SERUM HEMOLYTIC ACTIVITY OF THE ALTERNATIVE COMPLEMENT PATHWAY IN NORMAL DOGS

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The complement system, an important effector mechanism of innate immunity can be activated by three distinct mechanisms designated classical, alternative and lectin pathways. The functional activity of complement can be assessed by hemolytic assays that are valuable screening tools for complement component deficiencies. The aim of this study was to evaluate the alternative complement pathway in normal canine serum samples using the hemolytic assay (AH50). First the canine alternative pathway activation by erythrocytes from different animal species was evaluated. Erythrocytes suspensions (2,8% in borate Mg-EGTA buffer) from rabbit, chicken, sheep and guinea pig were incubated with 1:10 dilution of canine serum for 30 min at 37°C and the percentage of lysis were evaluated by supernatant analysis at 492 nm. Guinea pig erythrocytes were more effective than rabbit, chicken and sheep for canine alternative pathway activation (hemolysis of 66.6%, 12.7%; 12.6% and 10.7%, respectively) and were used in the AH50 assay. A standard AH50 method was used to evaluate ten serum samples obtained from apparently healthy adult dogs and the values ranged from 7.8 to 222.2 AH50/ml. The next step should be the evaluation of AH50 levels in dogs with different diseases.

KEY WORDS: complement, dog, hemolytic assay

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IMMUNOLOGICAL DATA OBTAINED OF *Paracoccidioides brasiliensis* EXOANTIGENS FROM CULTURES IN NGTA (NEOPEPTONE, GLUCOSE, THIAMINE AND ASPARAGINE) MEDIUM. PRODUCTION AND STANDARDIZATION OF ANTIGENS AND HIPERIMMUNESERA

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Paracoccidioides brasiliensis (Pb) is a dimorphic fungus that causes paracoccidioidomycosis, a common systemic mycosis in Latin America. The diagnosis is performed with the demonstration of the fungus from clinical materials by optical microscopy or by isolation in culture. It is accomplished by serological tests. The purpose of this study is the production of crude antigens from Pb to be used in serology. Yeast cells of Pb 113 were subcultured in NGTA agar medium by four times, at 36ºC during five days. After growth of the cultures suitable viability and concentration were determined. Five ml of the suspension were inoculated into 200 ml of NGTA medium and incubated at 36ºC, during 20 days with agitation. The cells were then killed with thimerosal 1:5000 and separated from crude antigen by filtration, concentrated 10 times and kept at 4ºC. The obtained antigen was studied by gel immunodiffusion tests, complement fixation (CF), electrophoresis with SDS-PAGE and immunoblot (IB) methods with sera of patients of several mycoses as paracoccidioidomycosis, histoplasmosis, aspergillosis, lobomycosis, other infectious diseases, healthy donors and hiperimmune sera of *Pb, Hc, Af* and *Ca*. Results showed three lines of precipitation in double immunodiffusion and five lines in immunoelectrophoresis tests. Titles of 1:4 in immunodiffusion, 1:4 in counterimmunoelectrophoresis and 1:256 in CF were obtained. Sera of patients with other diseases, heterologous antisera and sera of healthy donors presented no reaction. SDS-PAGE and IB showed different bands of glycoproteins between 19 to 105 kDa and better expression of gp43. It was demonstrated that this medium produced crude antingens with less protein contaminantion and better visibility of bands of the glycoproteins. As compared with Negroni modified medium these studies indicate that NGTA medium used in this research is cheaper and easier to work, presenting good results in serology and production of antigens and hiperimmunesera.

KEY WORDS: paracoccidioidomycosis, antigens, hiperimmunesera, serology

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HIGH T3 LEVELS ALTERED THE ACTIVITY OF FACTOR B OF COMPLEMENT SYSTEM

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Previous studies have shown that hypothyroidism affects the complement system (CS), increasing the lytic activity of the alternative pathway (AP). In accordance to this observation the aim of the present study was to investigate if the hyperthyroidism induced in rats by triiodotironine (T3) would also affect complement. Rats received 0.15 to 50μg of T3 by gavage during periods of 3-14 days. The lytic activity of CS was evaluated by hemolytic assay measuring time required for 50% of lysis to occur (t1/2). The activity of factor B was evaluated using serum depleted of B factor (RB) in a hemolytic assay. The lytic activities of classical/lectin pathways were not significantly altered at any time or T3 dose evaluated. The dose of 0.15μg reduced AP activity (increased t 1/2 values) after 7 and 14 days of treatment. These alterations showed statistical significance (Annova and Dunnet test). The effect on AP activity was dependent of the dose and the period of treatment. The factor B activity was altered in the conditions of reduced AP. Hyperthyroidism induced by T3 affects CS reducing AP lytic activity due to reduced activity of factor B; this effect is opposite to that observed in hypothyroidism. The effect of the T3 level on CS may be considered in certain conditions such as in autoimmune processes involving the thyroid gland.

KEY WORDS: Complement System, hyperthiroidism, factor B

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MACROPHAGES AND B-1 CELL CYTOKINES PROFILE IN AN *IN VITRO* MODEL OF TOLERANCE TO LPS

