Imunofarmacologia

05.001

ESTUDO COMPARATIVO DA ATIVIDADE CITOTÓXICA *IN VITRO* DO CHÁ VERDE (*Camellia sinensis* L. Kuntze) E 5-FLUROURACIL SOBRE LINHAGEM LEUCÊMICA PRÓ-MIELOCÍTICA HUMANA. Figueirêdo, CAV, Valadares, MC E Queiroz, MLS - Departamento de Farmacologia/ Hemocentro, UNICAMP, Campinas, SP, Brasil

INTRODUÇÃO: A *Camellia sinensis* (GTE) tem despertado grande interesse da comunidade científica em função de suas propriedades antimicrobianas e antitumorais. Diante disso, neste trabalho investigamos a atividade citotóxica do extrato do GTE frente à linhagem leucêmica promielocítica humana (HL-60) comparando com o agente citotóxico 5-Flurouracil (5-FU).
MÉTODOS: O extrato do GTE foi estudado nas concentrações de 12.5-500µg/mL. O 5-FU foi analisado nas concentrações de 1.9-250µg/mL. A avaliação da citotoxicidade foi feita pelo método de redução do MTT-tetrazólio (Mosmann, J: 89:271:1986). Paralelamente foi realizado o teste de exclusão com azul de Tripan.
RESULTADOS: Os resultados demonstraram que o GTE apresenta marcada ação citotóxica dose-dependente *in vitro* frente a linhagem pró-mielocítica humana HL-60 com IC_{50} de 100µg/mL. Nas concentrações de 500µg/mL e 300µg/mL o GTE apresentou citotoxidade semelhante à encontrada com o 5-FU. Estes resultados foram confirmados pelo método de exclusão com Azul de Tripan.
DISCUSSÃO: Neste estudo verificamos uma atividade antiproliferativa dose-dependente *in vitro* do GTE tanto pelo método de MTT quanto pelo método de exclusão por Tripan. Nas maiores doses utilizadas verificou-se que a atividade citotóxica apresentada pelo GTE é semelhante ao 5-FU, sendo portanto um candidato em potencial na terapêutica de leucemias. Maiores estudos estão em andamento em nosso laboratório avaliando a ação mielotóxica do GTE.
Apoio: CAPES/CNPq

05.002

EFFECTS OF Crotalus durissus terrificus VENOM ON MYELOPOIESIS. V. Lopes-Cruz1, G.Z. Justo1, C. Bincoletto1,2, M.L.S. Queiroz1 1Departamento de Farmacologia/Hemocentro, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), C.P. 6111, CEP 13083-970, Campinas, SP, Brasil; 2Universidade de Mogi das Cruzes, Centro Interdisciplinar de Investigação Bioquímica, CEP 08790-911, Mogi das Cruzes, SP, Brasil.

Background: The venom of the South American rattlesnake Crotalus durissus terrificus (CdtV) is a mixture of many proteins such as crotoxin (CTX), which represents the major neurotoxic component of the venom. Recent data demonstrated that CdtV and CTX inhibit some immune and inflammatory reactions. Since, mature inflammatory cells are derived from bone marrow evaluation of hematopoiesis may be an early indicator of toxicity. Thus, the aim of this study was to investigate the effects of Cdt crude venom on the growth and differentiation of bone marrow hematopoietic cells (CFU-GM) in mice. Methods: Male BALB/c mice, 8-10 weeks old, were treated with 2.5-50 mg/kg of CdtV by the i.p. route, for 3 consecutive days. Twenty-four hours after the last dose, animals were killed and bone marrow cells were used for the clonal culture assay. Results and Conclusions: Our results demonstrated a dose dependent reduction in bone marrow CFU-GM numbers ($P<0.01$), which are in line with the CTX-induced changes in peripheral phagocytes numbers reported by Cardoso et al. (Med Inflamm 10:125, 2001). Moreover, the increased levels of inflammatory cytokines and corticosteroids induced by CTX (Cardoso et al., Med Inflamm 10:125, 2001) might be responsible, at least in part, for the myelosuppression observed in this study. Financial support: FAPESP and CNPq.

05.003

EFEITO IMUNOMODULADOR DE PROTÓTIPOS À FÁRMACOS ANTIINFLAMATÓRIOS EM MODELOS DE INFLAMAÇÃO AGUDA E CRÔNICA. MAGNA. S. Alexandre-Moreira[1](PQ)*, Christina M. Takiya[2] (PQ), Bernardo M. O. Pascarelli[2] (IC), Lídia M. Lima[1] (PQ), &, & Eliezer J. Barreiro[1](PQ) [1]LASSBio-Deptª de Fármacos-Faculdade de Fármacia-UFRJ.[2] Deptª. de Histologia e Embriologia (ICB)-UFRJ.

Introdução: A racionalização planejada de novos derivados visou o desenvolvimento de protótipos com atividade antiinflamatória e/ou imunomoduladora, como inibidor de PGHS-2, para os compostos LASSBio 591 e 651 e inibidores duais de PDE e regulador da atividade de TNF-α para o protótipo LASSBio 468[1]. Objetivos: Este trabalho visa avaliar o efeito imunomodulador de LASSBio 591, 651 e 468 em modelos de inflamação aguda e crônica. Métodos e Resultados: Ratos wistar machos (120-170g) foram injetados com *M. tuberculosis* para indução da síndrome de artrite. Após 14 dias estes animais foram submetidos a tratamento (v.o) com LASSBio 591, 651 e 468, dexametasona e nimensulida. Alguns parâmetros foram analisados (peso corpóreo, escore artrítico). As substâncias foram ainda avaliadas em modelos de pleurisia em ratos induzida por carragenina[3]. Os resultados evidenciaram uma atividade antiinflamatória para LASSBio 591, 651 e 468.
Tabela 1.

Tratamento/ Dose (300 µmol/Kg)	% de inibição do edema em modelo de artrite induzida por adjuvante	% de inibição da produção de PGE_2 no exsudato pleural	% de inibição do no. total de células na cavidade pleural
Nimensulida	87*	97*	-
Dexametasona	50*	-	-
LASSBio 468	26*	-	-
LASSBio 591	40*	42*	90,1*
LASSBio 651	47*	65*	8.91*

Conclusões: Resultados preliminares indicam que LASSBio 591, 651 e 468 possuem efeito imunomodulador e antiinflamatório em modelo de artrite induzida por adjuvante e pleurisia em ratos.
Apoio financeiro: EUROFARMA, FAPERJ, CNPq
[1] Lima et al. *Bioorg. Med. Chem. Lett.* (2002) *in press*
[2]Newbould.Brit. J. Pharmacol. (1963)21:127-136
[3]Salvatore et al. Bioch. Pharmacol. (2002)65:785-795

05.004

AVALIAÇÃO DA MIELOTOXICIDADE DE UMA NOVA CLASSE DE COMPLEXOS ORGANOMETÁLICOS - PALADOCICLOS. 1,2*Rocha CO; 1Januário J; 1Cruz RF; 1Caires ACF; 1,2 Bincoletto C. 1.Centro Interdisciplinar de Investigação Bioquímica - Universidade de Mogi da Cruzes. 2.Departamento de Farmacologia, UNICAMP.

Introdução: A descoberta da Cisplatina estimulou a pesquisa de drogas antitumorais mais seletivas. Como a mielotoxicidade normalmente limita a dose da quimioterapia, permitindo a sobrevivência de alguns clones de células malignas, sua determinação é imprescindível no desenvolvimento de novos fármacos. Neste trabalho, avaliamos o comprometimento de células progenitoras da medula óssea para granulócitos/macrófagos (CFU-GM) na presença de um novo composto contendo paládio como metal de transição (paladociclo). Métodos: Células da medula óssea de camundongos Swiss sem tratamento e de animais tratados com 1 mg/L do composto paladociclo por 4 dias, foram removidos para os estudos in vitro (25, 10, 5 ou 1 mg/L) e in vivo, respectivamente. O crescimento e a diferenciação celulares foram avaliados através da técnica de cultura clonal. Resultados: A dose de 1mg/L não alterou o número de progenitores CFU-GM da medula óssea nos estudos in vitro e in vivo. Por outro lado, uma diminuição dose-dependente no número de CFU-GM foi observada nos estudos in vitro com doses superiores a 5 mg/L ($p<0.05$, ANOVA - Tukey-Kramer). Discussão: A mielotoxicidade deste composto é dose-dependente. Porém, a dose de 1 mg/L, a qual não apresentou efeitos mielotóxicos, aumentou a sobrevida de animais portadores do tumor de Ehrlich em estudos paralelos realizados em nosso laboratório, sugerindo que este composto apresenta atividade antitumoral com doses menores daquelas mielotóxicas. Agradecimento: CNPq, FAPESP, UMC.

05.005

AVALIAÇÃO DA CITOTOXICIDADE DE COMPLEXOS ORGANOMETÁLICOS CONTENDO PALÁDIO COMO METAL DE TRANSIÇÃO – PALADOCICLOS. 1,2Rocha CO; 1Caires ACF; 1,2Bincoletto C. 1Universidade de Mogi das Cruzes, Centro Interdisciplinar de Investigação Bioquímica, CEP 08790-911, Mogi das Cruzes, SP, Brasil. 2Departamento de Farmacologia/Hemocentro, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), C.P. 6111, CEP 13083-970, Campinas, SP, Brasil; e-mail: trindade@umc.br

Introdução: Os primeiros relatos do uso de metais ou de compostos contendo metais na terapia contra cânceres e leucemias datam dos séculos dezesseis e dezenove. O uso destes compostos ficou praticamente esquecido até os anos 60, quando a atividade antitumoral do complexo inorgânico, cis-diamina-dicloroplatina(II) (cisplatina), foi descoberta. Isto conduziu ao desenvolvimento de novas drogas citotóxicas e citostáticas. Objetivando o desenvolvimento de drogas antitumorais mais seletivas com menor incidên-

cia de toxicidade sistêmica. Neste trabalho, avaliamos a citotoxicidade de 5 Complexos Ciclopaladados (CCP) em duas linhagens de células leucêmicas, HL60 (leucemia promielocítica humana) e K562 (eritroleucemia humana). Métodos: A citotoxicidade celular dos CCP foi avaliada através do teste de redução do 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium (MTT). A viabilidade celular foi estudada através do método de exclusão com Azul de Trypan. Resultados: Os 5 CCP apresentaram um potencial citotóxico similar contra as duas linhagens leucêmicas estudadas Discussão: Os resultados sugerem que os CCP possuem atividade citotóxica que segue uma curva dose-dependente merecendo assim, contínuas investigações quanto ao seu potencial antitumoral. Agradecimento: CNPq, FAPESP, UMC.

