The Journal *of* Immunology

Sandfly Maxadilan Exacerbates Infection with *Leishmania major* and Vaccinating Against It Protects Against *L. major* Infection

Robin V. Morris, Charles B. Shoemaker, John R. David,
Gregory C. Lanzaro and Richard G. Titus

References
This article **cites 36 articles**, 20 of which you can access for free at:
http://www.jimmunol.org/content/167/9/5226.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Sandfly Maxadilan Exacerbates Infection with *Leishmania major* and Vaccinating Against It Protects Against *L. major* Infection[1]

Robin V. Morris,* Charles B. Shoemaker,[†] John R. David,[†] Gregory C. Lanzaro,[‡] and Richard G. Titus[2]*

Bloodfeeding arthropods transmit many of the world's most serious infectious diseases. *Leishmania* are transmitted to their mammalian hosts when an infected sandfly probes in the skin for a bloodmeal and injects the parasite mixed with its saliva. Arthropod saliva contains molecules that affect blood flow and modulate the immune response of the host. Indeed, sandfly saliva markedly enhances the infectivity of *L. major* for its host. If the salivary molecule(s) responsible for this phenomenon was identified, it might be possible to vaccinate the host against this molecule and thereby protect the host against infection with *Leishmania*. Such an approach represents a novel means of controlling arthropod-borne disease transmission. Here, we report that a single molecule, maxadilan, in sandfly saliva can exacerbate infection with *L. major* to the same degree as whole saliva, and that vaccinating against maxadilan protects mice against infection with *L. major*.   *The Journal of Immunology,* 2001, 167: 5226–5230.

Members of the genus *Leishmania* are sandfly-transmitted protozoan parasites that cause leishmaniasis in their vertebrate hosts. Within the vertebrate host, *Leishmania* reside within phagocytic cells and induce a spectrum of diseases ranging from mild cutaneous to lethal visceral forms. When infected with *Leishmania*, especially with those parasites that induce cutaneous disease, most humans mount an effective immune response that resolves the infection and confers solid immunity to reinfection (1). This suggests that among parasitic diseases of humans, leishmaniasis would be one for which a vaccine could be developed with relative ease. However, there is no safe and effective vaccine for the disease. Moreover, in certain experimental models for infection with *Leishmania*, the same parasite antigenic epitope can activate either a protective type 1 response or an exacerbative type 2 response, which means that immunizing with a given epitope of *Leishmania* may protect certain individuals but lead to disease exacerbation in others (2). Thus, novel approaches toward vaccinating against leishmaniasis need to be explored.

It is now apparent that the saliva of blood-feeding arthropods contains molecules that enhance blood flow (3) and inhibit the immune response of the host (4). While enhanced blood flow insures the feeding success of the arthropod, inhibiting the immune response of the host may prevent the host from becoming sensitized to the bite of the arthropod. However, there is now mounting evidence that the saliva of an arthropod vector can also enhance the infectivity of pathogens that the arthropod transmits (5–10). Therefore, injecting arthropod-borne pathogens by syringe does not mimic natural transmission.

We originally showed that infection with *Leishmania major* was dramatically enhanced in mice coinjected with the parasite plus sandfly saliva. Cutaneous lesions caused by the parasite were severalfold larger than lesions on control mice, and parasite burden in those lesions could be as much as several thousand-fold higher (5). Indeed, saliva completely reversed the outcome of infection in *L. braziliensis*-infected mice (4, 11). We proposed that the protein in saliva responsible for its disease-exacerbating qualities is a vasodilator, and that this vasodilator is related to a mammalian neuropeptide (12). Subsequently, the gene encoding the salivary vasodilator was cloned (13). This salivary vasodilator, termed *Lutzomyia longipalpis* sandfly maxadilan (MAX),[3] appears to be functionally related to the mammalian neuropeptide, pituitary adenylate cyclase-activating polypeptide (PACAP). Both MAX and PACAP are vasodilators and inhibitors/modulators of an inflammatory and an immune response, and both signal at least through the PACAP type 1 receptor (4, 14, 15).