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In this work we aimed to characterize the production of cytokines and nitric oxide (NO) by macrophages and B-1 cells using an *in vitro* model of tolerance to LPS. Cell cultures were obtained by peritoneal lavage of Xid (B-1 cells free) and Balb/c mice. Macrophages (MA), macrophages + B-1 (FA), and B-1 cells were maintained in culture (10^6cells/mL) and the media was changed daily. Three groups were studied: GC- control; G1- LPS (10mg/mL) added in the last 24h of cells culture; G2- LPS (10mg/mL) added in the last 48h of cells culture. Cytokines (pg/ml) and NO_2 were measured in the supernatant. We found high levels of TNF-α in the G1 for B-1 (97±47), MA (1877±404) and FA (871±129) when compared to GC (34±3; 117±23; 51±7 respectively) and G2 (29±2; 65±5; 63±2 respectively). B-1 cells IL-6 production was increased in G1 (88±15) compared to GC (5±1) and G2 (14±2). For FA, the groups were also markedly different: GC (50±20) < G1 (142±8) < G2 (341±29). No differences were found for MA. IL-10 concentration in G1 was increased for FA (210±26) and MA (236±47) when compared to GC (99±20; 85±29 respectively) or G2 (99±5; 106±19 respectively). B-1 showed higher levels in the G1 (168±24) and G2 (172±22) groups, when compared to GC (72±7). In FA, we observed an increase in NO_2 (μM) quantification when G2 (4±0,1) was compared to GC (3,7±0,05). In MA, G1 (4,8±0,2) and G2 (4,5±0,2) were higher than GC (3,8±0,5). No difference was found for B-1. Our data suggest that the three cell populations develop tolerance to LPS *in vitro* and that B-1 cells can modulate the role of macrophages to LPS, reducing TNF-α production and sustaining high IL-10 production, after the second LPS challenge.

KEY WORDS: Tolerance, LPS, Macrophages, B-1 cell

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INTERNAL TEAT SEALANTS FOR DAIRY COWS: A REPLACEMENT FOR A MECHANISM OF INNATE IMMUNITY

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When the mammary gland of dairy cows stops producing milk in the interval between two lactations, a keratin plug is formed in the teat canal. This plug has bacteriostatic properties and blocks the entry of microorganisms in the gland. Many cows, especially high production animals, delay the formation of this plug for over two weeks. To prevent infections during this period, antibiotic treatment after the last milking before the dry period is used in all cows, but this can lead to residues in milk and increase antibiotic resistance. To imitate this innate immunity mechanism, internal tet sealants, based on paraffin and bismuth subnitrate, were developed. They are applied into the teat canal immediately after drying off, and remain in place during the dry period (usually 60 days). The objective of this study was to evaluate the effect of one sealant (MASTBLOCK, IRFA Química e Biotecnologia) on the rate of self-cure during the dry period. We compared the effect of this sealant when used alone, in association with an antibiotic (gentamicin) and the antibiotic alone. All mammary quarters (N=105) were infected with mastitis-causing bacteria at drying off. In each cow, one quarter was infused with the sealant alone (T1, N=24), one was infused with antibiotic followed by the sealant (T2, N=29), and two received antibiotic only (T3, N=52). Milk cultures were done one week before drying off, at drying off, 8 and 21 days after the beginning of the next lactation. Self-cure rate was not significantly different ($p>0.01$, Fischer's Exact Test) among T1 (87,5%), T2 (93,1%) and T3 (90,4%). These results suggests that there is no difference among the use of an antibiotic or an internal teat sealant, under the conditions of this study.

KEY WORDS: cow, mastitis, keratin plug

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PRIMING WITH *Mycobacterium avium* INCREASES IMMUNOGENICITY BUT NOT PROTECTIVE EFFICACY OF DNAHSP65 VACCINE

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The efficacy of BCG vaccine (attenuated *Mycobacterium bovis*) against pulmonary tuberculosis varies enormously in different populations. Previous studies have revealed that most protective antigens expressed by the antituberculous vaccine are conserved in *M. avium*, supporting the hypothesis that exposure to environmental mycobacteria generates a cross-reactive immune response that interferes with BCG efficacy. In this work we investigated the effect of a prior sensitization with heat killed *M. avium* on both, the immune response and the protective efficacy induced by a DNAhsp65 vaccine (pVAXhsp65) in murine experimental tuberculosis. BALB/c female mice (6-8 weeks old) were used. Animals were initially, sensitized with $2x10^8$ heat killed CFU of *M. avium* by subcutaneous route and then immunized with 3 doses of pVAXhsp65 (100μg/15 days apart) by intramuscular route. Control groups were injected with saline, pVAX (4 doses), pVAXhsp65 (4 doses), *M. avium, M. avium* plus pVAX (3 doses) or *M. avium* plus pVAXhsp65 (3 doses). Blood samples for antibody evaluation were collected 12 days after last DNA dose. Fifteen days after last DNA dose the animals were infected with $1x10^4$ viable CFU of H37Rv *M. tuberculosis* by intratracheal route. Thirty days after challenge the animals were sacrificed and the bacterial burden was determined by the number of UFC in the lungs. Histological sections of lungs were also analysed. Priming with *M. avium* triggered a significant increase in the induction of IgG1 and IgG2a anti-hsp65 by pVAXhsp65 in comparison to all control groups. However this priming did not decrease the bacterial burden in the lungs. In addition, this prior sensitization with *M. avium* decreased the parenchyma preservation observed in the group immunized only with pVaxhsp65. These results reinforce the deleterious effect of environmental mycobacteria in antituberculous vaccines.

KEY WORDS: DNA vaccine, pVAXhsp65, mycobacteria, *Mycobacterium avium*

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EFFECT OF IL-15 ON FUNGICIDAL ACTIVITY AND H_2O_2 RELEASE BY HUMAN NEUTROPHILS CHALLENGED WITH HIGH VIRULENT STRAIN OF *Paracoccidioides brasiliensis*

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Phagocytic cells play a critical role against *Paracoccidioides brasiliensis*. Some papers have shown the effects of activator and suppressive cytokines on macrophages and monocytes response to fungus challenge. However the works focusing the involvement of these mediators on neutrophils antifungal functions are scarcer. Interleukin-15, a key immnoregulatory cytokine of the innate immune response has been shown to activate certain antimicrobial functions of neutrophils. Thus, the purpose of this work was to test the effects of IL-15 on fungicidal activity and H_2O_2 release by human neutrophils challenged with virulent strain of *P brasiliensis*. Peripheral blood neutrophils obtained from 20 healthy donors were incubated for 18 h with increasing concentrations of IL-15 (6.25, 12.5, 25, 50 and 100 ng/mL) and then challenged with high virulent strain of *P brasiliensis* (Pb18) by 4 h. After, the cells were evaluated for H_2O_2 release and fungicidal activity, by counting of colony forming units after plating. No IL-15 incubated cells exhibited a very low fungicidal activity. However, after incubation with this cytokine a significant increase in neutrophils fungicidal activity was detected. Moreover, H_2O_2 levels released by IL-15 incubated cells were higher than those detected for neutrophils alone. Human neutrophils activated with IL-15, increased fungicidal activity against *P. brasiliensis* associated with high levels of H_2O_2.