05.006

CYTOTOXIC EFFECT OF PROPOLIS ON DIFFERENT *Leishmania* SPECIES. Campos, T.B[1*].; Monteiro, M.C[2].; Silva, O.S[1].; Paulino, N[3].; Blazius, R.D[1].; Cunha, F.Q[2]. & Romão, P.R.T[1]. Lab de Imunoparasitologia[1] e Lab. de Pesquisa e Desenvolvimento de Biofármacos[3], Universidade do Sul de Santa Catarina (UNISUL), Tubarão, SC, Brasil; Dpto. de Farmacologia[2], Fac. de Medicina de Ribeirão Preto-USP, Ribeirão Preto-SP. [1]* Iniciação Científica.

Leishmania causes a spectrum of diseases ranging from self-healing ulcers to disseminated and often fatal infections, depending on the species involved and host's immune response. Adequate protective vaccines against trypanosomatid infections have yet to be developed, and drugs currently available for chemotherapeutic intervention are mostly unsatisfactory. Thus, one of the priorities in tropical medicine research has been the development of efficient drugs for treatment. It has been demonstrated that Propolis possesses many biological activities: antibacterial, antiviral, fungicidal and antitumoral. The aim of this study was to investigate the effect of Propolis against different *Leishmania* species (*L. amazonensis*, *L. braziliensis*, *L. donovani* and *L. major*). The cellular viability was measured by parasite mobility using a Neubauer chamber. The addition of Propolis (30-300 µg/ml) directly to promastigotes of different species of *Leishmania* resulted in dose-dependent parasite killing. The survival percentage of *Leishmania* species above treated with Propolis (30-300 µg/ml) ranged from 15 to 98%. *L. braziliensis* was more susceptible to the toxic effect of Propolis than the others species. This effect was more evident with Propolis at concentration of 30 µg/ml. In this concentration *L. braziliensis* showed a 60% of killing. In contrast, the viability of the other species was not modified when compared with the control (2% of killing). We are currently investigating the effect of Propolis and isolated compounds on amastigote forms of parasites and studying the course of infection in BALB/c mice infected with *L. amazonenis* and treated with Propolis and purified compounds.
Financial suport: CAPES/CNPq/FAPESP

05.007

ESPRAIAMENTO E FAGOCITOSE DE MACRÓFAGOS PERITONEAIS ATIVADOS POR ONCO-BCG: EFEITOS DA CYHALOTHRINA, UM PIRETRÓIDE TIPO 2. Abbud Righi, D.; Palermo Neto, J. Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo – São Paulo/SP.

Objetivos: Avaliar os efeitos cyhalotrina sobre a atividade de macrófagos ativados e obtidos da cavidade peritoneal de ratos. Métodos e Resultados: 30 ratos Wistar machos, foram divididos ao acaso em 3 grupos iguais: 1 grupo controle (C) e 2 grupos experimentais (E1 e E2). Os animais dos grupos experimentais receberam por via oral,: E1 = 1mg/kg/dia; E2 = 3mg/kg/dia de cyhalothrina durante 7 dias, respectivamente. Os animais do grupo controle foram tratados de igual forma com o veículo. Todos os animais receberam duas doses de 0,25 ml de Onco-BCG, pela via i.p. com intervalo de 7 dias, para ativação dos macrófagos peritoneais. As administrações de cyhalotrina e/ou veículo iniciaram-se 4 dias após a primeira administração de BCG; 3 dias após a segunda dose de BCG, coletaram-se os macrófagos peritoneais. Os resultados (médias ± SD) mostraram que a porcentagem de macrófagos espraiados dos animais dos grupos E1 e E2 foi menor que aquela do grupo C; os dados do grupo E2 foram menores que os do grupo E1(C = 81,15 ± 3,35; E1= 64,65 ± 2,34; E2 =57,6 ± 2,86). Observou-se diminuição do percentual de fagocitose, tanto nos animais dos grupos E1 e E2 em relação aos do grupo C; como nos animais do grupo E2 em relação aos do grupo E1 (C = 84,45 ± 2,92; E1= 65,7 ± 4,87; E2 =55,2 ± 1,87). Conclusão: A cyhalotrina, interfere com a atividade de macrófagos peritoneais. Uma que vez que observamos anteriormente ser este praguicida ansiogênico (Anais Fesbe 2001, resumo 01.083) é possível que a diminuição da atividade dos macrófagos agora observada, esteja relacionada a este fato. Apoio financeiro: FAPESP.

05.008

EFEITOS DO TRATAMENTO COM HALOPERIDOL SOBRE A ATIVIDADE DE MACRÓFAGOS PERITONEAIS DE RATOS. Lourenço, GA1,2; Dorce, VAC2; Palermo-Neto, J1 - 1 Depto de Patologia Experimental e Comparada FMVZ-USP; 2 Lab. de Farmaco Instituto Butantan

Introdução: No modelo de tumor experimental de Ehrlich, o tratamento com haloperidol promove diminuição da taxa de crescimento do tumor e aumento da atividade de macrófagos. Fêmeas apresentam maior incidência de doenças autoimunes e maior resposta imunológica. Estudamos então os efeitos do tratamento com haloperidol sobre a atividade de macrófagos peritoneais de ratos machos e fêmeas estimulados com onco-BCG oral.
Métodos: Ratos Wistar machos e fêmeas (n=6), estimulados com onco-BCG oral, receberam os tratamentos: Agudo - uma injeção de 2mg/kg de haloperidol 1 hora antes da coleta do lavado peritoneal. Prolongado - injeções diárias de haloperidol 2mg/kg por 21 dias. Abstinência - idem ao tratamento prolongado com abstinência de 72 horas antes da coleta. Efetuou-se lavado peritoneal com PBS estéril, e procedimentos para quantificação da produção de NO, H2O2 e da porcentagem de espraiamento e fagocitose.
Resultados: Macrófagos de ratos machos e fêmeas, com tratamento agudo e prolongado, apresentaram aumento na produção de NO e água oxigenada, além de aumento da porcentagem de espraiamento e fagocitose. Animais machos em abstinência, apresentaram níveis semelhantes aos animais controle em todos os itens analisados; entretanto, as fêmeas em situação de abstinência apresentaram uma pequena diminuição de todos os parâmetros, quando comparadas com o grupo controle. O tratamento com haloperidol aumentou a atividade de macrófagos, havendo uma discreta diferença entre machos e fêmeas em abstinência.
Apoio Financeiro: CNPq, FAPESP 99/04228-7, Fundação Butantan

05.010

***Schistosoma mansoni* INFECTION IN MIP-1a-DEFICIENT MICE.** Adriano L. S. Souza1, Ester Roffê1, Vanessa Pinho1, Danielle G. Souza1, Cíntia A. J. Pereira2, Michele M. Barsante3, Deborah A. N. Correa2, Mauro M. Teixeira1. 1 – Lab. Imunofarmacologia, Depto de Bioquímica e Imunologia, ICB, UFMG 2 – Depto de Parasitologia, ICB, UFMG 3 – Lab. Imunobiologia, Depto de Bioquímica e Imunologia, ICB, UFMG

Introduction: MIP-1a is a CC chemokine whose role in granulomatous response has been demonstrated in experimental models of embolization. MIP-1a concentrations are elevated in plasma of patients with hepatosplenic schistosomiasis. However, there is no data reporting the role of MIP-1a gene deletion (MIP-1a-/-) during Schistosoma mansoni infection. Methods: 8-week-old male and female C57/BL6 MIP-1a-/- mice were infected with 25 cercariae and sacrificed after 9 or 14 weeks, for evaluation of acute and chronic disease, respectively. The number of eggs was counted in the feces and in liver, spleen, intestine and lungs after KOH digestion. The organs were fixed for histopathological analysis and frozen for cytokine detection by ELISA. Differential leukocyte counts were performed in blood smears. Cells from spleen and mesenteric lymph nodes were harvested for proliferation assay in response to parasitic antigens. Infected and uninfected C57/BL6 wild type mice served as controls. Results: Preliminary results show no difference in the parasitological evaluation i.e., number of worms in the portal system and eggs sequestered in the tissues of knock-out and wild type mice at the acute phase of the disease. However, a smaller number of eggs was found in the liver of MIP-1a-/- mice at the chronic phase, which paralleled a reduced number of paired worms. A higher number of eosinophils and neutrophils was also found in the systemic circulation of knock-out mice at the chronic phase of the disease as compared to

wild type controls. Conclusion: MIP-1a appears to play a role during S. mansoni infection in mice. Further studies are needed to determine the mechanism of the protective action of MIP-1a gene deletion. Support: Capes, CNPq, WHO (TDR A10337)

05.011

MYOCARDIAL INFLAMMATION DURING T. CRUZI INFECTION IN RATS. Roffê E1, Souza ALS1, Prates EG1, Souza DG1, Pinho V1, Talvani A1, Camargos EP2 & Teixeira MM1. 1Immunofarmacology, Department of Biochemistry and Immunology and 2Neurobiology, Department of Morphology, ICB, UFMG, Belo Horizonte, Brasil

Introduction: During T. cruzi infection, establishment of inflammation is necessary to control the intense parasitism observed during the acute infection. However, chronic myocarditis does not correlate with the intensity of the parasitism. In an attempt to define the molecular mechanisms responsible for the inflammation during acute and chronic myocarditis, we examined the inflammatory infiltrate and expression of inflammatory mediators during infection of adult rats with T. cruzi. Methods: Holtzman rats, 90 days old, were infected with 104 tripomastigotes of the T. cruzi CL-Brener strain /50g and sacrificed on days 12, 15, 19, 30, 60 and 120 after infection. Parasitemia, myocardial histopathology and expression of inflammatory mediators (RT-PCR and ELISA) were assessed on these days. Results/discussion: The parasitemia was very low, beginning 9 days (2x105 parasites/mL) after infection and peaking around day 13 (5x105 parasites/mL), being negligible at day 15. We observed a moderate inflammatory infiltrate, composed predominantly of mononuclear cells and many amastigote nests in the myocardium, mainly at day 19 after infection. There was frequently a correlation between the expression of parasite nests and inflammatory cells. Moreover, the expression of chemokines and pro-inflammatory cytokines paralleled the inflammatory infiltrate. Future studies will define the functional role of the identified molecules in driving cardiac inflammation. Supported by: CAPES.

