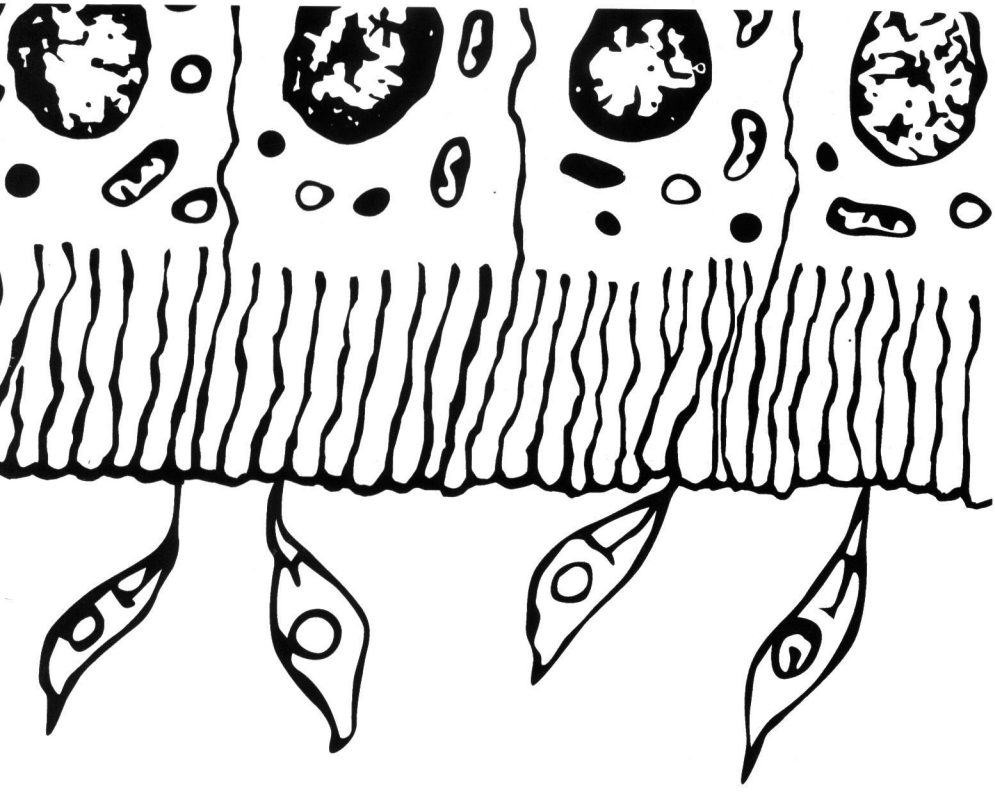

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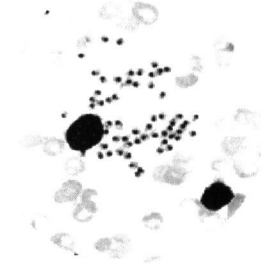

WORKSHOP ON CANINE VISCERAL LEISHMANIASIS

CPqGM-FIOCRUZ (SALVADOR, BA, BRAZIL)

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Immunity against *L. infantum* infection and the pathology of visceral leishmaniasis: key questions for research in this field.