KEY WORDS: Interleukin 15, *P. brasiliensis*, human neutrophils.

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THE ROLE OF NITRIC OXIDE (NO) IN ALLERGIC AND CARRAGEENAN-INDUCED PLEURISY IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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SHR differs from Wistar normotensive (NT) regarding allergic inflammation, and abnormalities in NO production/effect has been shown in SHR. Here we compared both strains response to allergic and carrageenan-induced pleurisy and the contribution of NO. Pleurisy was induced in male rats (250-300g) by carrageenan (CA, 200µg) or anaphylaxis (ANA, 1mg ovalbumin into sensitized rats - 10µg of ovalbumin in 10mg of $Al(OH)_3$). Pleural exudates were collected 30min and 4h and measured as indicative of increased vascular permeability (iVP, as mL). To determine the total (performed in Turk) and differential (Giemsa stained) cell counts (as $x10^3$ cells/mm^3), the cavity was washed with 2 mL of PBS. The inhibitor of NO synthesis (L-NAME 30mg/kg) was injected iv 30min before pleurisy. In ANA pleurisy iVP after 30 min was lower in SHR compared to NT (0.6±0.02 *vs* 0.9±0.05, respectively). This difference was no longer observed after 4h. At this time, the cell infiltration was not significantly different between strains (43±2.0 in SHR and 30±2 in NT). In both strains, PMN leukocytes accounted for more than 85% of the exudate cells. Pre-treatment with L-NAME did not modify these responses. The pleurisy to CA was observed only in the 4^{th}h after its injection, and in this case iVP in SHR (0.7±0.03) were similar to NT (0.8±0.03) as well as the number of cells (61±3.1 in SHR and 64±2 in NT). L-NAME inhibited iVP by 55% in both strains and cell influx by 50% in SHR and 35% in NT. Inflammation in SHR differs from NT as a function of the nature of the stimulus. Endogenous NO contributes to CA-induced pleurisy but not to ANA.

KEY WORDS: pleurisy, anaphylaxis, carrageenan, nitric oxide, inflammation, hypertension.

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MAST CELLS ARE NOT ACTIVATED BY *Tityus serrulatus* VENOM (TSV).

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In Brazil *T. serrulatus* are responsible by the fatal accidents and it was experimentally suggested that mast cells (MC) have a role in the lung edema, an important feature observed in severe cases of envenomation. Our goal was to study the role of MC in airways edema and haemorrhage induced by Tsv, and also to investigate if MC are activated by Tsv. Male Wistar rats (160-200g) were used in this study. The increase in vascular permeability and haemorrhage in the airways were evaluated by extravasation of Evans blue dye and cianometahaemoglobin methods respectively, 0.5 and 1h after iv injection of Tsv (200µg/kg, diluted in NaCl 0,9%). Control groups received NaCl 0,9%. The role of MC was evaluated pre-treating (30 min) the animals iv with pirilamine (6 mg/kg) or ketotifen (5 mg/kg). The effect of Tsv on MC was studied by histology of mesenteric MC, and by the release of 5-hydroxytryptamine (5-ht, measured by HPLC) by mesenteric and peritoneal MC *in vitro*, and *in vivo* in broncho-alveolar lavage after poisoning. In airways Tsv induced an increase of dye extravasation (trachea 80%, upper bronchi 100%, inner bronchi 325%, lungs 528%) and haemoglobin concentration (trachea 138%, upper bronchi 85%, inner bronchi 186%, lungs 250%), both not modulated by pirilamine or ketotifen. 5-ht was not found in *in vitro* challenged MC or in broncho-alveolar lavage. Our data shown that MC do not have a role in the increased vascular permeability and haemorrhage in the airways after Tsv injection. The lack of a role for MC in this model of scorpionism is probably due to an incapacity of Tsv to induce MC degranulation.

KEY WORDS: scorpion venom, mast cell, vascular permeability, haemorrhage

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IN VITRO AND *IN SITU* ACTIVATION OF THE COMPLEMENT SYSTEM BY THE FUNGUS *Lacazia loboi*

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Since activation of the complement system (CS) by fungal cells represents an important defense mechanism of the host, and since so far no study exists evaluating the participation of the CS in Jorge Lobo's disease and its activity on the fungus *Lacazia loboi*, the etiological agent of this mycosis, we carried out the present study. *L. loboi* was obtained from the footpads of BALB/c mice inoculated 10 months earlier. The viability index of the fungus was 48%. Fungal suspensions (2 x 10^6 cells) were incubated at 37°C with a pool of inactivated serum from patients with the mycosis or with sterile saline for 30 min. Next, the tubes were incubated for 2 h with a pool of noninactivated AB^+ serum (as a complement source), serum diluted in EGTA-$MgCl_2$ (to block the classical route of CS activation), serum diluted in EDTA (to block the two routes of CS activation), and inactivated serum. After centrifugation, fungal cells were resuspended in 0.5 ml sterile saline. The viability index of *L. loboi* was evaluated by staining with fluorescein diacetate/ethidium bromide and the remaining suspension was cytocentrifuged. The slides were submitted to immunofluorescence staining using fluorescein isothiocyanate-conjugated human anti-C3 monoclonal antibody. The results revealed that 96% of the fungi activated the CS by the alternative route and no significant difference in *L. loboi* viability was observed after CS activation. In parallel, frozen histological sections from 11 patients with the mycosis were analyzed regarding the presence of C3 and IgG by immunofluorescence staining. C3 deposits were observed in the fungal wall in 100% of the lesions analyzed and IgG deposits in 91%. The results suggest that the CS is activated in Jorge Lobo's disease and may contribute to the host's defense through macrophage-mediated *L. loboi* opsonization and phagocytosis.

