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The FML-vaccine (Leishmune®) against canine visceral leishmaniasis: A transmission blocking vaccine

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Abstract

Transmission blocking vaccines are one of the control strategies for vector-transmitted protozoan diseases. Antibodies raised in the vaccinated host prevent the development of the parasite in the insect vector, interrupting the epidemiological cycle. The FML antigen of *Leishmania donovani* in combination with saponin (FML-vaccine and Leishmune®) induced 92–97% of protections against zoonotic visceral leishmaniasis. We assayed the ability of FML to inhibit *Leishmania donovani* and *Leishmania chagasi* procyclic promastigote-binding to dissected *Lutzomyia longipalpis* midguts. We found a dose-dependent inhibition, more pronounced on *L. donovani* (80%) than on *L. chagasi* promastigotes ($p < 0.001$). On the other hand, the Fab-IgG serum fraction of Leishmune® vaccinated dogs (IgG2 predominant), also inhibited parasite binding in a dose-response ($p < 0.0001$) with an equally potent effect against *L. donovani* or *L. chagasi* ($p = 0.061$). The transmission blocking properties of the Leishmune® vaccine was also assessed by an in vivo membrane assay, with sand flies fed with 1.5×10^7 amastigotes, human blood and, vaccinated or normal control dog sera. Significantly higher values were found in rate of infection ($p < 0.025$) and intensity of infection (number of parasites/insect) ($p < 0.05$) of control sand flies, making a very reduced infection index (20.7%) in the vaccine group. Our results disclosed that the Leishmune® vaccine is a TBV, and that the dog antibodies present in sera, even 12 months after vaccination, lead to a significant effective protection of 79.3%.

Keywords: Transmission blocking vaccines; IgG2; Efficacy; *Lutzomyia longipalpis*; Canine visceral leishmaniasis; Kala-azar; TBV; Sand fly; Membrane-feeding assay

1. Introduction

Human visceral leishmaniasis or kala-azar is a canid zoonosis. Peri-domestic sand flies acquire the etiological agent (*L. chagasi* or *L. infantum*) by feeding on infected fox's skin and transmit it to dogs. The subsequent transmission to humans by sand flies causes human visceral leishmaniasis (VL), which is a severe disease, fatal if not treated by the onset of the symptoms [1]. Five-hundred thousand new human kala-azar cases are registered annually, most of them (90%) in India, Sudan, Bangladesh and Brazil. A protective prophylactic vaccine against human disease is not yet available. The best performance was obtained with a first gen-

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eration vaccine that gave 12% protection among individuals that skin-tested positive for leishmanial antigen [2]. Furthermore, chemotherapy against kala-azar is highly toxic and not always effective [1]. Since the disease is a canid zoonosis, the reduction of dog infectivity to sand flies by prophylactic vaccination would reduce the human incidence of the disease [3]. We have described the development of a prophylactic vaccine against canine visceral leishmaniasis, based on saponin and the FML antigen (fucose mannose ligand) of *L. donovani* [4–7]. The FML-vaccine showed 92–95% specific protection (76–80% vaccine efficacy) in Phase III trials against natural visceral leishmaniasis in Brazil [5,6]. Vaccination reduced both morbidity and incidence of the canine disease [5,6]. This effect lasted for at least 3.5 years and was concomitant with the reduction of the human incidence of the disease in the area [6]. We also showed that the FML-vaccine also has an immunotherapeutic effect when administered to *Leishmania donovani* or *Leishmania chagasi* infected dogs while they were still asymptomatic [8]. The decrease in the canine and human incidence of visceral leishmaniasis in the vaccinated area [5,6], and the maintenance of normal proportions of CD4 and CD21 lymphocyte levels in the blood of vaccinated dogs [8] indicate that dog vaccination with the FML-vaccine reduces dog infectivity to sand-flies [9,10]. Recently, we proved that dogs treated with Leishmune® (FML-licensed vaccine) are not infectious [11], as indicated by a complete absence of clinical signs and of parasites in skin, lymph node and blood PCR amplified samples ($p < 0.01$). Exposed untreated controls on the other hand, were symptomatic (25%), lymph node (56.7%) and blood *Leishmania*-DNA PCR (15.7%) positive, showing also positive immunohystochemical reactions in skin (25%).