Taken together, these observations suggest that MAX is indeed responsible for the disease-exacerbating qualities of sandfly saliva, a hypothesis that is tested here. Moreover, when mice are infected with *L. major* in numbers equivalent to those injected by a naturally infected sandfly (10–100), the parasite does not survive unless it is coinjected with sandfly saliva (5). This suggests that if mice were vaccinated with MAX, they would be protected against a challenge with *L. major* plus sandfly saliva. This novel approach for vaccinating against a pathogen is also examined here.

Materials and Methods

Mice, L. major, *and infection with the parasite*

Young adult female CBA/CaH-T6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Stationary phase promastigotes of *L.*

*Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; [†]Department of Immunology and Infectious Diseases, Harvard University School of Public Health, Boston, MA 02115; and [‡]Department of Pathology and Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX 77555

[2] Address correspondence and reprint requests to Dr. Richard G. Titus, Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523. E-mail address: Richard.Titus@colostate.edu

[3] Abbreviations used in this paper: MAX, *Lutzomyia longipalpis* sandfly maxadilan; PACAP, pituitary adenylate cyclase-activating polypeptide.

0022-1767/01/$02.00

major (LV39 (MRHO/Sv/59/P) were used. Mice were injected s.c. with 10^5 *L. major* ± varying doses of salivary gland lysate or MAX in one hind footpad. These mouse experiments were approved by the Institutional Review Board of Colorado State University.

Monitoring lesion development and parasite burden in the lesion

Lesion development was followed by measuring with a caliper the thickness of the infected footpad compared with the control contralateral uninfected footpad. Parasite numbers were determined in infected footpads using a published limiting dilution assay (16).

Sandfly salivary gland lysate and synthetic maxadilan

Salivary glands of *Lutzomyia longipalpis* (Belo Horizonte, Brazil, Lapinha Cave isolate) were collected and lysed by freezing and thawing as described (5). Synthetic maxadilan was prepared by the Biopolymers Laboratory, Harvard Medical School. The 63-mer amino acid sequence used was based on the predicted sequence of mature, secreted MAX (Ref. 17; CDATCQFRKAIEDCRKKAHHSDVLQTSVQTTATFTSMDTSQLPGS GVFKECMKEKAKEFKAGK).

Vaccinating against MAX

Groups of mice (*n* = 5) were injected s.c. at the base of the tail with 25 μg of synthetic MAX emulsified in CFA. Ten days later the mice were injected i.p. with 25 μg of MAX emulsified in IFA. Two weeks later the mice were boosted i.p. with 25 μg of soluble MAX. Another group of mice (*n* = 5) was injected with adjuvant or vehicle alone. Three days after the soluble boost with MAX, the titer of anti-MAX Ab in the sera of the MAX-sensitized mice was determined (see *MAX ELISA*). If the titer was sufficient (Table III), mice were challenged with either *L. major* or *L. major* + 0.5 salivary gland lysate (see *Results*).

MAX ELISA

Three days after the soluble boost with MAX, blood was collected from mice and the anti-MAX serum titer was determined by an ELISA. Briefly, ELISA plates were coated with synthetic MAX (10 μg/ml) using standard techniques (18) and developed with alkaline phosphatase-labeled goat anti-mouse IgG (H and L chain) and *p*-nitrophenyl phosphate (catalog no. 075-1806 and 50-80-01, respectively; Kirkegaard & Perry Laboratories, Gaithersburg, MD).

Cytokine ELISA and Griess reaction

Ten days after mice were injected s.c. at the base of the tail with 25 μg of synthetic MAX emulsified in CFA, the draining inguinal and para-aortic lymph nodes were removed and a single-cell suspension was generated. The cells were placed into culture (5 × 10^6/ml in 24-well plates, Costar 3524; Corning Glass, Corning, NY) in DMEM (18) + 0.5% normal mouse serum. Experimental cultures then received 10 μg/ml synthetic MAX; control cultures received diluent alone. The supernatants and cells were harvested after 3 days of culture. Supernatants were analyzed for their content of IFN-γ and NO by published methods (18, 19). The cells were purified over Percoll gradients (18) and analyzed by flow cytometry.