KEY WORDS: complement system, *Lacazia loboi*, Jorge Lobo's disease.

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EFFECT OF HIDROALCOHOLIC EXTRACT OF *Tamarindus indica* L. ON REACTIVE OXYGEN SPECIES PRODUCTION BY HUMAN STIMULATED NEUTROPHILS

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The neutrophil (PMNL) has the ability to release toxic products such as proteolytic enzymes and reactive oxygen species (ROS) capable of killing invading pathogens. Despite of the benefits of the ROS, they also produce deleterious effect to the organism in different types of illnesses like rheumatoid arthrits. The role of PMNL in ROS production in the physiopathology of various inflamatory diseases has drawn interest on the discovery of new compounds to modulate this process. In the present study the activity of hidroalcoholic extract of *T. indica* was assessed on luminol- and lucigenin- enhanced chemiluminescence (CL) produced by human neutrophils stimulated with particles of serum- opsonized zymosan (OZ). The total production of ROS by PMNL was measured by the luminol- enhanced CL (Lum CL) assay and the production of the superoxide anion ($O_2^{\cdot-}$) by the lucigenin- enhanced CL (Luc CL) assay. Zymosan-induced ROS production was significantly inhibited by tamarind extract and had concentration-dependent inhibitory effects on luminol- and lucigenin-dependent CL (their IC_{50} values were 248.5 ± 16.62µg/mL and 296.5 ± 35.55µg/mL, respectively). The extract did not demonstrate toxicity to the neutrophils at the studied concentrations, in both used assays: activity of lactate dehydrogenase (LDH) and Trypan Blue dye exclusion test. Our results showed that the hidroalcoholic crude extract of *T. indica* has a strong inhibitory activity on neutrophils oxidative metabolism. Moreover, research is under way to characterize the main compounds of the extract related with the studied biological activity.

KEY WORDS: neutrophil, *Tamarindus indica* L., reactive oxygen species

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VASCULAR MODULATION FOR NUCLEOTIDE RECEPTORS (P2Y) AFTER TLR4 ACTIVATION

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Abundant sources for extracellular ATP in the vessel wall make a pathophysiological role probable in the development of a lot of diseases. Thus, the purpose of this study is to evaluate the modulation by ATP in the vascular reactivity and the superoxide quantification in isolated rats aorta after LPS injection. LPS (10mg/kg i.p.) was injected in Wistar rats 8h and 16h before the experiment, the control group received saline solution and were anesthetized with chloral hydrate 10% i.p. The average arterial pressure (femoral) was evaluated. For the quantification of mRNA and nitrate, aortas had been homogenized in liquid nitrogen. In the study of vascular reactivity *in vitro* and for O_2^- we used thoracic aorta clean and unbroken. Statistical analysis was made by ANOVA. The MAP (Mean Arterial Pressure) reduced in 8h and 16h. mRNA for iNOS and TLR4 increased in 8h; no difference was observed in 16h. Nitrate and O_2^- increased in 16h, ATP presence reduced O_2^- in 16h. This reduction is predominantly for P2Y receptors; therefore in the presence of P2Y antagonist (Reactive Blue) the ATP practically does not reduced this quantification. In the presence of P2X antagonist (PPADS) the reduction was of 34,57%. It was observed, in the vascular reactivity, difference in the maximum relaxation to ATP when we compare 16h with control. Purinergic substances reduce O_2^- in septic aorta, mainly via P2Y receptors. This action may modulate (i*n vivo*) O_2^-, indirectly NO, iNOS and also arterial pressure.

KEY WORDS: Vascular reactivity, Superoxide

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CORRELATION OF SPECIFIC IMMUNITY AND DISEASE ACTIVITY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the brain and spinal cord that is mediated by $CD4^{+}$ T lymphocytes specific to myelin components. EAE is widely used as an experimental model for human multiple sclerosis. The Lewis strain of rats is very susceptible to induction of EAE by inoculation of purified myelin suspended in Complete Freund's adjuvant (CFA). The purpose of the present study was to investigate the correlation between disease activity and specific immunological parameters. Female Lewis rats (4–6 weeks old) were immunized, in the left footpad, with 100μL of an emulsion containing 50μg of MBP associated with 50 μl of ACF (Incomplete Adjuvant plus 5mg/ml of *M. butyricum*). Non immunized rats were used as a control group. Animals were daily evaluated for both, clinical score and weight. Rats were submitted to euthanasia during acute and recovery phases, characterized by high clinical scores and absence of clinical signs, respectively. Specific anti-myelin antibody levels and cytokine (IFN-γ and TNF-α) production induced by myelin and ConA were evaluated. All immunized animals developed the disease and showed a significant lost in weight. Anti-myelin antibody levels (IgG1 and IgG2b) increased during recovery phase. On the other hand, production of IFN-γ and TNF-α by spleen and lymph nodes stimulated with myelin were higher in the acute phase of the disease. Differences in TNF-α levels correlated better with disease activity. These results show clear differences in immunity to myelin during distinct phases of EAE.

