by using sheep red erythrocytes (E^s) pre-sensitized with rabbit antibodies anti-E^s in GVBS buffer (containing 15 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, and 1.8 mmol/L sodium barbital), or antibody non sensitized rabbit red erythrocytes in GVBS-MgEGTA (containing 2 mmol/L $MgSO_4$, and 10 mmol/L EGTA), to analyze the functional state of the AP and CP complement activation, respectively. The serum levels of the individual components C3, and C4 were immunochemically quantified by ELISA using specific monoclonal antibodies (mAbs) as probes. The LP activation was analyzed by mannose-binding protein (MBL) consumption using mAbs in ELISA. NHS samples treated with buffer or zymosan were included as controls. **Results:** We observed that *P. brasiliensis* activates and significantly reduces the hemolytic activity of NHS when AP and CP were analyzed. The fungal action was dose and time dependent. Levels of C3 and C4 in the respective samples were also significantly reduced when compared to the controls. We observed that samples that activated CS (hemolytic assay and ELISA for C3 and C4) had also consumed MBL. **Conclusions:** These results indicate that *P. brasiliensis* yeast forms, of the virulent strain Pb18, activate the AP and LP of CS in NHS samples *in vitro*, and suggest that these pathways may influence fungi/host cell interactions, *in vivo*. Since either the CP or the LP lead to C4 consumption, MBL-associated serine proteases (MASPs) consumption are now being measured in order to discriminate the participation of both/one pathways. **Financial support:** UENF, FAPERJ, CNPq

01.018 - INTRACELLULAR PARASITISM OF *PARACOCCIDIOIDES BRASILIENSIS* VIABLE SMALL FORMS IN MONONUCLEAR CELLS FROM PATIENTS WITH CHRONIC PARACOCCIDIOIDOMYCOSIS

Ricci, L. C.[1]; Blotta, M. H.[2]
[1]UNICAMP - Depart. de Microbiologia e Imunologia; [2]UNICAMP - Depart. Patologia Clínica

Introduction and Objectives: Ultrastructure of peripheral mononuclear blood cells (PBMC) from patients with paracoccidioidomycosis (PCM) and unaffected individuals had been studied, showing significant ultrastructural differences between these cells. After cultures of both PBMC groups on lymphocyte transformation test (LTT) by phytohemagglutinin developed with autologous or heterologous plasma many alterations in the ultrastructure of lymphocytes from patients with both plasma and also in controls could be observed. When controls lymphocytes were cultured on LTT with heterologous plasma (from patients) blasts showed ultrastructural alterations, different degrees of cytoplasmatic and nuclear alteration, such as organelle dissolution, vacuoles, amorphous masses, deformed nuclei, and absence of nucleoli. Morphological and ultrastructural abnormalities were observed when compared to those obtained from the same PBMC control from LTT with control plasma. However these morphological differences in lymphocytes from patients with PCM and same cells from control after LTT with heterologous plasma were never understood until date. The aim of this work is to prove that these ultrastructural alterations observed in the lymphocytes and blasts from patients with PCM and in controls from LTT with heterologous plasma are caused by an explosive intracellular (i.c.) infection of PBMC by viable forms of *P. brasiliensis* also present in the patients plasma. **Methods:** PBMC from six patients with the chronic form of PCM and from six normal persons were collected, washed, counted and separated in six sterile eppendorfs tubes for further experimental approaches that included LTT cultures, indirect immune reactions against three different antiserums and analysis of the fungus i.c. viability by inoculating lysades of PBMC in Vero culture cells and in conventional media. Free forms of *P. brasiliensis* in the plasma were also researched. **Results:** PBMC and blasts from patients with PCM and unaffected individuals blasts after LTT cultures with heterologous plasma showed serological positive reactions with all sera. The viability of i.c. forms were comproved by intense growing of the fungus in Vero cells after just 18 h p.i.. Different free forms of the fungus were observed in the plasma of these patients. **Conclusion:** To our knowledge, the results of this study reveal the most important pathogenic mechanism ever elucidate from *P. brasiliensis* comprising i.c. facultative parasitism in PBMC from patients with PCM, disease which etiological agent expresses unlimited pathogenic resources. Results that open many avenues to researches for the knowledge associated with PCM as the great diversity of clinical manifestations, alterations detected in immunity associated with regulatory T cells subsets, composition of the mononuclear cell subsets, recidives, immunosuppression and much more. **Financial support:** FAPESP, FAEP/UNICAMP, CAPES