Flow cytometry

Cells were labeled with the following Abs: FITC anti-mouse CD4 or FITC anti-mouse CD8 (557307 and 553030, respectively; BD PharMingen, San Diego, CA), FITC anti-mouse I-A^K MHC class II, private (MM3501; Caltag Laboratories, Burlingame, CA). Appropriate control Abs were purchased from the same suppliers. Labeled cells were then analyzed using methods described elsewhere (20).

Statistical analyses

Data for lesion progression were analyzed using ANOVA for repeated measure and for parasite burdens using unpaired *t* tests.

Results

MAX substitutes for sandfly saliva and exacerbates infection with Leishmania major in mice

To test whether MAX would substitute for whole saliva, we admixed varying amounts of synthetic MAX with 10^5 *L. major* promastigotes and injected them s.c. into a hind footpad of CBA mice. Three nanograms of MAX markedly (*p* < 0.002 compared with control mice infected with *L. major* alone) exacerbated lesion de-

velopment to the same degree as the lysate of 0.5 sandfly salivary gland (*p* < 0.002, Fig. 1*a*). One-half of one sandfly salivary gland is the dose that we reported exacerbates infection with *L. major* (5).

Interestingly, MAX had a biphasic dose-response effect on lesion development. As expected, a lower dose (1 ng) of MAX exacerbated infection with *L. major* to a lesser degree than 3 ng of MAX (Fig. 1*a*). However, a higher dose of MAX (10 ng) also exacerbated infection with *L. major* to a lesser degree (Fig. 1*a*). We have reported that doses of <0.5 sandfly salivary gland exacerbate infection with *L. major* to a lesser degree (21), but we had not previously tested doses above 0.5 salivary gland. Therefore, we tested a wide range of doses of sandfly salivary gland lysate (Fig. 1*b*). Importantly, the same phenomenon was observed. Compared with controls (saliva-free), exacerbation of infection increased with increasing doses of saliva, up to a maximum of 0.5 and 1.0 gland equivalents. Interestingly, higher doses (2.0 gland equivalents) had a reduced effect (Fig. 1*b*). The doses of salivary gland lysate that significantly enhanced lesion development were 0.5 and 1.0 gland extract; *p* < 0.001 and 0.002, respectively (Fig. 1*b*).

Finally, because 3 ng of MAX and 0.5 salivary gland lysate exacerbated infection equally, we determined whether similar parasite burdens were present in the two groups of mice. Both MAX (423-fold increase) and saliva (193-fold increase) markedly enhanced parasite burden (*p* < 0.001, Table I). This degree of enhancement of infection is similar to what we previously reported for whole saliva (5).

Vaccinating against MAX protects mice against infection with Leishmania major

Because MAX exacerbated infection with *L. major*, we hypothesized that vaccinating against it might neutralize the disease-enhancing effects of whole saliva and thus protect vaccinated mice against infection with *L. major*. CBA mice were injected with synthetic MAX (25 μg) emulsified in Freund's adjuvant and were then boosted with soluble synthetic MAX; other mice received adjuvant or diluent alone. Mice were then challenged s.c. in one
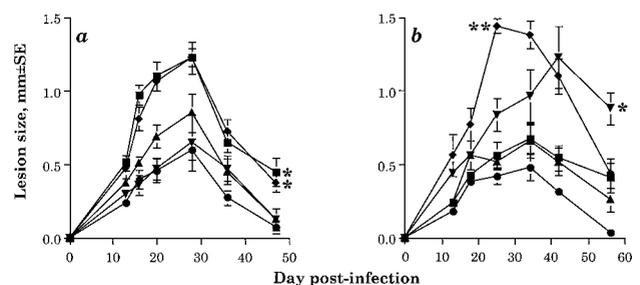