KEY WORDS: experimental autoimmune encephalomyelitis, IFN-γ, TNF-α, Lewis rats

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SCREENING FOR CHARACTERIZATION OF MURINE MONOCLONAL ANTIBODIES AGAINST HUMAN LIMPHOCYTES T ANTIGENS USING JURKAT CELLS

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The hybridoma technology, developed in the mid-1970s by Köhler and Milstein (1975), made it possible to produce virtually limitless quantities of highly specific murine monoclonal antibodies (mAbs) that recognize distinct epitopes of cellular antigens. There is currently available a large number of such reagents which detect different molecules on both normal and neoplasic cells from various lineages. The process of immunization in Balb/C was done with human T-cell leukaemia for production of mAbs allows the isolation of reagents with a unique, chosen specifity. Jurkat cell is a cell line derived from human T-cell leukaemia derived from vigorously proliferating leukemic T-cells, is a widely used model of human T lymphocytes and used to determine the screening to characterizes mAbs produced in our laboratory. The aim of this study was make a screening to characterize the specificity mAbs against antigens of T cells using Dot Blot technique. Whole lysate Jurkat cells had been used like antigen for electrophoresis in SDS-PAGE and Dot Blot techniques. In electrophoresis load up to 10μL of lysate per 1.0mm of well width for gels 0.45mm thickness, gradient 8-25% and rainbow molecular weight standards. In Dot Blot the nitrocellulose membrane had been incubated with whole lysate Jurkat cells overnight for coating. After incubate the membrane in primary antibody (mAbs produced) diluted in Blotto at room temperature. The membrane was incubated for with horseradish peroxidase (HRP) conjugated secondary antibody, diluted to 1:500 in Blotto and the reaction had been revealed. From 20 mAbs tested, 12 presented positive reactions in Dot Blot, showing that this mAbs have specificity against Jurkat antigens cells. Techniques like Western Blotting will be use to identify the mAbs specificity to a determinate protein through molecular weight and flow citometry tests will comparing the fluorescence intensity media expressed in our mAbs versus commercial mAbs.

KEY WORDS: screening for mAbs, jurkat cells, dot blot

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EPIDEMIOLOGIC PROFILE OF BLOOD DONORS FROM ACRE BLOOD CENTER WITH POSITIVE IRREGULAR ANTIBODIES SCREENING

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According to the actual hemotherapy law in Brazil, blood donors should be analyzed to define blood type and antibody presence. The detection and identification of irregular antibodies contribute to transfusion safety, avoiding undesirable hemolytic reactions. For describing the epidemiologic profile of blood donors from Acre Blood Center (ABC), Irregular Antibody Screening (IAS) was done by microplate techniques utilizing saline medium, low ionic force buffer and enzymatic medium utilizing papain enzyme. Of 22.976 blood donations received by ABC, from 2002 to 2004, 24 were detected on Irregular Antibodies Research (IAR). Within this 24, IAS positive donors, 19 (79.2%) were included on this study in which 11 (57.9%) were male donors on the age range of 26 to 35 years. Blood transfusion was related by 5 (26.3%) of them, 3 (15.8%) had tattoos 2 (10.5%) related illicit drugs use. All the eight women included on this study related previous pregnancy. Related to irregular antibody screening, the analysis of concordance of results obtained by ABC and Botucatu Blood Center (BBC) revealed 63.2% concordance of results, however, when this comparison was done to the identification of the referred antibodies, 3 (15.7%) were totally concordant, 5 (26.3%) were partially, 2 (10.5%) were non-concordant and in 9 (47.4%) the comparison was not available because one of both hemotherapy centers didn't identify the specific antibody (ABC or BBC). The most frequent irregular antibodies detected on ABC's samples by BBC were, anti-D, anti-C, probably anti-c, anti-E, probably anti-Lewis and antibodies for low-frequency antigens, however, in seven serum samples it was impossible to identify. The frequency of IAS positive donors on the studied period was 0.1%, demonstrating lower differences and some divergences among the results found on other Brazilian states. The epidemiologic factors evidenced as major relevance on this research were related to pregnancy history and blood transfusion.

KEY WORDS: blood donors, irregular antibody screening (IAS), haemotherapy

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PROTEOMIC EVALUATION OF THE MOUSE UNK CELL PLASMA MEMBRANE

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The unique behavior of *Natural Killer* cell present in the uterine environment (uNK) during pregnancy suggests the expression of specific receptors not shared by circulating NK (cNK) cells. In mice, the uNK cell membrane expresses glycoconjugates with carbohydrate moiety containing N-acetyl D-galactosamine at terminal portion reactive to *Dolichos biflorus* (DBA) lectin. This lectin has been considered the most useful way to distinguish uNK from cNK. In the present work it was aimed to isolate proteins from mouse uNK cell plasma membrane and performed a proteomic evaluation to identify the proteins reactive to DBA lectin. Mouse uNK cells was isolated from pregnant uterus with lectin-biomagnetic beads and obtained the homogenate of plasma membrane enriched fraction. The plasma membrane protein homogenate was submitted to 2D electrophoresis and blotted to PVDF membrane and reacted with DBA lectin. The DBA positive corresponding spots were excised from the gels and submitted to tryptic digestion and the respective peptide masses were acquired with a MALDI-TOF-MS mass spectrometer. Analyses were performed in reflectron mode with positively charged ions. Database searches were done with MASCOT program (http://www.matrixscience.com) using NCBI and SWISS-PROT databases to achieve a panel of candidate proteins. Among the candidate proteins, it was noticed the HSP-70 and COG4 as potentially committed on uNK cells activities at maternal-fetal interface.

KEY WORDS: Uterine-NK cell, proteome, Reproductive Immunology

FINANCIAL SUPPORT: Graduate Student scholarship recipient from FAPESP.

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SHORT-TERM ACUTE EXERCISE IN LOW AND MODERATE INTENSITIES CHANGES MONOCYTE HISTOPHYSIOLOGY IN RATS.

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Nowadays it is usually described the physical exercise like an improved of health. However, strenuous exercise has been associated to transitory immune suppression, rising infection susceptibility due to stress it causes in organism. Recent studies have described positive effects in innate immune system function associate to exercise. Therefore, the objectives of this study were investigating the effects of short-term low and moderate acute exercise on rat monocyte histophysiology. The experimental groups were divided: sedentary control, Low acute exercise (L) during 5, 10 and 15 minutes and Moderate acute exercise (M) with the same 5, 10 and 15 minutes. The exercise pattern was swimming. The counting of the total circulating monocytes was calculated using the leukocytes total number and differential counting of a blood smear. The monocytes cellular and nuclear area was obtained applying an image analyzer system Image Pro Plus Version 4.0 for Windows. For statistical analysis it was applied ANOVA test followed by Tukey HSD test ($p \leq 0,05$). It was observed an increase in circulating monocyte number in all of the L exercised groups and 5M group, when they were compared with control. Cellular area of blood monocytes presented increase in the groups 5L, 10L, 5M e 10M and the monocyte nuclear area increased in 10L, 5M e 10M. The alterations observed in our study could be due to modulation in synthesis and expression cell protein. Probably, increase in adhesion protein expression. Thereafter, in another way the redistribution of monocytes subsets from the marginal pool could be occurred. Thus, physical exercise in different intensities and volumes can modulate the number and morphology of blood monocytes.

