

SHORT COMMUNICATION

## Dissociation between Vasodilation and *Leishmania* Infection-enhancing Effects of Sand Fly Saliva and Maxadilan

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*In this study, the ability of maxadilan and Lutzomyia longipalpis salivary gland lysate to enhance the infection of CBA mice by Leishmania major and of BALB/c mice by L. braziliensis was tested. No difference was observed between sizes of lesion in CBA mice infected with L. major and treated or not with salivary gland lysate or maxadilan, although they were injected in concentrations that induced cutaneous vasodilation. Although parasites were more frequently observed in foot pads and spleens of animals treated with maxadilan than in the animals treated with salivary gland lysate or saline, the differences were small and not statistically significant. The lesions in BALB/c mice infected with L. braziliensis and treated with maxadilan were slightly larger than in animals that received Leishmania alone. Such differences disappeared 14 weeks after infection, and were statistically significant only in one of two experiments.*

Key words: *Lutzomyia longipalpis* saliva - maxadilan - *Leishmania major* - *Leishmania braziliensis* - vasodilation

*Lutzomyia longipalpis* saliva has been shown to enhance *Leishmania* infection in mice (Titus & Ribeiro 1988). This effect is attributed at least in part to maxadilan (Qureshi et al. 1996), a polypeptide that produces vasodilation (Lerner et al. 1991, Lerner & Shoemaker 1992) and presents a range of immunomodulatory activities: it inhibits T-cell proliferation and delayed-type hypersensitivity in mice (Qureshi et al. 1996), decreases TNF- $\alpha$  release by macrophages and increases IL-6 and IL-10 production in response to LPS, both in vitro (Soares et al. 1998) and in vivo (Bozza et al. 1998). In addition, maxadilan inhibits the intracellular killing of *Leishmania* by macrophages (Soares et al. 1998). In this study, we tested the ability of maxadilan and salivary gland lysates of *L.*

*longipalpis*, with intense vasodilation activity, to enhance the infection of CBA and BALB/c mice with *L. major* (MHOM/IR-173) and *L. braziliensis* (MHOM3456), respectively.

Groups of 6 to 18 CBA or BALB/c mice were used in four separate experiments. Two different batches of maxadilan were used: one recombinant (provided by Dr John David, Harvard School of Medicine, USA) and one synthetic (Soares et al. 1998) (provided by Dr Richard Titus, Colorado State University, USA). As expected, each batch produced diarrhoea when injected intraperitoneally in mice and cutaneous hyperaemia when injected intradermally in rabbits. Salivary glands were isolated from *L. longipalpis*, and lysates prepared with these glands also produced skin hyperaemia in rabbits. The CBA and BALB/c mice were infected into the foot pads with  $10^5$  fourth in vitro-passage, stationary-phase, *Leishmania* promastigotes suspended in (1) phosphate-buffered saline containing 0.1% bovine serum albumin (PBS-0.1%BSA) alone, or (2) PBS-0.1%BSA containing a half acinus of *L. longipalpis* salivary gland (only CBA mice), or (3) PBS-0.1%BSA containing maxadilan (1, 5, 50 and 1000 ng per injection). The total volume of injected material was 25  $\mu$ l. The concentration of salivary gland lysate was 10-

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fold higher than that necessary to cause cutaneous vasodilation (as determined by dose-response experiments in three rabbits), and the concentrations of maxadilan ranged from 2 to 2,000-fold higher than that necessary to cause cutaneous vasodilation in the rabbits treated. The viability and virulence of *L. major* promastigotes was confirmed by their ability to produce lesions in BALB/c mice.

No differences in the levels of circulating anti-*Leishmania* antibodies as detected in ELISA (not shown), permanence of the parasite in the site of inoculation, or dissemination of *Leishmania* to spleen, liver and lung (Table), were observed between groups of CBA mice infected with *L. major* in the presence or absence of salivary gland lysate, after 9, 14 or 21 weeks of observation. One of the batches of maxadilan caused no change in the development of *L. major*-induced lesion in CBA mice (not shown). With the other batch a slight increase in the size of the lesion was observed after the 13th week of infection in one experiment (Fig. 1). Although the group treated with maxadilan had a higher frequency of positive foot pad cultures and dissemination of parasites to the spleen (Table) and higher levels of anti-*Leishmania* antibodies in ELISA (not shown), these differences were not statistically significant.

There was a slight increase in lesion size of BALB/c mice infected with *L. braziliensis* and maxadilan, compared with the animals infected with *L. braziliensis* alone (Fig. 2). This difference, however, was small and reached statistical significance in only one out of two experiments. In both groups of animals the lesion subsided after 14 weeks of infection.

