GALECTIN-3 INDUCES HUMAN MONOCYTE MIGRATION BY HAPTOTATIC MECHANISM

POLLI C. D.(1), TOLEDO K. A.(1), BERNARDES E. S.(1), ROQUE-BARREIRA M. C.(1)

(1)Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto-USP, SP, Brazil.

Galectin-3 (gal-3) has an important role in the inflammatory process, demonstrated by the fact that gal-3 deficient mice (gal3-/-) show decreased recruitment of inflammatory leukocytes in a peritonitis model. In addition, gal-3 is a powerful attractant of monocytes and macrophages. Since leukocyte migration stimulated by lectins was shown to be done through haptotaxis, we investigated if a such mechanism accounts for the human monocyte migration induced by gal-3, as well the participation of components of the extracellular matrix (ECM) in the process. Human monocytes were purified by Ficoll gradient from health voluntaries. The cells were incubated with biotinyl-rgal3 and binding was revealed by streptoavidin-PE. Flow cytometry assays showed that rgal-3 binds to human monocytes, an interaction that was selectively inhibited by lactose. By using Boyden microchamber and Transwell System, we demonstrated that gal-3 induces monocyte migration in a manner that depends on its concentration, as well on its sugar recognition ability. We also showed that the migration is due to a haptotatic mechanism, since the responses to sub-optimal doses were increased when the wells were coated with ECM components, especially with laminin and fibronectin. The interaction of rgal-3 and ECM components was confirmed through microplate assays, which demonstrated that binding to laminin is sugar recognition dependent, whereas interaction with fibronectin depends on protein-protein interaction. Galectin-3 induces human monocyte migration through a haptotatic mechanism, a process that involves gal-3 binding to ECM components, such as laminin and fibronectin.

KEY WORDS: Galectin-3, monocyte, migration, haptotaxis, extracellular matrix

CORRESPONDENCE TO:

Karina A. Toledo, Depto. de Biologia Celular e Molecular, FMRP-USP, Av. Bandeirantes 3900, CEP 14049-900, Ribeirão Preto, SP. Email: karina@rpm.fmrp.usp.br

INTERLEUKIN-15 ENHANCES FUNGICIDAL ACTIVITY OF HUMAN MONOCYTES INFECTED *IN VITRO* WITH *Paracoccidioides brasiliensis*

BANNWART C. F.(1), MARTINS R. A. R.(1), NAKAIRA E. T.(1), NASCIMENTO M. P. P.(1), SOARES A. M. V. C.(1), PERAÇOLI M. T. S.(1)

(1)Departament of Microbiology and Immunology, IBB, UNESP, Botucatu, SP, Brazil;

Innate immunity involving mainly monocytes and macrophages is considered an important defense mechanism against pathogenic fungi in systemic mycoses. Non-actived human monocytes lack fungicidal activity against *Paracoccidioides brasiliensis*, the etiological agent of paracoccidioidomycosis. Since interleukin-15 (IL-15) is closely associated with the innate immune response we investigated the effects of human IL-15 on monocyte activity against the isolate Pb18 of *P. brasiliensis* . Peripheral blood monocytes obtained from healthy individuals were preincubated for 24 hr with or without different concentrations of human recombinant IL-15 (12.5, 25 and 50ng/ml). Then, monocyte monolayers were challenged with Pb18 high virulent strain of the fungus by co-culture during 4hr in a ratio of 50:1 monocytes:fungus. Fungicidal activity of monocytes was evaluated by viable fungi recovery from co-cultures after plating in brain-heart infusion agar (BHI). The results demonstrated that IL-15 enhanced fungicidal activity in a dose-dependent pattern. The highest effect was observed after monocyte treatment with IL-15 at a concentration of 50 ng/ml. This effect was abrogated by addition of anti-IL-15 monoclonal antibodies to the co-cultures. Our results indicate that IL-15 upregulates the fungicidal activity of human monocytes against *P. brasiliensis*.