05.012

***Trypanosoma cruzi* INFECTION: INVOLVEMENT OF LIPID BODIES AND APOPTOTIC CELLS IN THE MACROPHAGE DEACTIVATION.** D'Ávila, H.1; Freire-de-Lima, C.G.; Castro-Faria-Neto, H. C.1; Bozza, P. T.1 1Lab. Imunofarmacologia - IOC - FIOCRUZ – Rio de Janeiro, Brasil

There is an intense neutrophil apoptosis during experimental Chagas disease. We have demonstrated that uptake of apoptotic cells exacerbates parasite replication in co-cultured macrophages infected with T. cruzi in a manner PGE2 and TGF b-dependent. Studies showed that lipid bodies, organeles arachidonic acid - rich, might function as specific sites involved in PGE2 synthesis during inflammation. In this work, it was investigated the role of lipid bodies in the PGE2 synthesis during T. cruzi infection and the relationship with uptake of apoptotic cells. Lipid body formation and PGE2 synthesis were investigated in peritoneal macrophages from C57Bl/6 and BALB/c mice co-cultured with T. cruzi and GIPL (glycoprotein of trypanosomatids), only or with apoptotic cells or anti-av antibody. The intracellular localization of COX-2 was investigated by imunocytochemistry. The results demonstrated that T. cruzi infection and GIPL induced lipid body formation in macrophages. and this phenomenon can be markedly enhanced with co-culture of apoptotic cells. The treatment of macrophages with apoptotic cells or anti-av antibody also induced lipid body formation and this mechanism was accomplished by a significant enhancement in the PGE2 production and co-localization of COX-2 in this lipid bodies. In conclusion, the T. cruzi infection and the uptake of apoptotic cells induced lipid bodies formation and this phenomenom seem to modulate the macrophage deactivation by PGE2 synthesis. Support: CNPq, FAPERJ, Howard Hughes Medical Institute and FIOCRUZ

05.013

EFEITOS DO INTERFERON-g NA FAGOCITOSE E NA PRODUÇÃO DE MEDIADORES INFLAMATÓRIOS POR MACRÓFAGOS MURINOS. Fernandes, B., Henriques, M.G.M.O., Sampaio, A.L.F. Laboratório de Farmacologia Aplicada – FarManguinhos – FIOCRUZ - RJ e-mail: asampaio@far.fiocruz.br

Macrófagos possuem um papel importante no desencadeamento e manutenção de reações inflamatórias através da liberação de mediadores inflamatórios. Neste estudo, investigamos se há correlação entre aumento da fagocitose de partículas de zimosan (ZIM), e a liberação da quimiocina KC e do óxido nítrico (NO), por macrófagos pré-estimulados in vitro com interferon-g (IFN-g) ou com LPS. Macrófagos peritonais de camundongos saudáveis (105 cél./mL) foram incubados com 106 partículas de ZIM/mL após o pré-tratamento (1h) com LPS ou doses crescentes de IFN-g (1-40 U/mL), sendo a fagocitose analisada 4h após a adição do ZIM. Nestas condições, o LPS não foi capaz de alterar a atividade fagocítica dos macrófagos, enquanto que a estimulação com IFN-g induziu, de forma dose dependente, um significativo aumento da taxa de fagocitose. Observamos, por microscopia confocal, um aumento significativo da expressão da molécula CD11b nas vesículas fagocíticas das células do grupo ZIM+IFN-g (20U/mL), quando comparado com o grupo controle ZIM. A incubação com o ZIM ou com ZIM+IFN-g não foi capaz de estimular a produção de NO em 4h. Entretanto, neste mesmo tempo de análise, a incubação com ZIM foi capaz induzir a produção de KC. O pré-tratamento com IFN-g (20U/mL) levou a um discreto, porém significativo, aumento desta resposta (de 8,50±0,13 no grupo ZIM para 9,18±0,13 pg/mL no grupo ZIM +IFN-g) entretanto, este aumento não foi dependente de dose. Nossos resultados demonstram que o IFN-g é capaz de aumentar a fagocitose, mas não a liberação de KC, de forma dose dependente, dissociando o aumento de fagocitose da produção da quimiocina KC. Nossos dados ainda sugerem que o aumento na fagocitose está relacionado a um aumento da expressão da molécula CD11b. Apoio: PIBIC-FIOCRUZ/CNPq

05.014

REQUISITE ROLE FOR MCP-1 ON gd T LYMPHOCYTE TRAFFICKING DURING INFLAMMATION. Penido C*, Vieira-de-Abreu A *, Bozza MT, Castro-Faria-Neto HC*, Bozza PT* *Laboratório de Imunofarmacologia, DFF, FIOCRUZ, Departmento de Imunologia, IMPPG, UFRJ, RJ, Brazil.

The mechanisms by which gd T lymphocytes migrate to inflamed sites are poorly understood. We have previously shown that LPS induces gd T cell accumulation in mouse pleural cavity within 24h. Here we investigated the role of CC chemokines in regulating gd T cell migration after LPS or BCG challenge. LPS-induced gd T cell influx was significantly inhibited by the in vivo administration of a viral encoded 35kDa CC chemokine neutralizing protein. Indeed, we showed that LPS stimulation increased the expression of mRNA and protein synthesis for MCP-1 in the pleural cavity of mice 6h after LPS injection. Pretreatment of mice with dexamethasone significantly inhibited gd T cell influx and MCP-1 production in the inflammatory site. Moreover, MCP-1 neutralization by specific mAb or its deletion in MCP-1 knockout (KO) mice lead to an impairment of gd T cell accumulation into the pleural cavity after LPS stimulation. LPS-induced gd T cell mobilization and MCP-1 synthesis in C3H/HeJ mice was significantly reduced when compared to control mice C3H/HeN, suggesting an involvement of TLR4 in this phenomenon. Interestingly, the i.t. injection of BCG also induced MCP-1 synthesis followed by gd T lymphocyte mobilization to the mouse pleural cavity. In addition, BCG-induced gd accumulation was significantly reduced in MCP-1 KO mice when compared to wild type mice. These data shows that LPS- and BCG-induced gd T cell migration to the pleural cavity of mice is requisitely dependent on the CC chemokine MCP-1. Support: FIRCA/NIH, CNPq and FAPERJ

05.015

ENDOTHELIN INDUCES LYMPHOCYTE AND NEUTROPHIL ACCUMULATION AND CONTRIBUTE FOR LEUKOCYTE RECRUITMENT IN LPS-INDUCED INFLAMMATION. André Luiz Franco Sampaio1, Giles Alexander Rae2, Maria das Graças Müller de Oliveira Henriques1. 1 Lab. of Applied Pharmacology, FarManguinhos - Oswaldo Cruz Foundation - Rio de Janeiro, RJ, Brazil 2 Dept. of Pharmacology, ICB, University of Santa Catarina – Florianópolis, SC, Brazil e-mail: asampaio@far.fiocruz.br

Endothelins participate in different aspects of inflammatory reactions including edema formation and leukocyte accumulation. Herein, we demonstrated a role for endogenous endothelins in granulocyte and T lymphocyte recruitment in a murine inflammation model. Pleurisy was induced in male Balb-c mice by endothelin-1 or LPS. Animals were treated in situ with BQ-123 (150pmol/cavity), 5 min before intratoracic stimulation. Pleurisy was evaluated 4 or 24h after intrathoracic stimulation. Accumulation of lymphocyte populations into mouse pleural cavity was analyzed by flow cytometry. Intrathoracic injection of endothelin-1 triggered a neutrophil accumulation at 4h, via ET-A receptor. Concomi-

tant with neutrophil recruitment we observed an increase of CD4+ and CD8+ T cell populations. Albeit the ability of ET-1 to trigger neutrophil accumulation, the blockade of ET-A receptors, by BQ-123, failed to change the 4h neutrophil accumulation triggered by LPS. On the other hand, treatment with BQ-123 inhibited neutrophil and eosinophil accumulation, triggered by LPS at 24h, suggesting a role for these peptides in the delayed leukocyte accumulation. The LPS-induced eosinophil accumulation into mouse pleural cavity, is highly dependent of gamma-delta+ T (gd+)cells but not of CD4+ and CD8+ T cells. In LPS-induced pleurisy, CD4+ and CD8+ T lymphocyte accumulation are significantly inhibited by ET-A receptor antagonism. Conversely, no significant changes in gd+ T cells accumulation were observed after BQ-123 treatment. These data demonstrate a pro-inflammatory activity of endothelin-1 and suggest that endothelins have a role in granulocyte accumulation in LPS inflammation. However, the precise role of endogenous endothelins in LPS-induced inflammation is under investigation. Support: CNPq

05.016

EFFECTS OF DEXAMETHASONE ON THE EXPRESSION OF ADHESION MOLECULES IN MURINE BONE-MARROW CELLS. 1,2Padua, M., 1,3Cheraim, A.B., 3Alves,L., 2Silva, C.F.S., 2 Luz, R.A., 3Elsas, P.X., 2Elsas, M.I.C. 1Departamento de Patologia,UFF, 2Departamento de Pediatria, Instituto Fernandes Figueira, Fiocruz; 3Departamento de Imunologia, Instituto de Microbiologia, UFRJ.