01.019 - PARACOCCIDIOIDOMYCOSIS INFECTION IS CHARACTERIZED BY A RAPID AND STRONG TH1 RESPONSE, WITH THE $CD8^+$ LYMPHOCYTES AS AN IMPORTANT SOURCE OF IFN-GAMMA

Mamoni, R. L.[1]; Burlandy-Soares, L.[2]; Blotta, M. H.[3]
[1,2,3]UNICAMP - Patologia Clínica

Introduction and objectives: The human infection with *Paracoccidioides brasiliensis* may result in three major outcomes: paracoccidioidomycosis-infection (PI), which is observed in healthy carriers living in endemic areas and the adult form (AF) and juvenile form (JF) of the disease. The aim of the study was to compare these groups in relation to the kinetics of mRNA expression of Th1 and Th2 cytokines and to characterize the cells responsible for their production. **Methods and Results:** The PI group (n=15) was identified by positive DTH test to *P. brasiliensis* antigen. The patients were grouped according to clinical presentation in AF (n=15) or JF (n=10). The time kinetics (0, 3, 6, 12, 24 and 48 hours of PHA stimulus) of mRNA expression for IFN-γ, TNF-α, IL4 and IL10 were analyzed by a semi-quantitative RT-PCR (normalized by b-actin mRNA). Protein expression was analyzed by flow-cytometry. We found that PI group (n=15) presents earlier and higher expression (cytokine/b-actin± SE) of mRNA than JF group (n=10) for IFN-γ (PI *vs.* JF: T_0 - 0.6±0.1 *vs.* 0.0; T_3 - 1.0±0.1 *vs.* 0.2±0.1; T_6 - 1.2±0.1 *vs.* 0.4±0.2) and TNF-α (PI *vs.* JF: T_3 - 0.6±0.1 *vs.* 0.3±0.1; T_6 - 0.5±0.1 *vs.* 0.1±0.1). On the other hand, JF group presents a significantly earlier and higher expression of mRNA (cytokine/b-actin±SE) than PI group for IL4 (JF vs. PI: T_0 – 0.2±0.05 vs. 0.0±0.0, T_3 – 0.79±0.09 vs. 0.3±0.08, T_6 – 1.2±0.1 vs. 0.5±0.1, T_{12} – 0.8±0.1 vs. 0.3±0.08, T_{24} – 0.81±0.1 vs. 0.4±0.07), IL10 (JF vs. PI: T_6 – 0.8±0.17 vs. 0.15±0.07, T_{12} – 1.0±0.16 vs. 0.2±0.08, T_{24} – 0.87±0.16 vs. 0.2±0.08). Similar results were found in relation to the AF group. Flow-cytometry analysis confirmed mRNA findings and showed that CD8+ lymphocytes have a prominent role in the production of Th1 cytokines, mainly IFN-γ. PI group presents a significantly higher percentage of lymphocytes expressing IFN-γ and TNF-að than AF and JF group: IFN-γ (PI: 25.8±1.8, AF: 14.6±2.6, JF: 7.6±1.3); TNF-α (PI: 17.5±1.9, AF: 7.5±1.6, JF: 3.2±0.9); whereas IL-10 expressing monocytes were found mainly in JF and AF group. **Conclusions:** These data showed that the resistance to the infection in the human PCM is related to a rapid and strong Th1 response, as exhibited by cells of the PI group, and that the $CD8^+$ cells can be an important source or Th1 cytokines as IFN-γ and TNF-α. **Financial support:** FAPESP

01.020 - ELEVATED LEVELS OF SERUM IL-18 AND STNFRII DISTINGUISH JUVENILE FROM ADULT FORM OF PARACOCCIDIOIDOMYCOSIS (PCM) AND ARE ASSOCIATED WITH DISEASE SEVERITY