KEY WORDS: monocytes, interleukin-15, *Paracoccidiodes brasiliensis*

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CORRESPONDENCE TO:
Camila Ferreira Bannwart, Departamento de Microbiologia e Imunologia do Instituto de Biociências, UNESP, Botucatu, SP. Email: cfbannwart@yahoo.com.br

HISTAMINE ACTS AS A REGULATOR MEDIATOR IN PULMONARY INFLAMMATION INDUCED BY *Mycobacterium tuberculosis* INFECTION

CARLOS D.(1), DE PAULA L.(1), RAMOS S. G.(2), SILVA C. L.(2), FACCIOLI L. H.(1)

(1)Faculdade de Ciências Farmacêuticas de Ribeirão Preto, FCFRP, Universidade de São Paulo, USP; (2)Faculdade de Medicina de Ribeirão Preto, FMRP, Universidade de São Paulo, USP.

Histamine is a biogenic amine synthesized from L-histidine by histidine descarboxylase and stored mainly within granules in mast cells. Besides its proinflammatory actions, histamine also exerts a variety of other regulatory functions such as in the early recruitment of macrophages and neutrophils at the site of infection by modulating chemokines and cytokines production. Moreover, histamine can also influence the balance of Th1/Th2 responses. The aim of this study was to investigate the participation of histamine in the inflammatory response and modulation of the immune response induced by *M. tuberculosis* infection. Balb-c mice were infected through the intratracheal route with 1 x 10^5 viable *M. tuberculosis* (H37Rv) and treated with pyrilamine (H1 receptor antagonist), cimetidine (H2 receptor antagonist) or thioperamide (H3/H4 receptors antagonist) through the subcutaneous route. On the 30^{th} day of infection, we observed intense inflammatory reaction in the bronchoalveolar space and an increase in chemokines and cytokines production in lung homogenates when compared to the control group. The treatment of infected mice with pyrilamine diminished the number of recruited neutrophils in the bronchoalveolar space and the release of IL-6 and KC while Th1 cytokines production, like IL-12 and IFN-γ, was positively regulated, which resulted in the reduction in the number of bacilli in the lungs. However, there was an amplification of the recruitment of neutrophils and mononuclear cells followed by the detection of high levels of TNF-α, KC, MCP-1, MIP-2 and Th2 cytokines production, such as IL-5 and IL-10, after treatment of infected mice with cimetidine. This event induced the spreading of bacilli in the intersticium. These findings suggest that histamine plays an essential role in the modulation of the immune responses to *M. tuberculosis* infection.

KEY WORDS: histamine, inflammation, tuberculosis

CORRESPONDENCE TO:

Daniela Carlos, Departamento de Análises Clínicas, FCFRP, USP, Avenida do Café s/n, Monte Alegre, Ribeirão Preto, SP, Brasil, CEP: 14040-903. Email: danicar@rpm.fmrp.usp.br

THE ROLE OF B-1 CELLS IN INFLAMMATORY RESPONSE AFTER LIPOPOLYSSACCHARIDE EXPOSURE

BARBEIRO D. F.(1), BARBEIRO H. V.(1), SORIANO F. G.(1)

(1)Emergências Clínicas – LIM 51 / FMUSP, São Paulo, SP, Brazil.

The origins and functions of B-1 cells are controversy. These cells migrate from the peritoneal cavity of mice and home to a distant site of inflammation to become macrophage-like cells. We investigated the role of these cells on the kinetics and inflammatory response to lipopolyssaccharide (LPS) exposure. Cytokines production (TNFα, IL6 and IL10 (pg/ml) were measure in serum (S), lung (L) and intestine (I) of Xid mouse (a strain deprived of B-1 cells) and Balb/c strain in 1,5; 4 and 6h after LPS injection (15mg/kg). *In vitro* studies with peritoneal cells kept in culture (10^6cells/ml) and the supernates were collected 24 h after LPS challenge. There was higher mortality in Xid (60%) when compared with Balb/c (0%) after 16h of LPS injection. Statistical analysis was made by ANOVA. When compared Xid with Balb/c, there was increased TNFαin 1,5h (I- 2045±356; 877±65) remained increased 4h (S- 6284±1469; 366±90, L- 1135±270; 247±27; I- 1045±326; 226,4±9) and there was no difference in 6h. Higher IL-6 levels in 1,5h (S- 11422±545; 2494±144) remained increased 4h (S- 10038±38; 1947±384, L- 12086±86; 6647±950; I- 5638±1456; 745±260) and 6h (S- 10365±365; 2469±115, L- 13979±919; 8445±662; I- 8508±1329; 864±350). Lower IL-10 concentrations in 1,5h (S- 102±13; 749±83, L- 205±15; 331±29; I- 184±11; 316±18), 4h (S- 387±71; 568±60, L- 210±7; 297±12; I- 205±15; 288±10) and 6h (S- 447±65; 329±59, L- 215±15; 283±10; I- 175±24; 334±37). In B-1 cells culture we found lower pro inflammatory cytokine production than macrophages and macrophages+B-1 with TNFα (97±15; 1877±403; 871±129 respectively) and IL-6 (87±15; 175±12; 142±8 respectively) and there was no difference in IL-10 production by three cells populations. Our data shown that B-1 cells absence increased pro inflammatory cytokines production while in B-1 presence, increased IL10 production, suggesting the role of B-1 cells in modulate inflammatory response.