FIGURE 1. Both sandfly salivary gland lysate and sandfly salivary MAX enhance infection with *Leishmania major* in mice. *a*, Groups of CBA (*n* = 5) mice were injected with *L. major* and varying doses of MAX or sandfly salivary gland lysate (●, Control saliva-free; ▲, 1 ng of MAX; ◆, 3 ng of MAX; ▼, 10 ng of MAX; ■, the lysate of 0.5 salivary gland). Lesion development (the increase in footpad thickness) was then followed over time. The asterisk denotes that mice injected with either 3 ng of MAX or 0.5 salivary gland lysate developed lesions that were significantly (*p* < 0.002) larger than lesions that developed on control (MAX- or saliva-free) mice. The results are representative of four independent experiments. *b*, Groups of CBA (*n* = 5) mice were injected with *L. major* and varying doses of sandfly salivary gland lysate (●, control saliva-free; ▲, the lysate of 0.1 salivary gland; ◆, 0.5 gland; ▼, 1 gland; ■, 2 glands). The asterisk denotes that mice injected with either the lysate of 0.5 or 1 salivary gland developed lesions that were significantly (*p* < 0.001 (∗∗) or 0.002 (∗), respectively) larger than lesions that developed on control (saliva-free) mice. The results are representative of three independent experiments.

Table I.   L. longipalpis *salivary gland lysate or synthetic maxadilan markedly enhance parasite burden in lesions of* L. major[a]

Treatment	No. of *L. major*/Footpad (mean ± SD)	Fold Increase in Parasite No.	*p* Value
L. major	12,036 ± 852	Not applicable	Not applicable
L. major + 0.5 salivary gland	2,322,948 ± 98,021	193	<0.001
L. major + 3 ng of MAX	5,091,228 ± 576,643	423	<0.001

[a] At 4 wk postinfection, triplicate mice were individually analyzed for the number of parasites in their cutaneous lesions. Results are representative of three independent experiments.

hind footpad with *L. major* parasites (10^5) or parasites admixed with salivary gland lysate (0.5 gland). Vaccinated mice were highly resistant to infection. Cutaneous lesions on vaccinated mice were 3- to 5-fold smaller, and these mice healed their lesions by day 50 of infection, whereas lesions on mice treated with adjuvant or diluent alone had not healed their lesions by day 65 of infection. As a result, lesions on vaccinated mice were significantly smaller (Fig. 2) than lesions on control mice. In addition, the parasite burden in lesions on vaccinated mice was markedly reduced (506.7-fold, $p < 0.001$, Table II).

Vaccinating against MAX induces anti-MAX Abs and a type 1 anti-MAX immune response

To investigate the possible mechanism(s) underlying the protection against infection seen in MAX-vaccinated mice, we characterized the anti-MAX response elicited in the mice. First, serum contained a high titer (1/6,400–1/25,600; Table III) of anti-MAX Abs. In addition, a cellular response was induced in vaccinated mice. We isolated inguinal and para-aortic lymph nodes from mice injected at the base of the tail with MAX emulsified in CFA. These lymph nodes drain both the base of the tail and the footpad where *L. major* parasites were subsequently injected. When these lymph node cells were stimulated with MAX in vitro, the cells released substantial quantities of both IFN-γ and NO (Table III). We also determined the phenotype of the responding cells because several cell types can release IFN-γ. A flow cytometric analysis revealed that the cells were composed principally of CD4 cells (82%) with some CD8 cells (10%) and I-A+ cells (4%, Table III). Thus, both a humoral and cellular anti-MAX response were induced in treated mice (Table III), and this may explain how immunization not only negated the exacerbative effect of the saliva, but also reduced the

severity of disease below that seen in animals that were challenged with *L. major* but no saliva (compare groups ■ and ● in Fig. 2).

Discussion

It is now clear that the saliva of arthropod vectors contains vaso-modulatory and immunomodulatory factors (3, 4). These effects would benefit the arthropod vector in its quest to locate blood and may help to keep blood flowing without incurring a host inflammatory response. In addition, the arthropod vector can deliver the pathogen it transmits to the same skin site where it has salivated. Thus, the pathogen would also encounter, and could possibly benefit from, a host skin site that is profoundly altered by the effects of vector saliva. Indeed, this may explain why arthropods are such efficient vectors for disease.

