SHORT COMMUNICATION

Effect of *Lutzomyia whitmani* (Diptera: Psychodidae) Salivary Gland Lysates on *Leishmania (Viannia) braziliensis* Infection in BALB/c Mice

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Previous reports showed that Lutzomyia longipalpis *saliva exacerbate* Leishmania braziliensis *infection in mice. The sand fly* Lu. whitmani *is one of the vectors of* L. (Viannia) braziliensis (LVb), *a causative agent of cutaneous leishmaniasis in the State of Ceará, Brazil. To determine whether saliva of* Lu. whitmani *could increase the infectivity of* LVb *in mice, we inoculated groups of BALB/c mice with* LVb *promastigotes in the presence or absence of the salivary glands lysate from* Lu. whitmani. *We found that coinjection with* Lu. whitmani *saliva increased size but not longevity of cutaneous* LVb *lesions in BALB/c mice, since the formed lesions gradually resolved. The mechanism(s) by which* Lu. whitmani *saliva might exacerbate* LVb *infection in BALB/c mice is speculated.*

Key words: *Leishmania braziliensis* - saliva - *Lutzomyia whitmani* - BALB/c mice

Leishmaniases are parasitic diseases caused by protozoan parasites of the genus *Leishmania* that produce either tegumentary (cutaneous, mucocutaneous, diffuse) or visceral clinical presentations in man and are responsible for significant morbidity and mortality in many areas throughout the world (Pearson et al. 1999). The mammalian host becomes infected with *Leishmania* when the sand fly vector, *Phlebotomus* species in the Old World and *Lutzomyia* species in the New World, probes the skin of parasitized host for a blood meal and injects the parasite admixed with its saliva (Titus et al. 1994).

Previous studies have shown that salivary gland lysates of *Lu. longipalpis* and *P. papatasi* markedly enhance the infectivity of *L. major* in mice (Titus & Ribeiro 1988, Theodos et al. 1991). This exacerbating effect on the course of cutaneous leishmaniasis applies not only to infection with *L. major*, causative agent of Old World cutaneous leishmaniasis, but also to infection with other parasites causing cutaneous leishmaniasis in the New World, such as *L. mexicana* (Theodos et al. 1991) and *L. braziliensis* (Samuelson et al. 1991, Lima & Titus 1996). Additionally, it has been shown that the enhancement of infectivity is not due to a direct effect of the saliva on the parasite but rather to an effect on the host. Sand fly saliva has several pharmacological activities, including vasodilation, inhibition of platelet aggregation, and inhibition of coagulation (Ribeiro 1987). Besides, sand fly saliva may also have immunomodulatory effects related to the pathogenesis of leishmanial infections (Hall & Titus 1995, Qureshi et al. 1996, Mbow et al. 1998).

We report here the effect of salivary gland lysate of *Lu. whitmani* on the course of *L. (V.) braziliensis* infection in BALB/c mice. The rationale for extending these observations to a model using *L. (V.) braziliensis* plus salivary gland lysate from *Lu. whitmani* is that this sand fly is the most important natural vector for *L. (V.) braziliensis* in Ceará, Brazil (Queiroz et al. 1994). Moreover, *L. (V.) braziliensis* is transmitted in nature neither by *Lu. longipalpis* nor *P. papatasi*, the sand fly species targeted on saliva studies up to now.

The *L. (V.) braziliensis* strain (MCAN-BR-92-19914) used in this study was maintained in Schneider's insect medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated fetal calf serum (Sigma), 2% sterile normal human urine, 100 U/ml penicillin (Sigma), 100 µg/ml streptomycin (Sigma) and 2 mM L-glutamine (Gibco