KEY WORDS: B-1 Cells, inflammation.

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CORRESPONDENCE TO:

Denise Frediani Barbeiro. Avenida Dr. Arnaldo 455, sala 3134, Faculdade de Medicina, USP, São Paulo (SP), LIM 51, CEP 01246-903.

Email: denisedna@hotmail.com

SERUM LEVELS OF CYTOKINES IN PATIENTS WITH CHRONIC CHAGAS' DISEASE AND THE TREATMENT WITH BENZONIDAZOLE

PERESI E.(1), CALVI A. S.(1), MEIRA D. A.(1), CARVALHO T. B.(1), ORIKAZA C. M.(1), CAETANO P. K.(1), NAGOSHI L.(1), LIMA C. R. G.(1), PICKA M. C. M.(1), MARCONDES-MACHADO J.(1)

(1)Department of Tropical Diseases, School of Medicine, UNESP, Botucatu, SP, Brazil.

Chagas' disease, caused by *Trypanosoma cruzi,* affects 5 million people in Brazil. Chronic phase of infection is mostly asymptomatic, known as indeterminate form, but digestive and/or cardiac forms may also develop. Serum cytokines in indeterminate disease shows Th0 profile, which is important to eliminate parasitism and to reduce the onset or aggravation of the cardiomyopathy and/ or digestive forms. Benzonidazole, a trypanosomicidal agent, is the best choice for treatment and seems to modify the balance between proinflammatory and antiinflammatory mediators in chronic disease. Twenty-seven indeterminate chronic form patients were evaluated, before and after treatment with benzonidazole, by serum levels of TNF-α, IFN-γ, IL-2 , IL-4, IL-10 and TGF-β. Sixteen of them were male and eleven female, with age range 30-66 years. Seventeen blood donors were controls for normal values of cytokines. Levels of TNF-α, IFN-γ, IL-4, IL-10 and TGF-β ($p<0.05$), but not of IL-2 ($p>0.05$), were higher in patients than in controls, before and after treatment. Patients IFN-γ, IL-2, IL-4, IL-10 and TGF-β serum levels showed higher values before than after treatment ($p<0.05$). There was no difference between TNF- α levels before and after treatment with benzonidazole ($p>0.05$). These results suggest a modulation of cytokine secretion by treatment, with reduction of IFN-γ, IL-2, IL-4, IL-10 and TGF-β levels, although not to normal values. No significant change ($p>0.05$) was seen with treatment, in the serum levels of TNF- α, a cytokine that mediates macrophage microbicide activity that results in acute and chronic tissue inflammation during Chagas' disease.

KEY WORDS: Chagas' disease; cytokines; benzonidazole.