To test this hypothesis, we initially demonstrated that sandfly saliva dramatically enhanced the infectivity of *L. major* for mice (5). Here we show that a single sandfly salivary protein, MAX, can substitute for whole saliva and exacerbate infection with *L. major* to the same degree as whole saliva (Fig. 1, Table I). Different doses of either MAX or saliva had different effects on infection with *L. major*. A pair of sandfly salivary glands contains ~1 μg of total protein (5), and the optimal effect with whole saliva is achieved with 0.5 gland, or 250 ng of total protein (5, 21). Because MAX is ~1% of the total protein of the salivary gland (22), one would have predicted that 2–3 ng of synthetic MAX would have optimal effects, as, in fact, was the case (Fig. 1). That lower doses of either MAX or saliva had less of an exacerbative effect on *L. major* infection was also expected (Ref. 21 and Fig. 1). That higher doses of either MAX or saliva were also less effective was not expected (Fig. 1). However, these observations were quite reproducible, and a dose of 2 or 3 ng of MAX or 0.5–1.0 salivary glands consistently yielded maximal effects.

The reason(s) why sandfly saliva and MAX display a biphasic effect on infection with *L. major* are currently unknown. However, there are many possible explanations for the phenomenon. For example, at different concentrations, MAX may be a homo- or heterodimer (with itself or other salivary proteins) that, as a result, interact with different forms of the PACAP receptor. There are at least eight forms, and each may signal different effects in the target cell (23–26).

The results presented herein represent the "proof-of-principle" experiments for our work with arthropod salivary proteins. We show that a dual-function protein (MAX) is responsible for the effects of sandfly saliva (Fig. 1, Table I), and that immunizing against MAX can protect against infection with *Leishmania* (Fig. 2, Table II). Moreover, the results suggest that MAX is the major molecule in sandfly saliva that exacerbates infection with *L. major* because vaccinating against this molecule neutralized the effects of whole saliva. However, other factors may also contribute to exacerbation because vaccinating against MAX may mask their effects. In addition, the results confirm and extend the literature regarding the immune response to *Leishmania* (27). That is, the multiple effects that MAX has on the immune system would be predicted to
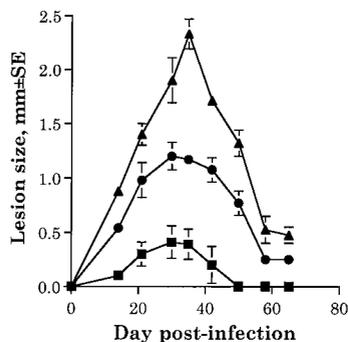

FIGURE 2. Vaccinating against MAX protects mice against infection with *Leishmania major*. Groups of CBA mice (*n* = 5) were injected with synthetic MAX emulsified in adjuvant followed by soluble MAX (● or ■) or with adjuvant followed by diluent alone (▲; see *Materials and Methods* for details). Group ● was then challenged with *L. major* while groups ■ and ▲ were challenged with *L. major* admixed with 0.5 salivary gland lysate. Results obtained with group ■ were significantly different from both groups ● (*p* < 0.005) and ▲ (*p* < 0.001). The results are representative of five independent experiments.

Table II. *Vaccinating against synthetic Maxadilan markedly protects mice from infection with* L. major[a]

Treatment	No. of *L. major*/Footpad (mean ± SD)	Fold Increase/Decrease in Parasite No.	*p* Value
Vaccinated against MAX challenged with *L. major*	924,240 ± 5,426	Not applicable	Not applicable
Sham-vaccinated challenged with *L. major* + saliva	24,465,123 ± 1,244,768	+26.5	<0.01
Vaccinated against MAX challenged with *L. major* + saliva	1,824 ± 12	−506.7	<0.001

[a] At 4 wk postinfection, triplicate mice were individually analyzed for the number of parasites in their cutaneous lesions. Results are representative of three independent experiments.

lead to exacerbation of infection with *L. major*. For example, MAX inhibits T cell activation (28), stimulates the production of cytokines that favor the development of an exacerbative type 2 response (Refs. 4 and 15; e.g., IL-6), and inhibits the production of molecules that are important in the destruction of *L. major* (Refs. 4 and 15; e.g., TNF-α, H_2O_2, and NO).