KEY WORDS: morphometry, swimming, innate immune system

FINANCIAL SUPPORT: FAPESP; PIBIC/CNPq; FAP/Unimep.

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Paracoccidioides brasiliensis INHIBITS H_2O_2 RELEASE BY HUMAN NEUTROPHILS

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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 PMNs to fungus challenge are poorly studied. Previous studies have been demonstrated that human PMNs fail to kill *P. brasiliensis*. Since, *P. brasiliensis* killing by human phagocytes is dependent on oxygen metabolites, the objective of this paper is to assess if the inability of human PMNs to kill *P. brasiliensis* is related to its capacity to evade oxidative metabolism. Peripheral blood PMNs ($2X10^6$/ml) obtained from 10 healthy donors were incubated with tissue culture medium alone, LPS, IFN-γ or IL-15 for 18 h, and evaluated for H_2O_2 release, before and after challenge with high (Pb18) virulent strain of *P. brasiliensis* ($2X10^4$ yeasts/mL) by 4h. *P. brasiliensis* yeast cells inhibits H_2O_2 release by unprimed PMNs. However, after PMNs priming with LPS, IFN-γ or IL-15 a significative H_2O_2 release in response to fungus was detected. The results suggest that *P. brasiliensis* evade oxidative metabolism, by inhibiting H_2O_2 release and that priming signals by cytokines is necessary to overcome this inhibition.

KEY WORDS: *P. brasiliensis*, H_2O_2, PMNs.

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THE TNF-α SECRETION BY MACROPHAGES INDUCED BY THE LECTIN MNCF IS MAINTAINED UNDER ANTI-INFLAMMATORY CONDITION.

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Glucocorticoids are known by their anti-inflammatory actions that include inhibition of the neutrophil migration, through mechanism that are poorly understood. Activated macrophages secret several inflammatory mediators, such as cytokines and neutrophil attractants. Among the macrophage derived attractants, a galactose-binding lectin, known as MNCF, is distinguished by its well demonstrated property of inducing neutrophil migration even in dexamethasone-pretreated mice. Several macrophage-derived inflammatory mediators stimulate macrophages themselves to secret cytokines. This fact motivated us to investigate if MNCF could exert similar activities on murine peritoneal macrophages, as well if the secreted products vary according the cells are obtained from mice that were treated, or not, with dexamethasone. MNCF was able to induce the secretion of IL-1β, IL-12p70, TNF-α, and NO by macrophages obtained from non-treated mice. When macrophages from dexamethasone-treated mice were stimulated with MNCF they did not produce IL-1β, IL-12p70 or NO, while TNF-αsecretion was completely preserved. So, under anti-inflammatory circumstances MNCF-stimulated macrophages remain able to secret the citokine TNF-αsuggesting that MNCF and TNF-α inflammatory actions can overcome the anti-inflammatory effects of glucocorticoids.

KEY WORDS: inflammation, lectin, MNCF, TNF-α, neutrophil migration, glucocorticoids

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ASPECTS OF THE IMMUNE RESPONSE AGAINST TOXIC PROTEIN IRRADIATED WITH ^{60}CO GAMMA RAYS

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Ionizing radiation has been successfully employed to modify the immunological properties of biomolecules. Very promising results were obtained when crude animal venoms, as well as isolated toxins, were treated with gamma rays, yielding toxoids with good immunogenicity. The obtention of modified antigens with lower toxicity and preserved or improved immunogenicity would be useful. Ionizing radiation has proven to be a powerfull tool to attenuate snake venoms toxicity without affecting and even increasing their immunogenic properties. However, little is known about the modifications that irradiated molecules undergo and even less about the immunological response that such antigens elicit. In the present work, we investigated the immunological behavior of bothropstoxin-I, a K49 phospholipase, before and after irradiation. Structural modifications of the toxin were investigated by SDS-PAGE. Aiming to compare the toxicity of the between native and irradiated forms of the toxin, an *in vitro* citotoxicity assay, using CHO cells, was performed. Isogenic mice were immunized with either the native or the irradiated toxin.The circulating antibodies were isotyped and titrated by ELISA. According to our data, irradiation promoted structural modifications in the toxin, characterized by higher molecular weight forms of the protein (aggregates and oligomers). The citotoxicity assay showed that the modified toxin was 5 folds less toxic than its native counterpart. Our data indicate that irradiated toxins were immunogenic and the antibodies elicited by them were able to recognise the native toxin in ELISA. These results indicate that irradiation of toxic proteins can promote significant modifications in their yours structures, but still retain many of the original antigenic and immunological properties of native proteins. Also, our data indicate that the irradiated protein induced higher titers of IgG2a and IgG2b, suggesting that Th1 cells were predominantly involved in the immune response.

KEY WORDS: Immunological ionizing radiation, bothropstoxin-1.