The results described herein conflict with those of other authors (Titus & Ribeiro 1988, Qureshi et al. 1996, Donnely et al. 1998, Mbow et al. 1998), and clearly show that the reported enhancing effect of sand fly saliva on *Leishmania* infection is not easily reproducible. The reason for this dis-

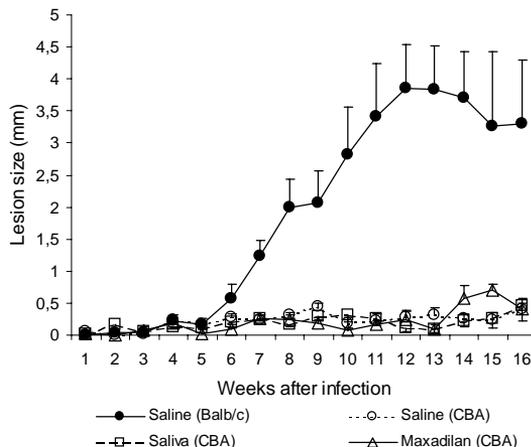


Fig. 1: effect of *Lutzomyia longipalpis* salivary gland and maxadilan on *Leishmania major* infection in CBA mice. Lines represent mean±SEM of lesion sizes. Only a small and transient increase in lesion size was observed in the CBA group treated with maxadilan (maxadilan) after 14 days of infection, compared with the CBA group infected with *L. major* only (saline). BALB/c mice were used as a control of *L. major* virulence.

crepancy is not clear, but may relate to the immune status of the host, which, due to environmental conditions, may or may not respond to an immunomodulating effect of sand fly saliva. The sand fly saliva and maxadilan preparations used in this study were certainly biologically active in terms of inducing vasodilation. The data presented herein show therefore, that the induction of vasodilation by sand fly saliva does not by itself suffice to significantly enhance *Leishmania* infection. They are in agreement with the observations that *L. longipalpis* salivary gland lysates with high vasodilation activity did not enhance experimental *L. chagasi* infection in dogs (Paranhos-Silva et al. 1993, Paranhos-Silva et al. manusc. in prep.) and are less effective in enhancing *Leishmania* infec-

TABLE

Presence of parasites in the foot pad and dissemination to the internal organs of CBA mice infected with *Leishmania major* alone, or associated with salivary gland of *Lutzomyia longipalpis* or maxadilan

Treatment	Positive cultures					
	FP <sup>a</sup>	LN <sup>b</sup>	Spleen	Liver	Lung	Any organ
Saline	6/13 <sup>c</sup>	6/13	1/13	0/13	0/13	9/13
Salivary gland	4/13	3/13	0/13	0/13	0/13	6/13
Maxadilan	10/13	5/13	4/13	0/13	0/13	11/13

a: foot pad; b: popliteo lymphonode; c: cumulative number of animals with positive cultures obtained from material collected after 9, 14 and 21 weeks of infection/total number of animals studied.

Statistical significance of the differences between group maxadilan vs saline (Fisher's exact probability test): any organ p = 0.645; foot pad infection p = 0.226; spleen infection p = 0.322

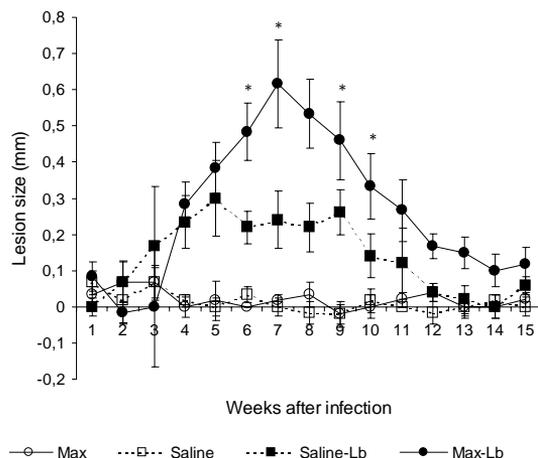


Fig. 2: maxadilán (Max) induced a small increase in the size of lesion produced by *Leishmania braziliensis* (Lb) in BALB/c mice. Such increase was statistically significant only from the 6th to the 10th week post-infection (\* p<0.05, one way ANOVA and Student-Newman-Keuls test)

tion than salivary gland lysates with low vasodilation activity (Warburg et al. 1994). They also indicate that, since the enhancing effect of maxadilán or salivary gland lysate on *Leishmania* infection may be small and inconsistent, a vaccine based in immunization against sand fly saliva may not have similar efficacy in different endemic regions.

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