



CYTED-RIMLEV

**WORKSHOP ON
CANINE VISCERAL
LEISHMANIASIS**

CPqGM-FIOCRUZ (SALVADOR, BA, BRAZIL)

MARCH, 28-30, 2012

Identification of novel *L. infantum* recombinant antigens candidates for canine visceral vaccines.

Cristiane Garboggini Melo de Pinheiro¹; Naiara Carvalho Teixeira¹; Laiz de Souza Paixão¹; Diane Ellen Cirino Machado Serra¹; Rafael Dhália²; Fabíola Barbieri Holetz²; Osvaldo Pompílio de Melo Neto²; Ernesto Torres de Azevedo Marques Júnior²; Lain Carlos Pontes-de-Carvalho¹; Geraldo Gileno de Sá Oliveira¹.

¹Fundação Oswaldo Cruz, Centro de Pesquisas Gonçalo Moniz, Laboratório de Patologia e Biointervenção, Salvador, Bahia, Brazil; ²Fundação Oswaldo Cruz, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil.

Zoonotic visceral leishmaniasis (ZVL) is a parasitic disease caused by *Leishmania infantum* (syn. *L. chagasi*). Domestic dogs are considered the main reservoirs of the parasite. An effective vaccine for the dog may contribute to control of the ZVL in man such as dogs. In a previous study, among other antigens, a fragment of kinesin (Lci2b) was selected from a cDNA library amastigote form of the *L. infantum*, using canine antibodies that showed specific cellular immune response. To evaluate importance of the repetitive domains of the kinesin in preventing the development of specific Th1 immune response two chimeric constructs were designed (Lci2-NT-CT-NH6 and Lci2-NT-5R-CT-NH6). Lci2-NT-CT-NH6 was elaborated with the non-repetitive N-terminus and C-terminus and Lci2-NT-5R-CT-NH6 was designed with an additional segment encoding five repetitive motives of the gene. In one experiment, BALB/c mice were immunized with either rLci2-NT-CT-NH6/saponin or rLci2-NT-5R-CT-NH6/saponin. In another experiment, BALB/c mice were immunized with two doses of a plasmid encoding the chimera Lci2-NT-CT-NH6 or Lci2-NT-5R-CT-NH6 and then with a dose of the corresponding each chimeric protein associated with saponin. Immunizations were performed at 21 days intervals between consecutive doses. The plasmid used (p43.2-LAMP) contained the ORF of a gene that encodes a transmembrane protein associated with lysosomes, known lamp, to promote antigen presentation via class II MHC. The humoral immune response was assessed in sera obtained ten days after the third immunization dose, through the detection of specific antibodies by ELISA. The cellular immune response was assessed four weeks after immunization by performing splenocytes proliferation assay and determination of cytokine production (IFN- γ and IL-5) by splenocytes, after stimulation *in vitro* with the recombinant proteins. Animals immunized with rLci2-NT-CT-NH6/saponin or rLci2-NT-5R-CT-NH6/saponin produced antigen specific antibodies of subclasses IgG1 and IgG2a. Splenocytes of such animals showed proliferation after stimulation *in vitro*, but failed to produce IFN- γ . Further, splenocytes from mice immunized with rLci2-NT-CT-5R synthesized IL-5. Mice immunized with p43.2-LAMP-Lci2-NT-CT (priming)-rLci2-NT-CT-NH6/saponin (boosting) ou p43.2-LAMP/Lci2-NT-

5R-CT (priming)- rLci2-NT-5R-CT-NH6/saponin (boosting) exhibited low specific humoral immune response, with IgG1 and IgG2a production. In addition, the former showed lymphoproliferative response and a tendency to produce IFN- γ and without producing IL-5. In conclusion, immunization with either rLci2-NT-CT-NH6/saponin or p43.2-LAMP/Lci2-NT-CT/rLci2-NT-CT-NH6/saponin, quimeric proteins lacking repetitive domains, have not induced specific Th1 immune response.

Financial support: CNPq, RENORBIO-Ministry of Science and Technology, INOVABIO-Ministry of Health, Programa Instituto do Milênio, INCT-DT, PAPES-FIOCRUZ.