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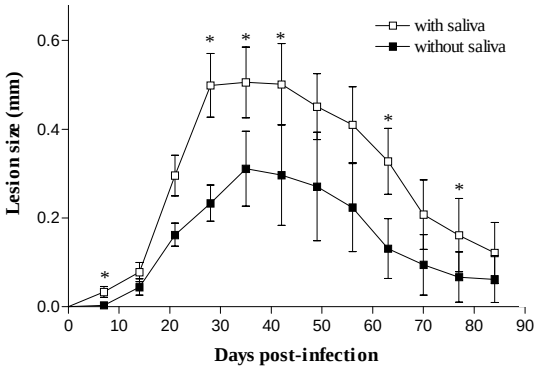
BRL, Grand Island, NY) at 26°C. Parasites were used at no later than the fourth passage. Salivary gland lysates were obtained from sugar-fed 3-5-day-old adult laboratory-reared, female *Lu. whitmani* from a closed laboratory colony maintained at Núcleo de Medicina Tropical/UFC and used as described previously (Titus & Ribeiro 1988).

When *L. (V.) braziliensis* promastigotes were inoculated with salivary gland lysate, the lesion appeared around seven days post-infection. They peaked in size at 28 days of evolution and persisted for up to 42 days, then regressed in a slower way than the lesions of the controls without saliva (Figure). Also, in the animals coinjected with *L. (V.) braziliensis* plus *Lu. whitmani* saliva, the lesions developed always larger than those of the controls did, which it was statistically significant ($p < 0.05$) at virtually all time point examined (Figure).



Enhancement of *Leishmania (Viannia) braziliensis* (LVb) infection in BALB/c mice by coinjection of parasites with *Lutzomyia whitmani* salivary glands lysates. Groups of nine BALB/c mice were injected subcutaneously in the right hind footpad with 10^7 stationary phase LVb promastigotes in 20 ml sterile saline with or without the lysate of 0.5 of one salivary gland per foot. Lesion sizes were measured weekly with a dial gauge caliper and expressed as the difference in thicknesses (mm) of the infected and contralateral uninfected footpads; values represent the mean ± SE; * $p < 0.05$.

In the BALB/c model of infection, *L. braziliensis* induces only transient and non-severe cutaneous lesion. Nonetheless, when *L. braziliensis* parasites are injected into BALB/c mice with sand fly saliva, they cause progressive skin lesions and infection is significantly enhanced as measured by lesion size, parasitic burden, and outcome of infection (Samuelson et al. 1991, Lima & Titus 1996, Donnelly et al. 1998). In this work we found that coinjection of *L. (V.) braziliensis* plus *Lu. whitmani* salivary gland lysate also increases the size of cutaneous lesions in BALB/c mice. However, while in the works using coinjection of *Lu. longipalpis* saliva plus *L. (V.) braziliesies* the cutaneous lesions persisted for the lifetime of the mice (Samuelson et al. 1991, Lima & Titus 1996), we showed that coinjecting saliva of *Lu. whitmani* plus *L. (V.) braziliensis* it formed spontaneously resolving cutaneous lesions. The mechanism(s) by which *Lu. whitmani* saliva exacerbates *L. (V.) braziliensis* infection in BALB/c mice may only be speculated, since most of the salivary factor(s) responsible for the enhancement of infectivity of *Leishmania* have not yet been identified. In the saliva *of Lu. longipalpis* one key molecule is the peptide maxadilan, which besides being a potent vasodilator may also have immunomodulatory properties (Lerner et al. 1991, Qureshi et al. 1996, Soares et al. 1998). *P. papatasi* lacks maxadilan, but its saliva contains large amounts of adenosine and 5'-AMP (Ribeiro et al. 1999). It has been demonstrated that adenosine is probably the factor in *P. papatasi* that interferes with the ability of activated macrophages to kill parasites (Katz et al. 2000).

So far as we are aware, the present report is the first to show that salivary gland lysate of *Lu. whitmani* enhance the infectivity of *L. (V.) braziliensis* in mice. Our data support other studies that have demonstrated that the vectors' saliva somehow modulates the long-term pathology of the disease. We hope with this work to contribute for a better understanding of the role of *Lu. whitmani* saliva in the natural transmission of *L. (V.) braziliensis* cutaneous leishmaniasis in this region of the world.

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