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## IM49 - ANALYSIS OF THE HEMOLYTIC EFFECT AND IN VIVO TOXICITY OF *QUILLAJA BRASILIENSIS* SAPONINS IN THE FORMULATION OF FML-VACCINE AGAINST VISCERAL LEISHMANIASIS IN MICE.

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FML+ Quil-A saponin led to 92%, ($p<0.005$) protection against visceral leishmaniasis in outbred mice. Saponins of *Quillaja saponaria* (Molina) (QuilA and QS-21) are used as potent adjuvants in commercial veterinary vaccines and experimental human vaccines. However, several undesirable effects were attributed to saponins. Aiming to improve the formulation with FML/ saponin, we comparatively analyzed the hemolytic potential and the *in vivo* toxicity of saponin containing fractions obtained from the bark (QBK), leaves (QBL) and branches (QBB) of the Brazilian plant: *Quillaja brasiliensis*. Balb/c mice were s.c. treated with 3 weekly doses of 200ug of each saponin fraction (QBK, QBL, QBB). Possible lethality and toxicity (pain, agitation, apathy) were monitored 0, 24, 48 and 72h after each injection. After the first injection (0h), pain reaction was observed in 2/3 QBK treated animals and in 3/3 of the QBL and QBB treated individuals. Scratching was evident in QBK animals and agitation in all individuals. All the effects disappear 24h after injection. Scratching was observed only in QBK treated animals during the first hours after the second saponin injection, while no undesirable effect was seen after the third injection. In contrast to what was described for saponins of *Quillaja saponaria*, the *Quillaja brasiliensis* saponins showed less toxicity and no lethality even using an enhanced dosage. Usually the dosage included in vaccine formulation is 100ug. The hemolytic activity of the *Quillaja brasiliensis* fractions was determined as the $HD_{50}$ (the concentration resulting in 50% haemolysis). THE $HD_{50}$ was 13,36 ug/ml for QBK, and > 500ug/ml for QBL and QBB. Our preliminary results indicated that in agreement with data obtained for *Q. saponaria* fractions, saponins contained in the bark fraction (QBK) while showing higher toxicity show also the higher immunogenic potential on the FML antigen of *L. (L.) donovani*. In vaccinated Balb/c mice, the highest IgG response was obtained in the FML+QBK treated group (Abs 492nm= 0.337 and 0.808, before and after infection, respectively). Also, significance in DTH response to f/t promastigote lysate was only found in FML+QBK vaccinated animals, 24h and 48h after antigen injection. Skin reaction in vaccinees was different from saline control ($p<0.01$) while no differences were found between adjuvant and saline control ($p>0.05$). As described for *Quillaja saponaria* (Molina), the bark fraction of *Quillaja brasiliensis* contains saponins with specific adjuvant potential on the FML antigen.

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## IM50 - PHENOTYPIC PROFILE OF PERIPHERAL BLOOD LEUKOCYTES AND SPLENOCYTES DURING CHRONIC *TRYPANOSOMA CRUZI* INFECTION IN DOGS

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Previous studies in our laboratory have demonstrated that two *T. cruzi* strains (Berenice-62 and Berenice-78) have distinct behavior in experimentally infected dogs. Be-78 infection is followed by a longer patent-parasitaemia, inducing myocarditis of variable degrees in contrast with Be-62 which elicits a short-parasitaemia and moderate heart-infiltrate. Despite these parasitological and histopathological findings, little is known about the immune response triggered by these two strains during the chronic infection in dogs. In this study peripheral blood (PB) leukocytes as well as splenocytes from dogs infected with Be-62 or Be-78 were analyzed using single color indirect immunofluorescence staining and flow cytometry. The data obtained from the *ex vivo* analysis demonstrated that in both compartments, PB and spleen, experimental Chagas' disease in dogs induce a decrease in the percentage of T cells (CD5+), with differential sub population profile depending on the *T cruzi* strain. In this context, lower levels of CD4+ cells and higher levels of CD8+ cells are observed in Be-78-infected dogs in comparison to Be-62. Despite the lower levels of CD21+ splenocytes detected in all infected dogs, a more drastic reduction of these cells was observed in the spleen of dogs infected with the Be-62 strain. However, in the PB compartment, lower levels CD21+ B cells were observed only in the Be-78 group. These changes on the potential for antigen presentation by B cells in different compartments may be important for the distinct profile of these strain-related disease outcome. Interestingly, the phenotypic analysis after antigen-specific stimulation *in vitro* demonstrated that the CD4/CD8 profile that was characteristic for the Be-78 strain was no longer observed. These results re-emphasize the data from other systems using intracellular pathogens, where that predominance of CD8+ cells is restricted to the *in vivo* context and can not be reproduced *in vitro* by using exogenous antigen sources. It is important to notice that although, the *in vitro* studies