Fig. 2 demonstrates that vaccinating against MAX can protect CBA mice against infection with *L. major*. We used the CBA model because infection in this mouse mimics infection with *L. major* in humans (i.e., both CBA mice and humans cure an infection with the parasite). In addition, using this model allowed us to compare our results with our previous work (e.g., Ref. 5). However, pilot studies with BALB/c mice are showing that vaccinating these mice also protects against *L. major* infection.

The results presented here suggest that arthropod vectors of disease are not simply "flying/crawling syringes." Rather, they play a dynamic role in the host/vector/pathogen relationship, and vaccinating against components of arthropod vector saliva holds promise as a novel approach toward vaccinating against vector-borne disease. Because arthropods transmit many pathogens, a single vector-based vaccine may help control the transmission of multiple diseases. Indeed, earlier work by others indicated that a vector-based vaccine might be effective. For example, vector saliva can modify the course of infection with bacteria (29, 30), viruses (8, 10, 31), and parasites (32, 33). Finally, recent work by Kamhawi et al. (34) showed that the bite of uninfected sandflies (this work used *Phlebotomus papatasi* sandflies; MAX is a protein found in *L. longipalpis* salivary glands) conferred resistance to a subsequent infection with *L. major*. Although the component in *P. papatasi* saliva that was responsible for the protection was not identified, these results suggested that a sandfly vector saliva-based vaccine for leishmaniasis might be feasible, and the results presented here demonstrate that this is the case.

For the experiments presented here we used the *L. longipalpis-L. major* experimental combination so that we could compare our results with those of previous publications and thus interpret the findings within the context of this previous work. We have not yet examined the effect that vaccination against MAX would have on infection with *L. chagasi*, a parasite which is vectored by *L.*

longipalpis (35). However, because *L. longipalpis* saliva enhances infection with *L. chagasi* (36) and because vaccination against MAX elicits a Th1 type response (Table III), it is likely that MAX-vaccinated mice would be protected against challenge against any species of *Leishmania*, so long as the parasite was coinjected with MAX.

We elected to vaccinate against MAX here because it is an immunomodulatory protein in sandfly salivary glands. However, there may be other proteins in salivary glands that are more immunogenic and thus more suitable for vaccine formulations. This is particularly important because the level of MAX expression differs between different geographical isolates of the fly (17), and MAX is not present in the salivary glands of the Old World fly, *P. papatasi*. Rather, the saliva contains large amounts of adenosine and AMP (37).

It is perhaps not surprising that it has proven so difficult to develop effective vaccines against vector-borne pathogens/parasites. These organisms often have very complex life cycles. Moreover, a parasite by definition is an organism that lives in or on another organism and often this parasitic existence lasts for the lifetime of both organisms. Therefore, it may be very difficult to develop pathogen-based vaccines that are long-lived and that induce sterile immunity to parasites. However, vaccines that target more than one facet of the life cycle of a parasite (e.g., the pathogen itself, vector salivary factors, vector-pathogen interactions) may prove to be effective.