Mamoni, R. L.[1]; Corvino, C. T.[2]; Blotta, M. H.[3]
[1,2,3]UNICAMP - Patologia Clínica

Introduction and Objectives: IL-18 is a pro-inflammatory cytokine of the IL-1 superfamily that exhibits broad functional effects in innate and acquired immune responses and which has been found in high levels in several chronic inflammatory and autoimmune diseases. Over-expression of IL-18 may promote early resolution of infection or could promote a detrimental exaggerated immune response. The aim of this study was to determine serum levels of IL-18 and other inflammatory markers (IL-12, sICAM-1, sTNFRI, sTNFRII) in patients with the JF in comparison to patients with the adult form of PCM as well as in infected (PI) and non-infected controls (C), and to assess their possible relationships to the severity of disease. **Methods and Results:** Twenty-one individuals of each group were analyzed. Sera samples were collected from all patients with PCM at active stage before treatment. The levels of IL-18, IL-12, sICAM-1, sTNFRI and TNFRII were determined by ELISA, using commercial kits. IL-18 (709.9±31.9 pg/mL) and sTNFRII (28.4±5.9 ng/mL) levels in patients with the JF of PCM were significantly higher than those in the AF (440.0± 53.0 pg/mL and 12.8±6.5 ng/mL) and controls (PI – 313.1±31.9 pg/mL and 8.0±0.7 ng/mL; C – 319.5±35.7 pg/mL and 7.7±0.7 ng/mL). In relation to sICAM-1, no difference was observed between JF (381.6 ± 44.6 ng/mL) and AF (324.8 ± 20.3 ng/mL) but both presented higher levels than controls (PI – 148.2 ± 11.4 ng/mL; C - 161.2 ± 11.4 ng/mL). sTNF RI levels in JF patients (1705.0 ± 192,8 pg/mL) were higher than controls (PI – 707.0 ± 44.6 pg/mL; C – 762.4 ± 62.5 pg/mL), but do not differ from AF group (3157.0 ± 420.8 pg/mL). IL-12 levels were also higher in patients (JF – 111.3 ± 18.9 pg/mL and AF – 118.0 ± 17.6 pg/ml) than in controls (PI – 51.36 ± 3.6 pg/ml and C – 46.6 pg/mL ± 4.1 pg/mL). IL-18 levels significantly correlated with sICAM-1 (r=0.71, p<0.0001), sTNFRI (r=0.65, p<0.0001), IL-12 (r=031, p=0.003), anti-*P. brasiliensis* gp43 antibody titer (r=0.4, p=0.007), specific IgE levels (r=0.34, p=0.04) as well as with clinical severity scores. **Conclusion:** sICAM-1, sTNFRI and IL-12 are elevated in PCM but do not discriminate between JF and AF. On the other hand, the results suggest the value of serum IL-18 and TNFRII levels as a parameter of PCM severity and may support a possible role for them in the pathogenesis of the disease. **Financial support:** FAPESP

01.021 - DENDRITIC CELLS OF PATIENTS WITH PARACOCCIDIOIDOMYCOSIS ARE NOT ACTIVATED BY GP43 OF *PARACOCCIDIOIDES BRASILIENSIS*

Baida, H.[1]; Ogusuku, S.[2]; Mendes-Giannini, M. J. S.[3]; Benard, G.[4]; Duarte, A. J. S.[5]; Romano, C. C.[6]

[1]Faculdade de Medicina da Universidade de São Paulo - Dermatologia; [2]Faculdade de Medicina da USP - Dermatologia; [3]Escola de Farmácia, UNESP, Araraquara, São Paulo, Brasil - farmácia; [4]USP - Laboratório de Alergia e Imunologia Clínica e Experimental, Faculdade de Medicina , São Paulo, SP, Brazil; [5]FM - USP - Dermatologia e Imunodeficiências; [6]USP - Dermatologia