Introduction: Systemic treatment of mice with dexamethasone, in a model of allergic inflammation, blocks eosinophil recruitment to inflammatory sites, thereby inducing increases in bone-marrow eosinophil numbers. This suggests that, inside the bone-marrow, eosinophils which resist killing by dexamethasone (thought to underlie peripheral eosinopenia), express increased levels of adhesion proteins, which prevent the release of hematopoietic cells to the circulation. Methods and Results: We evaluated the effect of dexamethasone on the expression of CD49d (VLA-4) in liquid culture of bone-marrow cells from naïve BALB/c and C57BL/10 mice, established in the presence of IL-5 (1ng/ml), alone or in association with dexamethasone (10-5 M-10-7 M). VLA-4 expression was evaluated after 7 days of culture by immunocytochemistry and flow cytometry analysis. Dexamethasone induced a significant increase in VLA-4 expression in BALB/c mice, relative to controls. Increase was restricted to the IL-5-dependent polymorphonuclear cell compartment (eosinophils). This effect was not observed in C57BL/10 mice, which nevertheless responded to dexamethasone by increased IL-5-dependent eosinophil production. Conclusion: Dexamethasone enhances VLA-4 expression in bone-marrow eosinophils depending on the strain analyzed. This can be dissociated from enhancement of eosinopoiesis by dexamethasone. Financial support: CNPq, PAPES/FIOCRUZ, CAPES, FIOCRUZ, FINEP,PIBIC-CNPq.

05.017

EXPRESSION AND In Vivo ANGIOGENIC EFFECTS OF JE/MCP-1 IN AN INFLAMMATORY ANGIOGENESIS MODEL. 1Barcelos, L.S., 1Talvani, A., 2Teixeira, A.S., 3Cassali, G.D. 2Andrade, S.P., 1Teixeira, M.M., Departamentos 1Bioquímica e Imunologia, 2Biofísica e Fisiologia e 3Patologia Geral, ICB-UFMG, BH-MG, Brazil

Introduction: Angiogenesis (AG) depends on a complex network of cells and mediators. Chemokines are thought to play an important role during AG. Here, we examined the expression of the CC chemokine JE/MCP-1 during inflammatory angiogenesis (IAG) in TNFR1-/- and wild-type (WT) mice and the ability of exogenous JE to modulate IAG. Methods: Sponges, a framework for tissue growth, were implanted in BALB/c or TNFR1-/- and WT C57Bl/6 mice and collected at various days for RT-PCR and/or ELISA. In some experiments, sponges were injected with recombinant JE (100 ng/sponge/day) from days 1-3, 6-8 and 11-13 and collected at day 14 post-implant for hemoglobin (Hb), myeloperoxidase and N-acetyl-glucosaminidase measurements, used as index for neovessels, neutrophil (PMN) and macrophage (Mac) influx, respectively. Results: We observed that JE mRNA and protein peaked at day 1, but significant levels were detected until day 14. Levels of JE and AG were significantly lower in TNFR1-deficient mice. Moreover, JE treatment induced a significant increase in Hb and Mo contents, but not PMN influx. Discussion: Thus, JE expression was very early, preceded Mac influx and appeared to be modulated by TNFa. Exogenous JE induced Mac recruitment and enhance AG. These results suggest a potential role for TNFa-induced JE production and consequently Mac influx. Supported by: CNPq, CAPES, FAPEMIG

05.018

ESTUDO COMPARATIVO ENTRE CAMUNDONGOS DEFICIENTES NO RECEPTOR E TRATAMENTO COM ANTAGONISTA DO PAF EM UM MODELO DE ISQUEMIA E REPERFUSÃO MESENTÉRICA. Guabiraba, R., Souza , D.G., Pinho, V. , Soares, A.C., Teixeira, M.M. Laboratório de Imunofarmacologia, Departamento de Bioquímica e Imunologia, ICB/UFMG - Belo Horizonte-Minas Gerais- Brasil

INTRODUÇÃO: O processo de isquemia e reperfusão mesentérica é acompanhado por resposta inflamatória aguda, caracterizada pelo aumento de permeabilidade vascular, recrutamento neutrofílico, hemorragia e produção de citocinas e mediadores, entre eles PAF. Neste trabalho temos por interesse comparar a resposta inflamatória após isquemia e reperfusão mesentérica entre camundongos PAFR -/- e convencionais tratados com antagonista do receptor. MÉTODOS: Camundongos PAF -/- e convencionais C57BL6 foram submetidos a 60 minutos de isquemia seguidos por 30 minutos de reperfusão da artéria mesentérica superior. O antagonista do receptor de PAF, UK 74505, foi administrado 10 minutos antes da reperfusão. RESULTADOS: Foi observada inibição do aumento de permeabilidade vascular e do recrutamento de neutrófilos nos animais PAFR -/- e nos tratados com antagonista do receptor na dose 1 mg/kg e diminuição de citocinas pró-inflamatórias. Animais PAFR-/- tiveram letalidade diminuída quando comparados com animais WT. Ao passo que lesões inflamatórias são inibidas por doses usuais do antagonista, a letalidade foi inibida apenas na dose de 10 mg/kg.CONCLUSÃO: Os resultados confirmam o papel do PAF na isquemia e reperfusão mesentérica e sugerem que doses elevadas de antagonistas do receptor do PAF são necessárias para um benefício máximo dessa estratégia. FINANCIADORES: CNPq, FAPEMIG

05.020

THE IMPORTANT MODULATORY ROLE OF IL-10 IN THE SYSTEMIC INFLAMMATORY RESPONSE AFTER REPERFUSION INJURY - LESSONS FROM GNOBIOTIC ANIMALS. Souza, D.G., Soares, A.C., Pinho, V.S., Vieira, L.Q. & Teixeira, M.M. - Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, ICB-UFMG- Belo Horizonte – Minas Gerais – Brasil.

Introduction: Reperfusion of an ischemic territory is commonly accompanied by significant neutrophil-dependent local and systemic injury. Several studies have suggested that bacterial translocation with subsequent activation of inflammatory cells may be important during ischemia and reperfusion (I/R) injury. Here, we have investigated the role of the intestinal microbiota during I/R injury in germ-free mice.
Methods: The superior artery mesenteric (SMA) of mice was made ischemic for a period of 60 min followed by varying periods of reperfusion. Results: There were fewer deaths and no increase of vascular permeability, neutrophil accumulation and haemorrhage after I/R in germ-free animals (GF) when compared with conventional animals (CV). Interestingly, GF presented much lower levels of inflammatory cytokines in tissue and serum, including TNF-α, MCP-1, MIP-2 and KC. In contrast, concentrations of IL-10 were greatly elevated in these animals. The "no-inflammation" phenotype of gnotobiotic mice was also seen after systemic injection of LPS. Moreover, pretreatment of animals with anti-IL-10 reversed the latter phenotype and was accompanied by tissue inflammation and lethality after I/R.
Conclusions: Thus, our results demonstrate that the intestinal microbiota is not relevant for I/R injury per se. However, the lack of intestinal bacterial induces a state of inflammatory hyporresponsiveness largely mediated by the secretion of IL-10.
Supported: CNPq, FAPEMIG

05.021

ROLE OF BRADYKININ FOR THE LOCAL AND SYSTEMIC INFLAMMATORY RESPONSE THAT FOLLOWS INTESTINAL REPERFUSION INJURY. [1]Souza DG, [1] Pinho V, [2]Lopes ES, [2]Pesquero JL, [3]de A. Castro MS & [1]Teixeira MM - Departamentos de [1]Bioquímica e Imunologia, [2]Fisiologia e Biofísica, [3]Farmacologia, ICB-UFMG- Belo Horizonte – Minas Gerais - Brasil

Introduction: Bradykinin appears to play an important role in the development and maintenance of inflammation. Here, we assessed the role of bradykinin for the injuries that occur after ischemia and reperfusion (I/R) of the territory irrigated by the superior mesenteric artery.
Results: Kallikrein activity increased after ischemia and peaked at 15 min after reperfusion.
A selective inhibitor of tissue kallikrein (TKI) inhibited kallikrein activity in a concentration-dependent manner. *In vivo*, pre-treatment with TKI prevented the extravasation of plasma and the recruitment of neutrophils. A bradykinin B2 receptor antagonist (HOE140) inhibited the increase in vascular permeability and the recruitment of neutrophils in the intestine and lungs following I/R. Similarly, a structurally distinct B2 receptor antagonist, FR173657, also prevented the injuries. In a model of more severe I/R injury, HOE 140 abrogated the increase in vascular permeability, neutrophil recruitment and haemorrhage. Furthermore, HOE140 significantly inhibited the elevations of TNF-??in tissue and serum and partially prevented lethality.
Conclusions: Thus, our results demonstrate that following intestinal ischemia and reperfusion injury there is an increase in tissue kallikrein activity and activation of B2 receptors. Our results suggest that B2 receptor antagonists may be useful adjunct therapy for the treatment of the severe injuries that follow intestinal ischemia and reperfusion.
Supported: CNPq, FAPEMIG

05.022

IL-1ß-DRIVEN ENDOGENOUS IL-10 PRODUCTION PROTECTS AGAINST THE SYSTEMIC AND LOCAL ACUTE INFLAMMATORY RESPONSE FOLLOWING INTESTINAL REPERFUSION INJURY. Souza, D.G., Guabiraba, R., Pinho, V., Soares, A.C.,. & Teixeira M.M. Laboratório de Imunofarmacologia, Departamento de Bioquímica e Imunologia, ICB-UFMG- Belo Horizonte – Minas Gerais – Brasil