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Visceral leishmaniasis (VL) is endemic in many parts of the world, including Brazil. In South America, most cases are caused by *L. chagasi/ infantum* (Lc). Most patients in Brazil are young children under the age of ten. A study conducted in an endemic area in Northeastern Brazil found that 7.5% of children under 15 had been infected by *L. chagasi*, yet the disease to infection ratio detected was only 1:6.5. Infected individuals may progress to a subclinical form of VL, or directly to an overtly classical form of the disease. Classical VL development is related to a host's inability to mount an effective Th1-type cellular immune response against Leishmania, reflected by the failure of immune cells to proliferate and produce INF-γ in response to specific Lsh antigens. However, cured individuals develop a resistance to reinfection, as well as DTH to *Leishmania,* and produce INF-γ upon the stimulation of their lymphocytes with parasite antigens. Patients with VL have been shown to produce Th1- and Th2-type cytokines. More recently, the regulatory cytokine IL-10 has emerged as the most promissory candidate acting as a suppressive factor related to the pathogenesis of human VL. Patients with severe VL have elevated serum levels of IL-10, a cytokine that is known to render macrophages unresponsive to activation signals, as well as to inhibit other T- cell functions. Plasma IL-10 from patients with VL has been shown to promote the intracellular growth of *Leishmania* in human macrophages. On the other hand, the neutralization of IL-10 resulted in the clearance of parasites from the splenic aspirate of patients with VL. Asymptomatic patients are identified by a positive DTH in cases of individuals with no signs of the disease. Dogs are known to be the main reservoir of Lc. As in cases of human infection, dogs may also present the full spectrum of infection manifestation. Recovery seems to be related to the ability to mount an effective Th1-type immune response. Most descriptions of the pathological aspects of VL come from autopsies of patients with severe forms of VL. The most remarkable lesions are found in hematopoietic organs and in the liver. These are characterized by a marked infiltration of heavily parasitized macrophages mixed with lymphocytes and plasma cells. Lymphoid organs, such as the spleen and lymph nodes, exhibit atrophy of the lymphoid follicles, plasmacytosis and macrophage infiltration. Occasionally, epithelioid granulomas can be seen. Hepatomegaly is a common finding. Liver histology reveals hyperplasia of Kupffer cells which may be heavily parasitized. Mononuclear cell infiltrate is present in the portal tracts, and small aggregates of inflammatory cells are seen within the liver parenchyma. Mortality is often due to bacterial infection. Disruption of the spleen architecture is a common finding in autopsies, and may result in a patient's inability to control bacterial infection. Changes in both the immune response and the

pathological aspects of VL are similar in human and canine forms of the disease. Understanding the relationship between immune response and patterns of tissue response may be important to assess the final outcome in VL. Studies employing murine models, and a few involving humans, suggest that the patterns of tissue response are correlated with different types of immune response. Thus, granulomas in mouse livers seem to be correlated with infection control. Well- formed liver granulomas, with few or no parasites, were the most notable finding in asymptomatic humans. Moreover, monomorphic macrophagic inflammatory infiltrate has been associated with susceptibility. However, even mice considered to be susceptible to Lc are able to control the infection. Thus, the murine model appears to reproduce the asymptomatic form of VL in humans and dogs, yet it limits the understanding of the entire spectrum of this disease in these species. With respect to humans, there are obvious ethical reasons to limit the study of the kinetics of VL. However, understanding the dynamics of the infection and its significance, in strict correlation with host immune response, is crucial to the understanding of VL pathogenesis and the development of immunization strategies and treatments. Therefore, studies using naturally infected dogs, compared with studies of experimentally infected ones, will be essential to further our understanding. Recent advances in the field of pathology including the incorporation of immunohistochemistry and molecular biology, will aid in the understanding of many aspects of this disease related to susceptibility or resistance. For example, nowadays it is possible to simultaneously determine the phenotypic profiles of immune cells and the cytokines they produce in an area of persisting parasites to compare these same parameters in other areas where parasites have been cleared. In addition to morphological analysis, the use of laser microdissection will provide tissue samples to evaluate gene expression and/or conduct proteomic analysis on serial sections. This data, integrated with clinical findings and follow-up, will be crucial to the development and evaluation of new vaccine candidates and treatments. Recently, we decided to perform a preliminary assessment of liver histological alterations in dogs naturally infected with Lc. We examined 39 animals from an endemic area in the state of Bahia, divided into the following four groups: a) 9 infected-symptomatic dogs; b) 10 infected-asymptomatic dogs; c) 10 non-infected- symptomatic dogs, and d) 10 normal dogs. Histological evaluation was performed in a blinded manner. The results showed that infected-symptomatic dogs differed significantly from the others with respect to the frequency of portal inflammation ($p < 0.003$), granulomas in portal tracts ($p < 0.04$) and parasitism (< 0.01). Only 1 out of 10 dogs in the infected-asymptomatic group had parasites in the liver, while 8 out of 9 infected-symptomatic animals had parasites. It is interesting to note that granulomas in infected-symptomatic animals were found to be permissive to parasites, while in the infected-asymptomatic group, only in one dog had parasites. These findings suggest that the evaluation of liver biopsies may provide important information on the evolution of VL and indicate that the functional analysis of granulomas is required to provide relevant information regarding the factors related to the mechanisms involved in parasite survival.