It became important then, to determine if the Leishmune®-FML-vaccine is actually a transmission blocking vaccine (TBV). The term "transmission blocking vaccine" (TBV) is used for the malaria vaccines that stimulate antibody production in humans against the sexual gamete forms of the parasite present in the midgut of the anopheles vector [12]. During a blood meal, these antibodies are acquired by mosquitoes and block the fertilization process and further development of the parasites in the vector, making the insect incapable of transmitting the disease [13]. Therefore, TBV are designed to raise antibodies against the gamete stage of the parasite present in the mosquito gut, and while they do not reduce disease in the infected person, prevent the spread of malaria through the community. Such antibodies would block further parasite development in rendering the vector non-infectious. There is a functional test to make the Phase I analysis of this kind of vaccines. Laboratory-raised mosquitoes are fed through a membrane with immune sera generated in vaccinated or infected animals. The mosquito gut can then be dissected to determine the number of infectious gametocytes that developed [12,14].

In the epidemiological cycle of the agents of visceral leishmaniasis (*Leishmania chagasi* and *Leishmania infantum*), the sand fly ingests amastigote-containing macrophages and monocytes from infected dogs during its blood meal. These amastigotes released into the sand fly midgut differentiate into flagellated, procyclic promastigotes and attach to the midgut epithelium [15]. The dividing procyclic promastigotes go into metacyclogenesis, acquiring virulence and transforming into non-dividing metacyclic promastigotes that detach from the midgut epithelium, migrate to the buccal cavity and infect a new vertebrate during the next blood meal [15]. The metacyclogenesis involves morphological changes of the parasite and biochemical transformation of its lypophosphoglycan (LPG) terminal exposed saccharide residues [16–18].

Being a surface antigen of *L. donovani* promastigotes and amastigotes [19,20], and a highly protective immunogen for canine vaccination [5,6,8], the FML antigen could also be specifically recognized by the midgut of the *Lutzomyia longipalpis* vector, acting as a parasite ligand to the midgut and the Leishmune® vaccine could be a TBV. In the present work, we assayed the possible transmission blocking vaccine potential of the Leishmune® vaccine by: (1) using the FML antigen to block the adhesion of *Leishmania donovani* and *Leishmania chagasi* procyclic promastigotes to dissected midguts of the *Lutzomyia longipalpis* sand fly vector; (2) testing the ability of antibodies raised in dogs after vaccination with the Leishmune® to block the adhesion of procyclic promastigotes to dissected sand flies midguts; and (3) assaying the reduction in the proportion of sand flies that become in vivo infected, when fed through a membrane with *Leishmania chagasi*, in the presence of sera taken from Leishmune® vaccinated dogs.

2. Material and methods

2.1. Leishmania promastigote culture

Promastigotes of *Leishmania (L.) donovani* (LD 1S/MHOM/SD/00-strain 1S) and *Leishmania (L.) chagasi* (IOC L-579) were grown in screw-capped tubes at 28 °C with a complex medium containing: brain heart infusion (37 g/l) (Difco, USA), hemin (0.01 g/l) and folic acid (0.02 g/l) (Sigma, SL, USA) supplemented with 10% heat inactivated fetal calf serum (FCS) (Nutricell, Campinas, Brazil). Procyclic promastigotes (exponential growth phase) were obtained after 24 h in culture. The parasites were washed twice in 0.01 M phosphate buffered 0.9% saline (PBS), centrifuged at $2760 \times g$ for 15 min and used for all interaction assays.

2.2. FML antigen

Isolation and chemical characterization of the fucose mannose ligand (FML) obtained from stationary-growth phase promastigotes of *Leishmania (L.) donovani* Sudan (LD 1S/MHOM/SD/00-strain 1S) were performed as previously