Introduction: IL-1ß may play a role in the inflammatory lesions which accompany ischemia and reperfusion injury to several vascular beds. Here, we examined whether IL-1ß participated in the cascade of events leading to TNF-a production and TNF-a-mediated intestinal ischemia/reperfusion (I/R) injury. Methods: The superior mesenteric artery (SMA) of rat was made ischemic for 120 min followed by 120 min of reperfusion. The effects of IL-1ß were studied by the administration of recombinant rat IL-1ß or anti-IL-1ra, the blockage of endogenous IL-1ß was achieved by the administration of recombinant human IL-1ra or anti-IL-1. The role of IL-10 was assessed by administration of recombinant rat IL-10 or anti-IL-10. Results: Surprisingly, IL-1 and aIL-1ra administration inhibited, whereas aIL-1 and IL-1ra increased the lethality, increase in vascular permeability, neutrophil accumulation, hemorrhage and cytokines levels induced by intestinal I/R injury. We sought to investigate the mechanisms of this inhibition and observed an increase in IL-10 and a decrease in TNF-a production in animals treated with IL-1b or aIL-1ra. In contrast, IL-1ß blockade diminished IL-10 levels and enhanced TNF-a levels. Treatment with IL-10 inhibited lethality and I/R injury whereas the injection of anti-IL-10 enhanced injury. More importantly, treatment with anti-IL-10 reversed the beneficial effects of IL-1b in I/R-induced lethality. Conclusion: Our results demonstrate a yet unreported beneficial effect of IL-1b in a model of I/R, an effect which was shown to be mediated by an increase in IL-10 production. Our results point to an important role of pro-inflammatory mediators in triggering endogenous anti-inflammatory networks in an acute systemic inflammatory response. Supported: CNPq, FAPEMIG

05.023

O ÓXIDO NÍTRICO (NO) MODULA A MIGRAÇÃO DOS EOSINÓFILOS DA MEDULA ÓSSEA AO PULMÃO DE RATOS ALÉRGICOS. *Mônia L.S.Lodo, *Rosana A.O.Costa, *Jerusa M.Jacheta, *Heloisa H.A.Ferreira.*USF,Bragança Paulista,SP.

Introdução: A inibição da síntese de NO pelo tratamento crônico com L-NAME causou redução no influxo de eosinófilo para o pulmão de ratos sensibilizados 48h após o desafio com a OVA (Ferreira, Eur.J.Pharmacol.358:253,1998). Agora investigamos se a diminuição no conteúdo de eosinófilos (eos) no pulmão destes ratos é conseqüência de alterações na emigração da medula óssea e/ou na mobilização dos eos do sangue periférico para o pulmão. Métodos: Ratos Wistar tratados com L-NAME (20mg/dia,30 dias) foram sensibilizados com OVA. Em 24, 48 e 72h após o desafio foi verificado na medula óssea do fêmur o número de eos nos diferentes estágios de diferenciação e a adesão à fibronectina em microplaca. Contagem total e diferencial dos leucócitos foi feita no sangue periférico e do lavado broncoalveolar (LBA). Resultados: 48h após o desafio, concomitante ao pico de eos no LBA, foi observado diminuição de 60% no número de eos na medula óssea e ausência no sangue periférico dos ratos controles (não tratados). Nos ratos tratados com L-NAME, simultânea à redução de 80% do conteúdo no LBA, nenhuma alteração no número dos eos foi observada na medula óssea ou no sangue. A adesão dos eos à fibronectina mostrou-se aumentada 64% nos ratos controles e 32% nos tratados com L-NAME, 24 e 48h após o desafio, respectivamente. Conclusão: Os resultados sugerem que o NO modula a expressão das moléculas de adesão envolvidas na migração dos eosinófilos, refletindo no tráfego destas células da medula óssea para o sangue periférico e, posteriormente, para o pulmão dos animais alérgicos. Financiador:PROPEP/USF

05.024

SELECTIVE EOSINOPHIL MIGRATION TO THE PERITONEAL CAVITY IN MICE CARRYING EGG WHITE IMPLANTS (EWI): ROLE OF ANTIGEN, CD4+ LYMPHOCYTES AND LEUKOTRIENES. 1,5Cheraim, AB, 2Elsas, PX, 3Oliveira, SHP de, 3Batistella, T, 4Russo, M, 1Elsas, MIG, 3Cunha, FQ. 1IFF/FIOCRUZ, 2Depto. Imunol., Inst. Microbiologia, UFRJ, 3Depto. Farmacologia, FMRP-USP, 4Depto. Imunologia, USP, 5Depto. Patologia, UFF.
Introduction: Subcutaneous heat-coagulated egg white implant (EWI) induces a chronic local eosinophilia. We investigated the mechanisms involved in eosinophil mobilization in this model. Methods and Results: EWI mice showed a time- and dose-dependent eosinophil accumulation in the peritoneal cavity after local challenge with ovalbumin. This was not blocked by pretreatment with nitroarginine, aminoguanidine, indomethacin, BN50021, or thalidomide. By contrast, it was significantly inhibited by CP105 (3mg.kg-1, s.c.), MK886 (1mg.kg-1, v.o), or dexamethasone (1mg.kg-1, s.c.), suggesting the involvement of leukotrienes. Eosinophil recruitment was also induced in EWI mice, but not naïve controls, by i.p. injection of culture supernatants from peritoneal cells harvested from EWI mice and stimulated ex vivo with ovalbumin (10μg.ml–1). This could be reproduced by injection of total peritoneal cells, as well as purified CD4+ (but not CD4-) lymphocytes stimulated with ovalbumin. MK886 blocked eosinophil recruitment induced by CD4+ cells. Conclusions: Eosinophil recruitment in EWI mice involves cytokine-producing, antigen-specific CD4+ cells, acting indirectly through leukotriene-producing resident cells. Finantial support: FAPESP, CNPq, FINEP, PAPES-FIOCRUZ, FIOCRUZ-FAPERJ.

05.025

MECHANISMS OF EOSINOPHILIA INDUCED BY EGG WHITE IMPLANT: AN ESSENTIAL ROLE FOR STRESS-INDUCED MEDIATORS. 1,4Cheraim, A.B., 1Neto, H.A.P., 2Silva, E.S., 2Araujo Pinto J.I., 3Cunha, F.Q., 1Elsas, M.I.G., 2Elsas, P.X., 1IFF/FIOCRUZ, 2Depto. Imunologia., UFRJ, 3Depto. Farmacologia., FMRP-USP, 4Depto. Patologia, UFF.

INTRODUCTION: Glucocorticoids enhance IL-5 responses in murine bone-marrow culture. Subcutaneous implant of heat coagulated egg white induces a local eosinophilia in mice. We analyzed the effects of EWI on bone marrow (BM) eosinopoiesis, and a role for glucocorticoid stress hormones. METHODS AND RESULTS: BM eosinophilia and BM responses to IL-5 were analized as parameters of BM eosinopoiesis in normal, implanted (EWI) and sham-implanted (SHAM) mice. Surgery itself induced BM eosinophilia relative to normal control mice, 24h and 15 d, but not at 30 d after surgery. At 15 and 30 days, but not at 24h, EWI induced significantly higher BM eosinophilia relative to SHAM controls. Similar effects were found for BM responses to IL-5. Pre-treatment with RU486 or metyrapone prevented the upregulation of BM eosinophilia in SHAM mice, 24 h after surgery. Tolerization to ovalbumin prevented the upregulation of BM eosinophilia in EWI mice, 15 days after implantation. These points to separate early (nonspecific) and late (specific) mechanisms modulating eosinopoiesis. Blockade of both early and late events by combining tolerance induction with glucocorticoid blockade by RU486 prevented the upregulation of BM eosinopoiesis in EWI mice. CONCLUSIONS: Antigen-specific and -nonspecific (stress) mechanisms interacts to upregulate BM eosinopoiesis in a model of cronic eosinophilia. FINANTIAL SUPPORT: PAPES-FIOCRUZ, FINEP, CAPES and PIBIC-CNPq

05.026

THE EFFECT OF ALLERGENIC SENSITIZATION ON THE MODULATION OF MURINE HEMOPOIESIS BY COX INHIBITORS. [1]Lintomen, L., [1]Gaspar Elsas, M.I.C., [2]Maximiano, E.S., [1]Paula Neto, H.A., [1]Garcia, K.M., [3]Vargaftig, B.B., [2]Xavier Elsas, P. [1]Depto. de Pediatria, IFF/FIOCRUZ, [2]Depto. de Imunologia, Inst. de Microbiologia/UFRJ, [3]Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur-INSERM U485, Paris, France

Indomethacin upregulates hemopoiesis and protects bone-marrow (BM) from radiation damage, while allergenic sensitization and challenge upregulate responses to hemopoietic cytokines in murine BM. We evaluated whether immunization affects BM responses to indomethacin. Semi-solid and liquid cultures were established from total BM cells of ovalbumin-sensitized, with or without intranasal challenge, or naive BALB/C mice. Semi-solid cultures were established with GM-CSF and liquid cultures with IL-5, alone or associated with indomethacin (10^{-7}-10^{-11} M) or aspirin (10^{-7}-10^{-8} M). Total myeloid colony numbers and numbers of eosinophil-peroxidase-positive cells were determined after a week. Indomethacin (10^{-7}-10^{-9} M) increased myeloid colony formation and eosinophil precursor responses to IL-5 in naive BALB/c mice. However, it had no effect on BM of ovalbumin-sensitized and challenged mice. Aspirin (10^{-7} M) had similar effects, equally abolished by sensitization. Indomethacin was effective on BM cells from sham-sensitized, ovalbumin-challenged, but not from sensitized, saline-challenged mice. The effect of indomethacin in naive BALB/c mice was abolished by transfer of immune plasma. This was not prevented by previous removal of antibody from immune plasma. Upregulation of hemopoiesis by indomethacin required adherent cells from naïve BM. The effect of indomethacin in naive BALB/c mice was also abolished by transfer of immune mononuclear cells.
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05.027

EFFECT OF ALLERGEN SENSITIZATION ON EOSINOPHIL PRECURSOR RESPONSES TO DRUGS AND CYTOKINES IN BONE-MARROW CULTURE. 1,2 Mendonça Sales, S.C.;2 Elsas, M.I.G.;1,2 Padua, M.;2Lintomen,L. 1,3Cheraim, A.B., 2Maximiano, E.S.,3 Elsas, P. 1Dep. de Patologia, UFF; 2Dep. de Pediatria, IFF, Fiocruz; 3Dep. de Imunologia, Inst. de Microbiologia, UFRJ.