CORRESPONDENCE TO:

Eliana Peresi, Departamento de Doenças Tropicais, Faculdade de Medicina, UNESP, CEP: 18600-000, Botucatu, SP. Email: elianaperesi@yahoo.com.br

ENHANCED NATURAL KILLER ACTIVITY AND PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN MICE SELECTED FOR HIGH ACUTE INFLAMMATORY RESPONSE (AIRMAX)

CASTOLDI L.(1,2), GOLIM M. A.(3), RIBEIRO O. G.(4), IBAÑEZ O. M.(4), ROMAGNOLI G. G.(1), KANENO R.(1)

(1)Department of Microbiology and Immunology, Institute of Bioscience of Botucatu, UNESP, Botucatu, SP, Brazil; (2)Department of Pathology, Faculty of Medicine of Botucatu, UNESP, Botucatu, SP, Brazil; (3)Hemocentric Division, Faculty of Medicine of Botucatu, UNESP, Botucatu, SP, Brazil; (4)Laboratory of Immunogenetics, Institute Butantan, São Paulo, Brazil

AIRmax and AIRmin strains of mice were developed by selective breeding based on their high or low acute inflammatory responsiveness. Previous reports have shown that AIRmax mice are more resistant than AIRmin for the development of a variety of tumors, including spontaneous metastasis of murine melanoma. Since natural killer activity is implicated in the immunosurveillance against tumor development, we analyzed the number and activity of NK cells ($CD49b^+$), T lymphocyte subsets and *in vitro* cytokine production by spleen cells of normal AIRmax/AIRmin mice. NK Cytotoxic activity was assessed by standard 4h ^{51}Cr – release assays and the expression of surface markers (CD49b, CD4, CD8, and CD3) was determined by FACS. *In vitro* production of IL-12p40, IL-10, IFN-γ and TNF-α were analyzed by ELISA. Analysis of lymphocyte subsets showed that AIRmax have a higher relative number of $CD49b^+$ cells than AIRmin (AIRmax ♂ 2.56 ± 0.71%; AIRmax ♀ 2.28 ± 0.75%; AIRmin ♂ 1.68 ± 0.51%; AIRmin ♀ 1.42 ± 0.50%), as much as NK activity against Yac.1 target cells (AIRmax ♂ 7.24 ± 2.031%; AIRmax ♀ 11.64 ± 7.39%; AIRmin ♂ 3.74 ± 1.64%; AIRmin ♀ 5.16 ± 5.53% specific lysis). The number of $CD3^+/CD8^+$ cells was also higher in AIRmax (AIRmax ♂ 15.40 ± 2.6%; AIRmax ♀ 9.08 ± 2.32%; AIRmin ♂ 11.88 ± 3.48%; AIRmin ♀ 8.47 ± 1.74%). These findings were associated with the ability of AIRmax spleen cells to *in vitro* produce higher levels of the pro-inflammatory cytokines TNF-α, IL-12p40 and IFN-γ but not the anti-inflammatory IL-10. Taken together, our data suggest that the selective breeding for achieve AIRmax and AIRmin strains was able to polarize a number of mechanisms associated with cytotoxic activity, that can be responsible for the antitumoral resistance observed in AIRmax mice.

KEY WORDS: inflammation, innate immunity, immunogenetics

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CORRESPONDENCE TO:

Ramon Kaneno, Departamento de Microbiologia e Imunologia do Instituto de Biociências de Botucatu, UNESP, Caixa Postal 510, CEP 18618-000, Botucatu, SP. Email: kaneno@ibb.unesp.br

EFFECT OF TWO DIFFERENT ORIGIN BETA-1,3 POLYGLUCOSES ON *Toxoplasma gondii* INFECTED MICE MACROPHAGE ACTIVITY

PICKA M. C. M.(1), CALVI S. A.(1), ORIKAZA C. M.(1), CARVALHO T. B.(1), NAGOSHI L. R.(1), CAETANO P. K.(1), PERESI E.(1), LIMA C. R. G.(1), MARCONDES-MACHADO J.(1)

(1)Department of Tropical Diseases, School of Medicine, UNESP, Botucatu, SP, Brazil.