References
1. Neva, F. A., and H. W. Brown. 1994. Blood and tissue protozoa of human beings. In *Basic Clinical Parasitology*, F. A. Neva and H. W. Brown, eds. Appleton & Lange, Norwalk, CT, p. 57.
2. Reiner, S. L., Z. E. Wang, F. Hatam, P. Scott, and R. M. Locksley. 1993. TH1 and TH2 cell antigen receptors in experimental leishmaniasis. *Science 259:1457*.
3. Ribeiro, J. M. 1987. Role of saliva in blood-feeding by arthropods. *Annu. Rev. Entomol. 32:463*.
4. Gillespie, R. D., M. L. Mbow, and R. G. Titus. 2000. The immunomodulatory factors of bloodfeeding arthropod saliva. *Parasite Immunol. 22:319*.
5. Titus, R. G., and J. M. Ribeiro. 1988. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. *Science 239:1306*.
6. Mbow, M. L., J. A. Bleyenberg, L. R. Hall, and R. G. Titus. 1998. *Phlebotomus papatasi* sand fly salivary gland lysate downregulates a Th1, but upregulates a Th2, response in mice infected with *Leishmania major*. *J. Immunol. 161:5571*.
7. Belkaid, Y., S. Kamhawi, G. Modi, J. Valenzuela, N. Noben-Trauth, E. Rowton, J. Ribeiro, and D. L. Sacks. 1998. Development of a natural model of cutaneous leishmaniasis: powerful effects of vector saliva and saliva preexposure on the long term outcome of *Leishmania major* infection in the mouse ear dermis. *J. Exp. Med. 188:1941*.
8. Jones, L. D., E. Hodgson, and P. A. Nuttall. 1989. Enhancement of virus transmission by tick salivary glands. *J. Gen. Virol. 70:1895*.
9. Labuda, M., L. D. Jones, T. Williams, and P. A. Nuttall. 1993. Enhancement of tick-borne encephalitis virus transmission by tick salivary gland extracts. *Med. Vet. Entomol. 7:193*.
10. Edwards, J. F., S. Higgs, and B. J. Beaty. 1998. Mosquito feeding-induced enhancement of Cache Valley Virus (Bunyaviridae) infection in mice. *J. Med. Entomol. 35:261*.

Table III. *Responses to maxadilan in vaccinated mice*

Parameter	Value (control values)[a]
Titer of anti-MAX Ab	6,400–25,600 (zero)
IFN-γ produced	8.02 ng/ml (zero)
NO produced	2.03 μM (zero)
Phenotype of responding cells	82% CD4+
	10% CD8+
	4% I-A+

[a] Control mice/cultures were not stimulated with MAX. Sera from nonvaccinated mice did not contain detectable anti-MAX Abs, and cells from these mice did not produce either detectable IFN-γ or NO when stimulated with MAX in vitro. Results are representative of two to five independent experiments.

11. Samuelson, J., E. Lerner, R. Tesh, and R. Titus. 1991. A mouse model of *Leishmania braziliensis braziliensis* infection produced by co-injection with sand fly saliva. *J. Exp. Med. 173:49.*

12. Ribeiro, J. M., A. Vachereau, G. B. Modi, and R. B. Tesh. 1989. A novel vasodilatory peptide from the salivary glands of the sand fly *Lutzomyia longipalpis. Science 243:212.*

13. Lerner, E. A., and C. B. Shoemaker. 1992. Maxadilan: cloning and functional expression of the gene encoding this potent vasodilator peptide. *J. Biol. Chem. 267:1062.*

14. Moro, O., and E. A. Lerner. 1997. Maxadilan, the vasodilator from sand flies, is a specific pituitary adenylate cyclase activating peptide type I receptor agonist. *J. Biol. Chem. 272:966.*

15. Soares, M. B., R. G. Titus, C. B. Shoemaker, J. R. David, and M. Bozza. 1998. The vasoactive peptide Maxadilan from sand fly saliva inhibits TNF-α and induces IL-6 by mouse macrophages through interaction with the PACAP receptor. *J. Immunol. 160:1811.*

16. Lima, H. C., J. Bleyenberg, and R. G. Titus. 1997. A simple method for quantifying *Leishmania* in tissues of infected animals. *Parasitol. Today. 13:80.*

17. Lanzaro, G. C., A. H. Lopes, J. M. C. Ribeiro, C. B. Shoemaker, A. Warburg, M. Soares, and R. G. Titus. 1999. Variation in the salivary peptide, maxadilan, from species in the *Lutzomyia longipalpis* complex. *Insect Mol. Biol. 8:267.*

18. Shankar, A. H., and R. G. Titus. 1995. T cell and non-T cell compartments can independently determine resistance to *Leishmania major. J. Exp. Med. 181:845.*