Introduction: Paracoccidioidomycosis (PCM) is a mycosis that evolves with variable degrees of cellular immune immunosuppression, according to the severity of the clinical presentation. Dendritic cells, the most potent antigen presenting cells, have been shown in human models to induce immune responses against many antigens and to interact with T and B lymphocytes and to modulate its responses. In this study, we analyzed the activity of human monocyte-derived dendritic cells stimulated *in vitro* with gp43 of *P. brasiliensis.* **Methods and Results:** The suspension of mononuclear cells was obtained through gradient of Ficoll-hypaque density from peripheral blood, washed for 2 times in RPMI 1640 and centrifugated in another density gradient of Percoll for the obtention of monocytes enriched suspension. The cells were cultivated in 6 wells plates for 7 days in the presence of GM-CSF and IL-4. After differentiation, the cells were cultivated for 18h with RPMI (medium) or stimulated with gp43. The following costimulatories molecules were analyzed by flow cytometry: CD1a, CD11c, CD86, CD14 and HLA-DR. In parallel, we used dendritic cells of healthy individuals as control. In addition, we also verify the cellular morphology by microscopy examination. The incubation of patients' DC for 18 h with gp43 did not modify the expression of costimulation markers in none conditions of the culture (media ± SE - CD1a: 1,39 ± 0,5 medium vs 3,37 ± 1,0 gp43), (CD86: 23 ± 6,7 vs 22,4 ± 5,6 gp43); (CD14: 2,4 ± 0,7 medium vs 1,6 ± 0,2 gp43) or (HLA-DR: 22,6 ± 4,8 medium vs 18,9 ± 7,4 gp43). Moreover, in the same experiments, we observed a decrease in Cd11c expression in gp43 stimulated cells (52,5 ± 9,5 medium vs 41,3 ± 8,1, $P< 0,05$). The DC of the controls stimulated with gp43 were activated because we observed increase in the expression of CD11c (20,9 ± 3,6 medium vs 29,4 ± 3,0, P<,05), CD86 (14,8 ± 3,7 medium vs 23,2 ± 4,8 gp43, P<,05) and HLA-DR (7,8 ± 0,8 medium vs 15,7 ± 3,8 gp43). **Conclusion:** Taken together, these results suggest that *P. brasiliensis* inhibits activation of immature DC of patients by a mechanism that involves decreased expression of the CD11c and this can result in dysfunction of the host-immune response. **Financial support:** FAPESP 03/10597-2, 03/06515-0; LIM-56 HC

01.022 - PROLIFERATIVE RESPONSE TO ANTIGEN FILTERED FROM *P. BRASILIENSIS* CULTURE AND METAPERIODATE-TREATED GP43 IN PARACOCCIDIOIDOMYCOSIS PATIENTS AND CURED INDIVIDUALS - COMPARISON WITH GP43

Ogusuku, S.[1]; Baida, H.[2]; Duarte, A. J. S.[3]; Mendes-Giannini, M. J. S.[4]; Kono, A. S. G.[5]; Benard, G.[6]; Romano, C. C.[7]

[1]Faculdade de Medicina da Universidade de São Paulo - Dermatologia; [2]Faculdade de Medicina da Universidade de São Paulo - Dermatologia; [3]FM - USP - Dermatologia e Imunodeficiências; [4]UNESP - Departamento de Analises Clinicas, Faculdade de Ciencias Farmaceuticas; [5]Faculdade de Medicina da Universidade de São Paulo - Divisão de Moléstias Infecciosas e Parasitarias ; [6]USP - Laboratório de Alergia e Imunologia Clínica e Experimental, Faculdade de Medicina , São Paulo, SP, Brazil; [7]USP - Dermatologia