Introduction: We previously described modulation of bone-marrow (BM) responses to eosinopoietic cytokines by allergen sensitization and challenge, as well by dexamethasone, prostaglandin E2, and indomethacin. Here we evaluated whether these factors interact in IL-5 stimulated BM cultures, and whether this affects responses to IL-13 and chemokines. Methods and Results: We evaluated the modulation of eosinophil precursor responses to IL-5 in liquid BM culture by different agents, comparing naïve and allergic BALB/c and C57BL/10 mice. C57BL/10 responded to allergen sensitization and challenge differently from BALB/c mice. Comparable responses to dexamethasone and prostaglandin E2 were observed in both strains. Indomethacin was effective in naïve but not immune BALB/c mice, and ineffective in C57BL/10 mice. Eotaxin showed opposite effects in naïve versus sensitized BALB/c, and had no effect in C57BL/10 mice. RANTES had an inhibitory effect on allergic C57BL/10, but no effect on BALB/c and naïve C57BL/10. MCP-5 had an inhibitory effect in C57BL/10, but no effect on BALB/c. MIP-1 either strain. IL-13 had stimulatory effects on naïve BALB/c, but not naïve C57BL/10, but presented inhibitory effects on sensitized mice of both strains.Conclusion: Bone-marrow responses to different modulatory agents depend on at least 3 factors: mouse strain, the immunological status of the host, and the agent itself. Financial support: CAPES, FINEP, PAPES/FIOCRUZ, CNPq.

05.028

BLOQUEIO FARMACOLÓGICO DA MIGRAÇÃO EOSINOFÍLICA INDUZIDA POR SOBRENADANTE DE CÉLULAS DA LÂMINA PRÓPRIA INTESTINAL DE RATOS IMUNIZADOS COM OVALBUMINA. Feitosa, R.F.G.*, Carubbi, E.K.**, Rocha, M.F.G.**, Lima, A.A.M*. *Depto. Fisiologia e Farmacologia/IBIMED/UPC-UFC e **Fac. Veterinária-UECE, Fortaleza, Ceará, Brasil.

Introdução: Nesse estudo investigou-se a participação de mediadores inflamatórios na migração de eosinófilos (ME) induzida por sobrenadante (SOB) de cultura de células da lâmina própria intestinal (CLPI) de ratos sensibilizados. Métodos: CLPI foram isoladas de ratos Wistar pré-sensibilizados com OVA (30μg/rato) + $Al(OH)_3$. O intestino delgado foi lavado e seccionado e as placas de Peyer descartadas. As CLPI foram isoladas por método enzimático e incubadas por 1 h com OVA, 10 μg/ml. A seguir, lavadas e incubadas por 2 h com Dulbecco. O SOB foi testado no modelo de migração celular. Ratos não sensibilizados foram pré-tratados com moduladores farmacológicos ou PBS, 30 min antes da injeção ip de SOB de CLPI. Os anticorpos (Ac) foram incubados com SOB 30 min antes da administração. Os animais foram sacrificados 4h pós-estímulo e o fluido peritoneal coletado para contagem total e diferencial celular. Valores expressos como média ± EPM. Resultados e Discussão: Dexametasona (0,5mg/kg; 0.429±0.07), quinacrina (20mg/kg; 0.395±0.05), indometacina (2mg/kg; 0.30±0.05), WEB2086 (20mg/kg; 0.213±0.01), ciproheptadina (2 mg/kg; 0.354±0.10) e metergoline (5mg/kg; 0.411±0.04), sc; loratadina (10mg/kg; 0.617±0.0.06), montelucaste sódico (10mg/kg; 0.374 ± 0.02), talidomida (90mg/kg; 0.64±0.03), vo; cetotifeno (10mg/kg; 0.375 ±0.06), ip; e Ac anti-TNFα (50μg/kg) inibiram ($p<0,05$) a ME induzida por SOB de CLPI, comparado ao PBS (1.062±0.15). Cromolina (20mg/kg), difenidramina (50mg/kg), pentoxifilina (90mg/kg), NDGA (50mg/kg), sc; meloxicam (10mg/kg; vo) e Ac anti-IL1β (100μg/kg), não inibiram a ME. Sugere-se o envolvimento de prostaglandinas, PAF, leucotrienos, histamina, 5-HT e TNFα na ME induzida pelo SOB da cultura de células totais da lâmina própria intestinal de ratos imunizados com OVA.

05.029

MODULAÇÃO FARMACOLÓGICA DA MIGRAÇÃO EOSINOFÍLICA INDUZIDA POR SOBRENADANTE DE MACRÓFAGOS DA LÂMINA PRÓPRIA INTESTINAL DE RATOS SENSIBILIZADOS COM OVALBUMINA. Feitosa, R.F.G.*, Carubbi, E.K.**, Rocha, M.F.G.**, Lima, A.A.M*. *Departamento de Fisiologia e Farmacologia/IBI-MED/UPC-UFC e **Faculdade de Veterinária-UECE, Fortaleza, Ceará, Brasil.

Introdução: O objetivo desse estudo foi investigar a participação de macrófagos (Mφs) intestinais, através do sobrenadante (SOB) dessas células isoladas da lâmina própria (CLPI) de ratos sensibilizados (RS), na migração eosinófílica (ME) em modelo de cavidade peritoneal. Métodos: CLPI foram isoladas de ratos Wistar pré-sensibilizados com ovalbumina (OVA; 30μg/rato) + $Al(OH)_3$. O intestino delgado foi lavado e seccionado e as placas de Peyer descartadas. As CLPI foram isoladas utilizando-se dispase (3mg/ml). A suspensão celular em meio Dulbecco foi distribuída em placas de 12 poços e incubada por 1,5 h. Os Mφs (10^6cél/ml) aderidos à placa foram lavados e estimulados por 1h com OVA, 10 μg/ml. A cultura de Mφs foi incubada por 30 min com bloqueadores farmacológicos na concentração de 10^{-5}M, antes da adição do estímulo. A seguir, os Mφs foram lavados e incubados por 2 h, sendo o SOB coletado para dosagens de IL-1β e TNFα por ELISA e migração celular. Ratos normais foram sacrificados 4h pós-estímulo e o fluido peritoneal coletado para contagem total e diferencial celular. Valores expressos como média (Eosinófilos x 10^6/ml) ± EPM. Resultados e Discussão: Dexametasona (0.43±0.11), indometacina (0.46±0.07), metisergida (0.31±0.02) e ciproheptadina (0.57±0.04), inibiram ($p<0,05$) a ME induzida pelo SOB de Mφs, comparados ao controle Dulbecco (1.33±0.14), enquanto cetotifeno não inibiu essa resposta. Nos resultados de ELISA, observou-se aumento de IL-1β e TNFα liberados por Mφs de RS, comparando-se aos ratos não sensibilizados e aumento de IL-1β liberada por Mφs em relação a liberada pelas CLPI. Esses dados sugerem que prostaglandinas, 5-HT, IL-1β e TNFα participam da migração eosinofílica induzida por SOB de Mφs da lâmina própria intestinal de ratos imunizados com OVA.

05.030

EFEITO DOS ANTAGONISTAS DOS RECEPTORES DE TAQUICININAS NO RECRUTAMENTO DE EOSINÓFILOS NA PLEURISIA ALÉRGICA. Ana Letícia Alessandri*, Maria Salete de A. Castro**, Danielle G. Souza*, Vanessa Pinho* e Mauro M. Teixeira* *Imunofarmacologia, Departamento de Bioquímica e Imunologia, **Farmacologia da Inflamação e Dor, Departamento de Farmacologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil

Introdução: As taquicininas são um grupo de neuropeptídeos que incluem a substância P, neurocinina A e neurocinina B. Neste estudo nós investigamos o papel dos antagonistas dos receptores de taquicininas e da depleção das terminações nervosas sensitivas sobre o recrutamento de eosinófilos em um modelo de imunização com OVA. Métodos: Camundongos balb/c, sensibilizados nos

dias 1 e 8, foram no dia 14 tratados de modo local ou sistêmico com os antagonistas de receptores de taquicininas e desafiados. Após 48h o recrutamento de eosinófilos foi avaliado. Para estudar o efeito da depleção das terminações nervosas sensitivas, os animais previamente sensibilizados, foram pré-tratados com capsaicina e desafiados no dia 20, sendo a contagem celular realizada 48h após o desafio. Resultados: O pré-tratamento local com o antagonista do receptor NK1 ou com capsaicina inibe o recrutamento de eosinófilos induzido por OVA. Os antagonistas dos receptores NK2 e NK3 não afetaram o recrutamento de modo significativo. Discussão: Estes resultados sugerem um envolvimento dos neuropeptídeos presentes nas terminações nervosas sensitivas no recrutamento de eosinófilos na pleurisia alérgica de camundongo, e que a ativação do receptor NK1 também é importante neste processo. Apoio: CNPq, FAPEMIG

05.031

EOTAXIN PRODUCTION BY PLATELET-ACTIVATING FACTOR IN AN ALLERGIC PLEURISY IN MICE. André Klein1, Vanessa Pinho2, Ana Letícia Alessandri2, André Talvani2, Takao Shimizu3, Satoshi Ishii3, Mauro Teixeira2. 1Division of Pharmacology, Federal University of Mato Grosso do Sul/CCBS,2Department of Biochemistry and Imunology, Federal University of Minas Gerais, ICB,3Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tokyo, Japan.