19. Hall, L. R., and R. G. Titus. 1995. Sand fly vector saliva selectively modulates macrophage functions that inhibit killing of *Leishmania major* and nitric oxide production. *J. Immunol. 155:3501.*

20. DeKrey, G. K., and R. G. Titus. 1999. A method for the isolation and analysis of leucocytic cells from leishmanial ear lesions in mice. *J. Immunol. Methods 228:1.*

21. Theodos, C. M., J. M. Ribeiro, and R. G. Titus. 1991. Analysis of the enhancing effect of sand fly saliva on infection with *Leishmania* in mice. *Infect. Immun. 59:1592.*

22. Lerner, E. A., J. M. Ribeiro, R. J. Nelson, and M. R. Lerner. 1991. Isolation of Maxadilan, a potent vasodilatory peptide from the salivary glands of the sand fly *Lutzomyia longipalpis. J. Biol. Chem. 266:11234.*

23. Shioda, S. 2000. Pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors in the brain. *Kaibogaku Zasshi. 75:487.*

24. Leceta, J., R. P. Gomariz, C. Martinez, C. Abad, D. Ganea, and M. Delgado. 2000. Receptors and transcriptional factors involved in the anti-inflammatory activity of VIP and PACAP. *Ann. NY Acad. Sci. 921:92.*

25. Vaudry, D., B. J. Gonzalez, M. Basille, L. Yon, A. Fournier, and H. Vaudry. 2000. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev. 52:269.*

26. Lundberg, P., I. Lundgren, H. Mukohyama, P. P. Lehenkari, M. A. Horton, and U. H. Lerner. 2001. Vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide receptor subtypes in mouse calvarial osteoblasts: presence of VIP-2 receptors and differentiation-induced expression of VIP-1 receptors. *Endocrinology 142:339.*

27. Launois, P., F. Tacchini-Cottier, C. Parra-Lopez, and J. A. Louis. 1998. Cytokines in parasitic diseases: the example of cutaneous leishmaniasis. *Int. Rev. Immunol. 17:157.*

28. Qureshi, A. A., A. Asahina, M. Ohnuma, M. Tajima, R. D. Granstein, and E. A. Lerner. 1996. Immunomodulatory properties of maxadilan, the vasodilator peptide from sand fly salivary gland extracts. *Am. J. Trop. Med. Hyg. 54:665.*

29. Wikel, S. K., and Bergman, D. 1997. Tick-host immunology: significant advances and challenging opportunities. *Parasitol. Today 13:383.*

30. Nazario, S., S. Das, A. M. de Silva, K. Deponte, N. Marcantonio, J. F. Anderson, D. Fish, E. Fikrig, and F. S. Kantor. 1998. Prevention of *Borrelia burgdorferi* transmission in guinea pigs by tick immunity. *Am. J. Trop. Med. Hyg. 58:780.*

31. Feinsod, F. M., A. Spielman, and G. L. Waner. 1975. Neutralization of Sindbus virus by antisera to antigens of vector mosquitoes. *Am. J. Trop. Med. Hyg. 24:533.*

32. Alger, N. E., J. A. Harant, L. C. Willis, and G. M. Jorgensen. 1972. Sporozoite and normal salivary gland induced immunity in malaria. *Nature 238:341.*

33. Alger, N. E., and J. Harant. 1976. *Plasmodium berghei*: sporozoite challenge, protection and hypersensitivity in mice. *Exp. Parasitol. 40:273.*

34. Kamhawi, S., Y. Belkaid, G. Modi, E. Rowton, and D. Sacks. 2000. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science 290:1351.*

35. Grimaldi, G., and R. B. Tesh. 1993. *Leishmania*ses of the New World: current concepts and implications for future research. *Clin. Micro. Rev. 6:230.*

36. Warburg, A., E. Saraiva, G. C. Lanzaro, R. G. Titus, and F. Neva. 1994. Saliva of *Lutzomyia longipalpis* sibling species differs in its composition and capacity to enhance leishmaniasis. *Philos. Trans. R. Soc. London 345:223.*

37. Katz, O., J. N. Waitumbi, R. Zer, and A. Warburg. 2000. