

EXPERIMENTAL INFECTIONS OF THE PRIMATE *CEBUS APELLA* (PRIMATES: CEBIDAE) WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS* USING DIFFERENT CONCENTRATIONS OF PROMASTIGOTES: EVALUATION OF THE HUMORAL AND CELLULAR IMMUNE RESPONSE

L. M. GARCEZ¹*, J. J. SHAW^{1,2}, F. T. SILVEIRA¹, P. M. DE LUCA³, S. C. F. MENDONÇA³

¹Leishmaniasis Program, Evandro Chagas Institute, Caixa Postal 3, 66017-970, Belém, Pará Brazil; ²Parasitology Department, Institute of Biomedical Sciences, São Paulo University, São Paulo. ³Dept of Protozoology, Oswaldo Cruz Institute, Rio de Janeiro.

These studies are part of a project to evaluate the immunogenicity and effectiveness of Leishvacin® in the experimental *Cebus apella* model. The principal object of the present experiment was to determine the minimum infective dose of *Leishmania (Leishmania) amazonensis* that would be suitable to challenge experimentally immunized animals. Four young laboratory bred monkeys were inoculated with 12 day old stationary phase cultures of *L. (L.) amazonensis* (IFLA/BR/67/PH8). The promastigotes were suspended in a glucose/saline solution and given as a single dose (I- $0,5 \times 10^6$, II- 10^6 , III- $1,6 \times 10^6$ e IV- $4,8 \times 10^6$) intradermally on the dorsal surface of the tail. A blood sample was collected from each animal just before it was inoculated and at 30 and 60 days after the infective inoculation. Plasma from each sample was stored for serological studies, the white cells were separated and cultured *in vitro* to evaluate cell mediated responses. Tissues samples were collected from the inoculation site 45 days post inoculation (p.i.) for direct parasitological and histopathological examinations and clinical examinations were performed at 15 day intervals. After 1 month monkeys II, III and IV each developed an erythematous papule (12-16mm diameter) at the inoculation site which increased in size, measuring 13-20mm in diameter by 2 months p.i. Animal number I only developed a small nodule 1.5 months p.i., (3mm diameter) that disappeared 2 months p.i. Amastigotes were only detected in the direct smears of animals III and IV. Specific IgG antibodies were measured using an ELISA test and all animals had positive titres at two months. (I: 40, II: 160, III: 1280 e IV: 1280). Titres were higher in the animals that received the larger inoculum. The lymphocyte proliferation tests showed that there was a cellular response one month after the inoculation and that it was greater with the homologous crude antigen. Although monkey IV showed a definite humoral response there were no significant *in vitro* cell mediated responses. The preliminary results show that a single inoculum of $1,6 \times 10^6$ *L. (L.) amazonensis* promastigotes is infective to *C. apella* and would seem to be best one to use as a challenge after vaccination with Leishvacin®. The immune response of monkeys is compatible to that observed in natural infections of *L. (L.) amazonensis* in man.

This investigation was supported by Evandro Chagas Institute, Primates Nacional Center and PCDEN Program.

CANINE VISCERAL LEISHMANIASIS: IMMUNOCYTOCHEMICAL STUDY OF THE MHC CLASS II ANTIGENS IN LIVER AND LYMPHOID ORGANS

TAFURI, W. G. L.¹, TAFURI, W. L.², BARBOSA, A. J. A.³, MICHALICK, M. S. M.¹, GENARO, O.¹, SILVA, J. C. F.¹, MAYRINK, W.¹ & NASCIMENTO¹, E.

1. Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais,

Av. Antonio Carlos 6627 Belo Horizonte

2. Departamento de Ciências Biológicas da Universidade Federal de Ouro Preto

3. Faculdade de Medicina da Universidade Federal de Minas Gerais

The MHC antigens are involved in cell adhesion in different phases of the immune cellular response. The aim of this work was to study the immunocytochemical expression of MHC class II antigens in the liver and lymphoid organs (spleen, lymph nodes and Peyer's patches) of thirteen 30 month old mongrel dogs experimentally infected and five naturally infected with *Leishmania chagasi*. Cryostat sections of liver, spleen, lymph nodes and Peyer's patches were stained by the peroxidase anti-peroxidase complex technique (PAP) The immunocytochemical labelling for MHC class II antigens showed the same topography in all organs examined in all animals of the same group or of different groups. However, the MHC class II expression appeared less intense in cervical and abdominal lymph nodes of naturally infected dogs. In leishmaniasis the cellular immune response is known to have remarkable importance, and macrophages presenting MHC II should play a central role in cellular response. In this work we have not observed significant differences in MHC II antigen expression in infected dogs. However, the lower expression in lymph nodes of naturally infected dogs could be indicate that *Leishmania chagasi* is able to downregulate the MHC II antigen expression.