Several mediators including stem cell factor (SCF), leukotriene B4 (LTB4), platelet-activating factor (PAF) and eotaxin mediates the eosinophil recruitment in experimental allergic models. We have demonstrated that SCF appeared to drive the local production of LTB4 wich cooperatet with eotaxin to induce eosinophil recruitment. However, it is not clear the mediator(s) underlying local eotaxin production and release. Here we investigate the ability of PAF in eotaxin production in allergic pleurisy. Naive BALB/c mice were injected in the pleural cavity (i.pl.) with PAF (10-11 to 10-9 moles), or ovalbumin (OVA)-immunized mice were challenged i.pl. with OVA. PAF receptor antagonist UK 74,505 (1mg/Kg) or anti-eotaxin (100 μg) were injected 1 hour prior to OVA or PAF, and the eosinophil recruitment was assessed at 48 hours after the challenge. Eotaxin was measured 1,3,6 or 24 h after PAF injection. PAF induced eosinophil recruitment and eotaxin production, and this eosinophilia is abolished with anti-eotaxin. UK 74,505 inhibited the eosinophil recruitment and eotaxin production in OVA-injected mice. In conclusion, the production of PAF in allergic reactions could function to facilitate the recruitment and activation of eosinophils by eotaxin release. Financial support CAPES/PICDT, FAPEMIG, CNPq, PROPP/UFMS

05.032

ROLE OF CCL22 (MDC) FOR THE RECRUITMENT OF EOSINOPHILS DURING ALLERGIC PLEURISY IN MICE. Vanessa Pinho*, Sandra H. Oliveira** Danielle G. Souza*, Ana Letícia Alessandri* & Mauro M. Teixeira* *Imunofarmacologia, Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; **Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, São Paulo, Brazil

Introduction:Eosinophils are important inflammatory cells in allergic diseases. In this study we have investigated the role of CCL22 on the recruitment of eosinophils in vivo and in vitro. Material and Methods: CCL22 was injected intrapleurally into naïve mice and eosinophil recruitment evaluated at different times after injection. The role of endogenous CCL22 in the allergic pleurisy induced by Ova was investigated by using anti-CCL22. To determine the ability of CCL22 to induce chemotaxis of murine eosinophils, we used a classic two-chamber assay. Results: CCL22 induced a dose- and time-dependent recruitment of eosinophils that was dependent on the release of platelet-activating factor (PAF) and CCL11. In an allergic pleurisy model, anti-CCL22 polyclonal Ab had no significant effect on eosinophil recruitment, either when given before allergen challenge or during the sensitization phases with antigen. In vitro, eosinophils did not migrate towards CCL22 but CCL22 was able to induce eosinophil degranulation, as assessed by the release of EPO. Discussion: Although exogenously added CCL22 may induce eosinophil migration in vivo via release of PAF and eotaxin, endogenous production of CCL22 does not drive eosinophil migration during allergic inflammation. However, CCL22 may be an important activator of eosinophils once these cells have migrated into tissue. Supported by: CNPq, FAPESP

05.033

CHARACTERIZATION OF EOSINOPHIL LIPID BODY FORMATION DURING ALLERGIC INFLAMMATION. Vieira-de-Abreu A, Assis EF, Gomes GS, Castro-Faria-Neto HC and Bozza PT. Immunopharmacology Laboratory, DFF, FIOCRUZ, RJ, BR

Central to the pathogenesis of allergic diseases are both the recrultment and subsequent activation of specific leucocytes incluing eosinophils at sites of inflammation. Lipid bodies are inducible, specialized cytoplasmic domains for eicosanoid-forming enzyme localization, which may have roles in enhanced paracrine inflamatory mediator production during inflammatory conditions. Howerer, little is known about the origins, composition or functions of eosinophil lipid bodies in vivo. The present study investigated the mechanisms and function of allergen-induced lipid body formation in leukocytes that migrate to the inflammatory site in vivo. Antigenic challenge in actively sensitized mice significantly induced a time-dependent lipid body formation in eosinophils, wich occurs in parallel to the recruitment of eosinophils to the inflammatory site. Interestingly, lipid body formation was drastically inhibited by specific neutralizing Abs to eotaxin, RANTES and CCR3. Moreover, the administration of chemokines like RANTES and eotaxin induced significant eosinophil lipid body formation. By immunocytochemistry we detected the presence of 5-LO, and interestingly, two citokines RANTES and MIF within eosinophis lipid bodies formed in vivo after allergen challenge. These results demonstrate that chemochines can modulate the formation of lipid body in eosinophils in allergic inflammation. Moreover, our results suggest that lipid bodies may act as intracellular sites for the production of lipid mediators and cytokines during allergic inflammation. Support: FIRCA/NIH, CNPq and FAPERJ

05.034

"IN VITRO" VASCULAR REACTIVITY OF AORTA AND PULMONARY ARTERY FROM RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS. 1Cabanillas, JGQ; 1Teixeira, CE; 2Teixeira, SA; 1de Nucci, G; 1Antunes, E & 2Muscará, MN. Departments of Pharmacology. 1UFCM – UNICAMP, Campinas and 2ICB - USP, São Paulo, SP.

OBJECTIVES: Experimental allergic encephalomyelitis (EAE) is an accepted animal model to study multiple sclerosis and involves changes in nitric oxide (NO) production at the central nervous system level. In this study we investigated the "in vitro" vascular responses to noradrenaline (NA) and sodium nitroprusside (SNP) of aortae (RA) and pulmonary arteries (RPA) obtained from rats with EAE. METHODS: RA and RPA were removed from Lewis rats with EAE (stage 3), cut into rings, mounted in 10 mL organ baths and connected to isometric force transducers. RESULTS: Concentration-response (CR) curves for NA (10 nM-100 uM) showed that both RA and RPA were contracted with pEC50 values of 6.82 + 0.05 and 6.82 + 0.13, respectively. CR curves of both RA and RPA rings obtained from the EAE animals were rightward shifted in relation to control tissues (pEC50 6.21 + 0.08 and 5.91 + 0.20, respectively). SNP (10 nM-100 uM) relaxed both vessel types in a concentration-dependent manner with pEC50 values of 6.76 + 0.02 and 7.19 + 0.06 for control RA and RPA, and 6.49 + 0.03 and 6.78 + 0.05 for RA and RPA from the EAE animals, respectively (mean rightward shift of 1.9 and 2.6-fold in relation to the control CR curves). CONCLUSION: These preliminary results show that vascular hyporreactivity occurs in rats with severe EAE. Whether these observations are due to NO overproduction, still remains to be established. Financial Support: FAPESP.

05.035

DENDRITIC CELLS RECRUITED TO THE LUNGS SHORTLY AFTER INTRANASAL DELIVERY OF *Mycobacterium bovis* BCG DRIVE THE PRIMARY IMMUNE RESPONSE TOWARDS A TH1-TYPE CYTOKINE PRODUCTION. Micheline Lagranderie[1], Marie-Anne Nahori[1], Anne-Marie Balazuc[1], Hélène Kiefer-Biasizzo[1], Jose-Roberto Lapa e Silva[2], Geneviève Milon[1], Gilles Marchal[1] and Boris B. Vargaftig[1] - Institut Pasteur, Paris, France, (2) Federal University of Rio de Janeiro, Brazil

We showed in a previous study that the intranasal (i.n.) delivery to BP2 mice (I-A^q)inhibits ovalbumin-induced eosinophilia and bronchial hyperreactivity. The present work has been performed to identify and characterize the leukocyte lineages involved in the polarization of the responses in the lungs of mice after i.n. delivery of BCG. The different APCs recruited into the lungs of mice shortly after i.n. delivery of BCG were analysed. Their capacity to drive the immune response towards a Th1-type cytokine production has been checked. In the bronchoalveolar lavage (BAL), the alveolar macrophages (AMs) were recruited 6h after BCG delivery, they were CD11c+, CD11b- and F4/80+. The number of cells isolated from the lung tissue increased later 48 to 96h after BCG delivery. The flow cytometric analysis indicated two major populations of potential APCs present into the lung tissues: one population CD11c-, CD11b+, F4/80+ and I-A^q- was considered to be interstitial macrophages (IMs) and a second population expressing CD11c+ and I-A^q+ antigens, negative for CD11b and F4/80 markers was considered to be leukocyte dendritic cells (DCs). To characterize more precisely lung DCs, we further examined the expression of MHC class II I-A^q and accessory molecules on these cells before and after overnight culture. Freshly isolated lung DCs (expressing CD11c) were found to up-regulate CD11b and CD40 antigens after overnight culture but remained negative for CD8α antigen suggesting that they were myeloid-derived. We also found that after i.n. delivery of BCG to mice, only AMs and DCs were loaded with BCG. The population of lung DCs collected shortly after i.n. delivery of BCG produced *ex vivo* higher amount of IL-12 than AMs. The lung DCs were potent inducers of naive CD4+ T lymphocyte priming, as assessed by IFN-γ production by these naive CD4+ T cells. Lung explants recovered from 4 to 12 weeks after BCG delivery and stimulated with anti-CD3 mAb produced sustained levels of IFN-γ. Our results suggest that AMs and particularly DCs by secreting IL-12 short-term after BCG delivery induced long-term Th1 immune response.

05.036

MODULAÇÃO FARMACOLÓGICA DA MIGRAÇÃO EOSINOFÍLICA INDUZIDA POR SOBRENADANTE DE MACRÓFAGOS DA LÂMINA PRÓPRIA INTESTINAL DE RATOS SENSIBILIZADOS COM OVALBUMINA. Feitosa, R.F.G.*, Carubbi, E.K.**, Rocha, M.F.G.**, Lima, A.A.M*. *Departamento de Fisiologia e Farmacologia/IBIMED/UPC-UFC e **Faculdade de Veterinária-UECE, Fortaleza, Ceará, Brasil.