FINANCIAL SUPPORT: CNPq

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EVALUATION OF MOUSE ANTI-MOUSE uNK MONOCLONAL ANTIBODY OBTAINED BY INTRA-SPECIE IMMUNE RESPONSE AGAINST MOUSE UTERINE NATURAL KILLER CELL

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In the human and rodents a transient accumulation of natural killer cells in the uterus exclusively during pregnancy, suggests this cell as specific subset of lymphocyte population related to uterine environment during pregnancy. The high incidence of these innate immune response cells in the pregnancy is still an unsolved question of immunology of reproduction. Previously it was reported the *Dolichos biflorus* (DBA) lectin reactivity to mouse uNK cells but not to cNK (blood circulating) cells, showing differences in membrane glycoconjugates expression among them. Based on this data it was performed the intra-specie inoculation of mouse uNK cells to induces the immune response. The splenocytes from mouse presenting immunoglobulin reactive to uNK cells in the serum were isolated to obtain the mouse-anti mouse uNK (*mam-uNK*) antibody producing lymphocyte clones. In the present work it was used one of this *mam-uNK* antibody to evaluate its specificity. It was collected uterus, spleen, lymph node, hart, liver and kidney from mice on 8th and rat uterus on 9th gestational day for paraffin embedding. Paraffin sections were processed for immunoperoxidase or immunofluorescence. Round shaped and dot-like positive reactions were detected in the cytoplasm of both mouse and rat uNK cells. The cytoplasm of hepatocytes, epithelial cells of kidney convoluted tubules, macrophages of spleen and lymph node showed positive reaction in small vesicles distributed in their cytoplasm. In the cardiac muscle cells cytoplasm were evident the labeling on thin tubular and round vesicular shaped structures. These reactions pattern strongly suggest the antigen recognized by mam-uNK monoclonal antibody distributed in the membranous organelles such as secretory granules, lysosome-endosome system and endoplasmic reticulum, but it dos not seems to be specific for uNK cells.

KEY WORDS: uNK cell, Monoclonal Antibody, Reproductive Immunology

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EFFECT OF PEPTIDOGLYCANS OF *Agaricus blazei* ON THE CANDIDACIDAL ACTIVITY BY MOUSE PERITONEAL MACROPHAGES

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The immunomodulatory activity of medicinal mushrooms is mainly attributed to the beta glucan fraction. In this work, we evaluate the effect of an acid-treated peptidoglycan fraction (ATF), obtained from the medicinal mushroom *Agaricus blazei*, on the candidacidal activity, the H_2O_2 and NO production, and the expression of the mannose receptor (MR) by peritoneal macrophages. Normal BALB/c mice received three intraperitoneal doses of ATF and after 48 hr, their resident peritoneal macrophages were assayed against *Candida albicans* yeast forms. Our results indicate that the treatment increases the fungicidal activity since less viable fungi were recovered as compared to the control (Control: 100,17 ± 13,27 CFUs; ATF: 80,17 ± 6,88 CFUs; $p<0,01$). This increased fungicidal activity was associated with a higher spontaneous release of H_2O_2 by macrophages (control: 1.0 ± 0.16; ATF: 2.0 ± 0.54; $p<0.01$), although nitric oxide (NO) production was not influenced by the treatment. Further, macrophages were cultured with FITC-labeled α-D-mannosylated BSA which attaches itself to the MR. We found that the treatment enhances the expression of the MR by peritoneal macrophages, which are involved in the attachment and phagocytosis of non-opsonized microorganisms. Our results suggest that ATF can increase the host resistance against some infectious agents through the stimulation of microbicidal activity of macrophages.

KEY WORDS: *Candida albicans*, Macrophage, Mushroom

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THE ERYTHROID PHENOTYPE OF BLOOD TYPE “O” DONORS IN COORDINATING BLOOD BANK – ACRE STATE – BRAZIL

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The ABO system was discovered in 1990 and remains until today the most important system in transfusional practice. The wrong ABO transfusion can result in death of the patient after a hemolytic reaction followed by immunological and biochemical changes. ABO antibodies are found in the serum of individuals, and are directed against the A and B antigens absent in the red cells. The period between January 26th and February 7th, 111 “O” type blood donors were selected from Acre Coordinator Blood Bank, from both sex and age range of 18 to 61 years to constitute a data bank of phenotypes and make a screening panel. Blood samples were collected without anticoagulant and 0.8% red blood cells suspension was prepared. From this suspension, 50μL were incubated with 25μL of known commercial anti-serum, on microplate, during 15 minutes at 37ºC and centrifuged at 910rpm-10minutes. The erythrocyte phenotype was done for the following systems: RHD (CcEe), Kell, Duffy and for the “N” antigen. From the amount-studied donors, 89.2% were RHD positive that proves the predominance of this antigen on the major of population. Related to RH system, the fenotype Cde/cDe appears on 53.2% of the population. To Kell system, 90.91% are k (Cellano) and 9.09% are Kell positive. The “N” antigen was found in 73.8% of the studied samples. The frequency of Duffy systems reveals the predominance of phenotype Fy (a+b-) diverging from the American Association of Blood Banks – AABB, Technical Manual, probably because of the race diversity in this region. The Kell system and “N” antigen are concordant to AABB.

KEY WORDS: blood donors, phenotype, transfusion

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RED BLOOD CELLS ALLOIMMUNIZATION IN ONCOLOGY PATIENTS IN PORTO VELHO, RONDONIA – BRAZIL

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Alloimmunization to erythrocyte antigens is a well-characterized complication in heavily transfused like oncology patients. The aim of this study was determine the alloimmunization occurrence in oncology patients in Rondonia State, region of known flow migratory with large red blood cells (RBC) antigens diversity. Eighty-seven samples was collected and distributed in the following groups: oncology patients without transfusion (n=19); healthful voluntary that had never received transfusion (n=26); oncology patients that had received transfusion (n=19) and voluntary participants that already had made transfusion use (n=23), being the same ones submitted to the irregular antibody screening and identification if positive. The participants were submitted to a questionnaire. It was observed a fewer antibody presence on oncology participants (5%) that had received transfusion comparing non-oncology participants (16%) that also had received transfusion. The oncology patients, despite to have a great number of transfusions and a short pass time between the transfusions and the examination had a fewer antibody presence, probably to immunity fall due base illness and treatment.