Introduction: Previous works with in vitro stimulation of peripheral blood mononuclear cells (PBMC) of patients with PCM using gp43 of *P. brasiliensis* had demonstrated low limphoproliferative responses that were restored with neutralization of IL-10 and addition of IL-12. These characteristics suggest an imbalance of the IL-12/IL-10 axis, a phenomenon observed mainly with gp43. Thus, in this study we examine the proliferative response of patients with the chronic form of the disease (CR) and of cured controls (CC) front to filtered of culture free of gp43 (Filtered) and gp43 treated with metaperiodate (Mgp43). **Methods and Results:** The suspension of mononuclear cells was obtained through gradient of Ficoll-hypaque density from peripheral blood, washed for 2 times in RPMI 1640. CC presented low response to antigens derived from fungus (average of 941 ± 157 for filtered, 1221 ± 245 cpm for Mgp43) always inferior to that observed with gp43 (8263 ± 1675), considering the values for non-stimulated cells (285 ± 106 cpm). We also observed in this group preserved proliferative responses to the metabolic antigen of *Candida albicans* (average of 9448 ± 1589) and for PWM (average of 28216 ± 3495). In CR patients, response for antigens derived from fungus was observed low (average of 683 cpm ± 457 for filtered, 416 ± 119 for Mgp43 and 694 ± 431 for gp43), always considering the background proliferation (average of 258 ± 78 cpm) and normal values of limfoproliferative for antigen not related to fungus (*Candida albicans*: average of 12070 ± 1743) and for mitogen PWM (average of 35255 ± 4176). We additionally studied the specificity of these antigens performing cultures of not sensitized healthy individuals to the fungus antigens and we did not find detectable proliferative responses. **Conclusion:** These data suggest that gp43 seems to be the antigen most representative in the cellular immunity. **Financial support:** FAPESP 03/10597-2,03/06515-0; LIM-56 HC.

01.023 - EVALUATION OF PHAGOCYTOSIS OF *P. BRASILIENSIS* IN DENDRITIC CELLS OF PATIENTS WITH PARACOCCIDIOIDOMYCOSIS AND HEALTHY INDIVIDUALS

Ogusuku, S.[1]; Baida, H.[2]; Duarte, A. J. S.[3]; Mendes-Giannini, M. J. S.[4]; Benard, G.[5]; Romano, C. C.[6]

[1]Faculdade de Medicina da USP - Dermatologia; [2]Faculdade de Medicina da Universidade de São Paulo - Dermatologia; [3]FM - USP - Dermatologia e Imunodeficiências; [4]UNESP - Departamento de Analises Clinicas, Faculdade de Ciencias Farmaceuticas; [5]USP - Laboratório de Alergia e Imunologia Clínica e Experimental, Faculdade de Medicina , São Paulo, SP, Brazil; [6]USP - Dermatologia

Introduction: Paracoccidioidomycosis (PCM) is a mycosis that evolves with variable degrees of cellular immune immunosuppression, according to the severity of the clinical presentation. The mechanism of cellular immunity seems to be crucial for the control and recovery of the disease. In this context, the dendritic cells present an important role, constituting a family of antigen presenting cells with capacity to interact with T and B lymphocytes and to modulate heir reactivities. In this study, we evaluate the in vitro phagocytosis of the dendritic cells (DC) of healthy individuals and patients with PCM challenged with *Paracoccidioides brasiliensis*. **Methods**: Suspension of mononuclear cells was obtained through gradient of Ficoll-hypaque density from peripheral blood, washed for 2 times in RPMI 1640 and centrifugated in another density gradient of Percoll for the obtention of monocytes enriched suspension. The cells were cultivated in 6 wells plates for 7 days in the presence of GM-CSF and IL-4. On day 7, we analyzed the differentiation of monocytes into dendritic cells verifying the expression of the following surface markers: CD11c, CD14, CD86 and HLA-DR by flow cytometry. Moreover, the cellular morphology by microscopy was also verified. The dendrític cells (DC) were incubated with *P. brasiliensis* marked with FITC for a period of 1h and 24h in eppendorf tubes, protected of the light. The analysis of phagocytosis was also carried out by flow cytometry. **Results:** Healthy individuals DC of stimulates with the fungus demonstrated 59.7% of phagocytosis when the cells were kept for 1h and 54.2% in the 24h of incubation. On the other hand, DC of individuals with active PCM showed a drastic reduction or absence of phagocytosis in the same periods (1,5% for 1 hour and 5,2% for 24h). We did not observe differences between controls and patients (37,13% versus 44,71%) for dextran phagocytosis index. **Conclusion:** These data suggest a deficiency of recognition and phagocytosis in DC of the individuals with PCM front to fungus, suggesting a possible mechanism of alteration in the innate immune response. However, it will be necessary to investigate and to characterize the recognition receptors, cytokines and costimulatory molecules in this response. **Financial support:** FAPESP 03/10597-2, 03/06515-0; LIM56 HC