Introdução: Macrófagos têm se destacado por sua capacidade de sintetizar e liberar IL-1 e TNFα as quais participam da imunofisiopatologia intestinal. O objetivo desse estudo foi investigar a participação de macrófagos (Mφs), através do sobrenadante (SOB) dessas células isoladas da lâmina própria intestinal (CLPI) de ratos sensibilizados (RS), na migração eosinófílica (ME) em modelo de cavidade peritoneal. Métodos: CLPI foram isoladas de ratos Wistar pré-sensibilizados com ovalbumina (OVA; 30μg/rato) + $Al(OH)_3$. O intestino delgado foi lavado e seccionado e as placas de Peyer descartadas. As CLPI foram isoladas utilizando-se dispase (3mg/ml). A suspensão celular em meio Dulbecco foi distribuída em placas de 12 poços e incubada por 1,5 h. Os Mφs (10^6cél/ml) aderidos à placa foram lavados e estimulados por 1h com OVA, 10 μg/ml. A cultura de Mφs foi incubada por 30 min com bloqueadores farmacológicos na concentração de 10^{-5}M, antes da adição do estímulo, com exceção de montelucaste, anti-leucotieno, que foi usado p.o. na dose de 10mg/kg, 30 min antes do SOB i.p. A seguir, os Mφs foram lavados e incubados por 2 h, sendo o SOB coletado para migração celular e dosagens de IL-1β e TNFα por ELISA. Ratos normais foram sacrificados 4h pós-estímulo e o fluido peritoneal coletado para contagem total e diferencial celular. Valores expressos como média (Eosinófilos x 10^6/ml) ± EPM. Resultados e Discussão: Dexametasona (0,43±0,11), indometacina (0,46±0,07), montelucaste (0,57±0,01), metisergida (0,31±0,02) e ciproheptadina (0,57±0,04), inibiram ($p<0,05$) a ME induzida pelo SOB de Mφs, comparados ao controle Dulbecco (1,45±0,14), enquanto cetotifeno não inibiu essa resposta. Nos resultados de ELISA, observou-se aumento de IL-1β e TNFα liberados por Mφs de RS, comparando-se aos ratos não sensibilizados e aumento de IL-1β liberada por Mφs em relação a liberada pelo *pool* de CLPI. Esses dados sugerem que TNFα, IL-1β, leucotrienos, prostaglandinas e 5-HT participam da migração eosinofílica induzida por SOB de Mφs da lâmina própria intestinal de RS com OVA.

05.037

ELECTRICAL AND MECHANICAL RESPONSES OF CORPUS CAVERNOSUM (CC) AND ANO-COCCYGEUS MUSCLE (AcM) FROM RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). 1Teixeira, CE; 1Cabanillas, JGQ; 2Teixeira, SA; 1de Nucci, G; 1Antunes, E & 2Muscará, MN. Departments of Pharmacology 1FCM - UNICAMP, Campinas and 2ICB - USP, São Paulo, SP.

OBJECTIVES: EAE, an accepted animal model of multiple sclerosis, is an autoimmune demyelinating disease involving nitric oxide (NO) overproduction. In this study we investigated the responses to both electrical field stimulation (EFS) and sodium nitroprusside (SNP) of CC and AcM from rats with EAE (stage 3). METHODS: EAE was induced in Lewis rats by a single s.c. injection of an emulsion containing guinea-pig myelin basic protein into each hind paw. CC and AcM strips were mounted in 10 mL organ baths and connected to isometric transducers. RESULTS: In tissues from control animals, EFS caused frequency-dependent contractions of both CC (2-32 Hz) and AcM (2-20 Hz), which were potentiated by 61 + 15% and 51 + 17% in the respective EAE tissues. In pre-contracted control tissues (10 μM noradrenaline for CC and 30 μM carbachol for AcM), EFS caused frequency-dependent relaxations which were reduced by 32 + 2% (CC) and 62 + 4% (AcM) in the EAE tissues. SNP relaxed control CC and AcM in a concentration-dependent manner (pEC50 values: 4.42 + 0.16 and 6.30 + 0.06, respectively). These responses were attenuated in EAE tissues and a 2.9-fold rightward shift of the AcM curves was observed. CONCLUSION: During late stages of EAE in rats, dessensitization of the tissues in response to SNP and enhancement of EFS-induced contractions occur. Whether endogenous NO production is involved in these responses, still remains to be elucidated. Financial Support: FAPESP.

05.038

AÇÃO DA CICLOHEXAMIDA E QUINACRINA NO EFEITO RENAL INDUZIDO PELO SOBRENADANTE DE MACRÓFAGOS ESTIMULADOS PELO VENENO DE *Crotalus durissus cascavella*. Martins, A. M. C., Almeida, A. C. P., Havt, A., Nobre, A. C. L., Bezerra, G. P., Bezerra, I. S. A. M., Fonteles, M. C., Lima, A. A. M., Monteiro, H. S. A. Depto Fisiologia e Farmacologia, UFC.

Objetivo: Estudar o papel do inibidor de síntese protéica; ciclohexamida (10^{-5}M), e de PLA_2; quinacrina (10^{-5}M), sobre o efeito tóxico renal induzido pelo sobrenadante de macrófagos (SOB.MφS) ativados pelo veneno de *C.d.cascavella*.
Métodos e resultados: Os macrófagos foram isolados e estimulados com o veneno de *C.d.cascavella*, por 1h. A seguir foram lavados e mantidos em cultura por mais 2 horas. O sobrenadante (1ml) foi testado em sistema de perfusão de rim isolado de rato, com solução de Krebs-Henseleit, contendo 6g% de albumina bovina à 38°, durante 120 min. Os 30 min iniciais do experimento foram considerados como controle interno e após esse período o SOB.MφS ativado pelo veneno de *C.d.cascavella* (10μg/ml) foi adicionado. Avaliamos os seguintes parâmetros: pressão de perfusão (PP), fluxo urinário (FU), ritmo de filtração glomerular (RFG) e percentual de transporte tubular de sódio (%TNa^+). A análise estatística foi feita pelo ANOVA (*$p<0,005$).
Resultados: No período de avaliação (90 min), observamos os seguintes dados:

	Controle	Veneno	Ciclohexa	Quina
PP (mmHg)	117,6 ± 1,4	137,0 ± 1,3*	114,1 ± 4,9	118,3 ± 1,5
FU ($ml.g^{-1}.min^{-1}$)	0,22 ± 0,22	0,38 ± 0,02*	0,18 ± 0,01	0,17 ± 0,07
RFG ($ml.g^{-1}.min^{-1}$)	0,70 ± 0,04	1,41 ± 0,17*	0,69 ± 0,05	0,71 ± 0,05
% TNa^+	77,0 ± 1,0	73,6 ± 1,0*	75,0 ± 0,52	76,2 ± 1,57

Conclusões: Os resultados sugerem a participação de PLA_2 nos efeitos tóxicos renais induzidos pelo SOB.MφS ativados pelo veneno de *C.d.cascavella*.
Apoio financeiro: CNPq/CAPES

05.039

THE EFFECT OF ALLERGENIC SENSITIZATION ON THE MODULATION OF MURINE HEMOPOIESIS BY COX INHIBITORS. 1Neto, H.A.P., 1Lintomen, L.,1Elsas, M.I.G., 2Maximiano, E.S., 1Garcia, K.M., 3Vargaftig, B.B., 2Elsas, P.X 1IFF/FIOCRUZ, 2Depto. de Imunologia,UFRJ, 3Unité de Pharmacologie Cellulaire, Institut Pasteur

Introduction:Indomethacin upregulates hemopoiesis and protects BM (BM) from radiation damage, while allergenic sensitization and challenge upregulate responses to hemopoietic cytokines in murine BM. We evaluated whether immunization affects BM responses to indomethacin. Methods and Results: Cultures were established from BM cells of: a) naive; b) ovalbumin-sensitized, saline-challenged; or c) ovalbumin-sensitized and –challenged BALB/C mice. Semi-solid cultures were established with GM-CSF and liquid cultures with IL-5, alone or associated with indomethacin (10-7-10-11M) or aspirin (10-7-10-8M).Total myeloid colony numbers and numbers of EPO+ cells were determined after a week. Indomethacin (10-7-10-9M) increased GM-CSF-stimulated myeloid colony formation and eosinophil precursor responses to IL-5 in naive mice. However, it had no effect on BM of ovalbumin-sensitized and challenged mice. Aspirin (10-7M) had similar effects, equally abolished by sensitization.Indomethacin was effective on BM cells from sham-sensitized, ovalbumin-challenged, but not from sensitized, saline-challenged mice. The effect of indomethacin in naive mice was abolished by transfer of plasma and cells from immune donors.This was not prevented by previous removal of antibody from immune plasma. Upregulation of hemopoiesis by indomethacin required adherent cells from naïve BM.
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05.040

EFFECTS OF CORTICOSTERONE ON THE PINEAL PRODUCTION OF INDOLEAMINES. Fernandes, P.A.C.M; Ferreira, Z.S. & Markus, R.P. Dep. Fisiologia, Instituto de Biociências, Universidade de São Paulo, Brasil.

Introduction: Diurnal variation of chronic inflammatory lesion induced by BCG inoculation is lost after pinealectomy, superior cervical ganglionectomy or adrenalectomy (ADx). Rhythmic production of the metabolite 6-sulphatoxymelatonin is not observed after adrenalectomy, and nocturnal administration of melatonin (MEL) recover diurnal variation. (Lopes et al., Inflamm. Res 50: 6, 2001). Aim: Investigate the influence of glucocorticoids on extraneuronal catecholamine uptake (ECU) and MEL synthetic pathway. Methods: Rats cultured pineals were exposed to 1 or 100μM corticosterone (CORT) for 1 h with MAO and COMT inhibitors iproniazid and tolcapone (ECU) or 48 h (MEL synthesis). The content of adrenaline (10μM, 30s) taken up by the gland, or MEL indoleamines precursors produced by noradrenaline (10nM, 5h) stimulation were measured by hplc. Values are expressed as ng/pineal and ng/well. Results: Adrenaline uptake (1.4±0.24, n=3) was not blocked by 1μM (1.73±0.65, n=3) or 100μM (1.69±0.34, n=3) of CORT. Noradrenaline (10nM)-induced N-acetylserotonin (NAS) production in glands (3.9 ± 0.8, n=8) and in the medium (13.99±2.93, n=8) was increased 2.5 fold by CORT (1μM or 10μM; 48h). A higher dose of corticosterone (100μM) had no effect. The production of 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid were not modified by any CORT dose. Conclusion: Our data indicate that ECU was not affected by 1h incubation of CORT, while a longer incubation of lower doses of CORT is able to increase the activity of arylalkylamine N-acetyltransferase, which converts serotonin to NAS, the immediate precursor of melatonin. Support: FAPESP, CNPq.