KEY WORDS: alloimunization, oncology, erythrocyte antigens

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IMMUNOGENICITY OF DNAHSP65 VACCINE AGAINST TUBERCULOSIS IN LEWIS RAT

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A DNA vaccine containing the gene of heat shock protein (pVAXhsp65) showed high immunogenicity and protective efficacy against tuberculosis in BALB/c mice. As this mycobacterial protein is highly homologous to the correspondent mammalian protein, an anti-hsp65 immunity could trigger or worsen an autoimmune disease. The purpose of this work was to evaluate the immunogenicity of DNAhsp65 in Lewis rat by using protocols already tested in mice. This analysis will allow a further study of the autoimmune potential of this vaccine in experimental autoimmune encephalomyelitis induced by myelin immunization in these animals. To test DNAhsp65 transcription, RNA was extracted from different tissues (muscle, bone marrow, thymus, spleen and lymph nodes) 3 and 7 days after immunization and assayed by RT-PCR. mRNA for hsp65 was not detected in any of the investigated samples. To test immunogenicity, the animals were immunized with pVAXhsp65 (3 doses/100μg each/intramuscular route) preceded or not by a BCG dose (100μg) by subcutaneous route (prime-boost protocol). Non-immunized rats or rats that received only vector (pVAX) were used as control groups. IgG1 and IgG2b anti-hsp65 were quantified in seric samples and cytokine levels (IFN-γ and IL-4) in supernatants from splenic cell cultures stimulated with recombinant hsp65 (rhsp65) or ConA. pVAXhsp65 did not induce significant amounts of antibodies nor increased the low levels of anti-hsp65 antibodies induced by BCG immunization. Levels of IFN-γ induced by rhsp65 were significantly higher in rats that received only pVAXhsp65 but significantly lower in rats submitted to the prime-boost protocol comparing to non-immunized animals. ConA stimulation induced similar IFN-γ levels in the different groups. No IL-4 was detected in culture supernatants. These results do not allow a definitive conclusion but they could de interpreted as low immunogenicity of these protocols in the rat or induction of a predominant cellular immune response.

KEY WORDS: tuberculosis, DNA vaccine, Lewis rat, hsp65, RT-PCR

FINANCIAL SUPPORT: FAPESP, CNPq.

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T. bahiensis VENOM INDUCES LEUKOCYTOSIS AND MYELOPOIESIS.

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In Brazil scorpions from *Tityus* genus are responsible by the majority of scorpion sting. There are no data about the effects of *T. bahiensis* venom (Tbv), but those reported for *T. serrulatus* include leukocytosis and cytokines in the serum. Our goal was to study the effect of Tbv on the production and mobilisation of leukocytes from bone marrow into blood. Tbv (200µg/kg) or saline (controls) were injected ip into male Swiss mice (26g) and after 0,5, 1, 3, 6 or 24h the animals were killed for performing clonogenic assays with cells from bone marrow (BMC). In brief, the cells were maintained at 37°C and 5% of CO_2 in semi-solid medium, with or without a mixture of colony stimulating factors (CSF). After 7 days the colonies (clusters with more than 50 cells) were counted with a stereoscopic microscope (40X). To investigate the effects of Tbv on blood leukocyte numbers, total and differential leukocyte counts were performed. Results showed that one hour after poisoning (n=6-12), leukocytosis (7±0,7 X 10±0,8; NaCl X Tbv, cellsX10^6/µL) and neutrophilia (2±0,2 X 3±0,3) were observed up to 24h (n=8-12; 6±0,7 X 18±0,8 and 2±0,2 X 7±0,5 respectively), when an increase number of lymphocytes (4±0,5 X 8±0,5), eosinophils (0,1±0,02 X 0,3±0,1) and monocytes (0,1±0,02 X 0,4±0,1) was found. The clonogenic assay with BMC obtained from 6 or 24h poisoned animals showed a synergism between Tbv and CSF (n=8-12, respectively 101±14 X 200±33 and 82±6 X 135±12). Tbv modifies the circulating leukocyte dynamics probably by mobilizing these cells from both the circulation marginal (1h after the Tbv injection) and from medullary pools (3, 6 and 24h).

KEY WORDS: scorpion venom, myelopoiesis, leukocytosis, inflammation

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ACTIVATION OF PHAGOCYTICS CELLS BY JACALIN

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Candidiasis is being recognized with increasing frequency in immunocompromised or severely debilitated individuals. The participation of macrophages and neutrophils has been demonstrated as essential for defence against this fungus. In previous works we observed that jacalin (JCA) extracted from Jaca seeds (*Artocarpus integrifolia*) increased the clearance of *Candida albicans* inoculated by i.p. route in mice. In this work we evaluated the peritoneal phagocytic cells and their phagocytic and candidacidal activities between 6h and 120 hs after administration of JCA. The mice received 500μg of JCA or PBS for 6, 24, 48, 72, 96 e 120 h and euthanized with ether. The phagocytic cells were obtained by washing the peritoneal cavity with 3ml RPMI-albumin and adhered on coverslips for 1h at 37ºC. *C. albicans* CR15 was added 5:1 phagocyte and incubated at 37ºC for 2 h. Half of samples were stained with May Grunwald Giemsa for analysis of phagocytosis and in half of samples, the phagocytes were lysed with sterile water and *C. albicans* plated in Sabouraud agar for determination of candidacidal activity. There was predominance of neutrophils after 6h of administration of JCA and as expected the candidacidal activity was higher than that obtained by administration of PBS (reduction of inoculum 59,6% for JCA versus 40,3% PBS).The maximum of candidacidal activity was observed after 72h administration of JCA when macrophages reduced 76% of inoculum while the non-stimulated reduced only 40%. In the same conditions 70% of macrophages were able to ingest mean number of 5 yeasts. In contrast, only 20% of the non-stimulated macrophages ingest mean number of 3 yeasts. These results suggest that JCA stimulated cytokines production (IFN-γ, TNF-α) which stimulated the functions of phagocytic cells.

KEY WORDS: Jacalin, phagocytes, *Candida albicans*

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