Toxoplasma gondii (T. gondii) is an obligate intracellular parasite and the mechanisms involved in its destruction are hydrogen peroxide (H_2O_2) and nitric oxide (NO) provided by activated macrophages. β-1,3 polyglucose is a polysaccharide of *Saccharomyces cerevisae* cell wall, with activating effects on macrophages.This study compared the effect of a home-made (glucan 1) and a manufactured (glucan 2) β-1,3 polyglucose on *T.gondii* infected mice macrophage activation, by measuring H_2O_2 and NO production. Ninety female BALB/c mice were divided into six groups: G1: *T. gondii* infection; G2: *T. gondii* + glucan 1; G3: glucan 1; G4: *T. gondii* + glucan 2; G5: glucan 2; G6: saline. Macrophages were obtained by peritoneal washing and incubated with or without LPS for 24 hours when H_2O_2 and NO production was assessed. The results showed that LPS stimulated macrophages of G1, G2, G3, G4 and G5 groups produced higher H_2O_2 levels than G6 ($p < 0,001$). Nonetheless, the production of this metabolite was higher in G2, G3, G4 and G5 than in G1 ($p < 0,001$), with increasing levels during treatment. Higher levels of H_2O_2 were produced by G2 macrophages than by G4.On the other hand, higher levels of H_2O_2 were observed with glucan 2 in single use (G5), than with glucan 1 alone (G3). As to NO, in G1, G2, G3, G4 and G5 macrophages there was higher production than in G6.Groups G2, G3, G4 and G5 showed higher NO production than G1, with constant increases from the second day on. Nevertheless, the highest levels of this metabolite were found in G2 and G4.These results show that glucan 1 + infection had higher effect on macrophage H_2O_2 production than glucan 2 + infection. When both were used alone, glucan 2 induced higher H_2O_2 production than glucan 1. There was no difference in NO production with glucan 1 and 2 either in single use (G3 and G5) or with infection (G2 and G4).

KEY WORDS: *Toxoplasma gondii*, β-1,3 polyglucose, H_2O_2, NO

CORRESPONDENCE TO:

Mariele Cristina Modolo Picka, Department of Tropical Diseases, School of Medicine, UNESP, Botucatu, SP, Brazil. Email: mariele@fmb.unesp.br

INTERFERON-GAMMA (IFN-γ) AND GRANULOCYTE-MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF) ENHANCE FORMATION OF MULTINUCLEATED GIANT CELLS DERIVED FROM MONOCYTES STIMULATED *IN VITRO* WITH *Paracoccidioides brasiliensis* ANTIGEN.

NASCIMENTO M. P. P.(1), SOARES A. M. V. C.(2), NAKAIRA E. T.(1), PERAÇOLI M. T. S.(2).

(1)Departamento de Doenças Tropicais e Diagnóstico por Imagem, Faculdade de Medicina de Botucatu; (2)Departamento de Microbiologia e Imunologia, Instituto de Biociências, UNESP, Botucatu, SP, Brazil.

Cell fusion is a central phenomenon during the immune response that leads to multinucleated giant cells (MGC) formation. These cells are a common occurrence at sites of granulomatous inflammation and may be induced *in vitro* by stimulation with cytokines. This study investigated the effect of INF-γ and GM-CSF on MGC formation *in vitro* from monocytes stimulated with *P.brasiliensis* antigen (PbAg). Peripheral blood monocytes obtained from healthy individuals were cultured for three days with or without stimulus such as: PbAg (100ug/mL) or PbAg plus IFN-γ (50, 100 and 300 IU/mL), or PbAg plus GM-CSF (50, 100, 200, 500 and 1000 pg/mL). The fusion index (FI) and the percentage of MGC formation were determined after cell fixing and May-Grumwald-Giensa staining. The results demonstrated that monocyte incubation with PbAg or IFN-γ or GM-CSF induces FI significantly higher than in control cultures without stimulus. Monocyte cultures stimulated with PbAg plus different GM-CSF concentrations showed FI significantly higher in comparison with cultures only stimulated with PbAg. The FI obtained in cultures stimulated with PbAg plus 300 UI INF-γ were significantly higher than those observed in cultures only stimulated with PbAg or with different concentrations of INF-γ. Together, these data suggest that IFN-γ and GM-CSF play a positive modulatory effect on MGC generation after monocyte stimulation with *P.brasiliensis* antigen.

KEY WORDS: *Paracoccidioides brasiliensis*, multinucleated giant cells, INF-γ and GM-CSF

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CORRESPONDENCE TO:

Magda Paula Pereira do Nascimento, Departamento de Microbiologia e Imunologia do Instituto de Biociências, UNESP, Distrito de Rubião Júnior s/n, Botucatu, SP. Email: magdapaula@terra.com.br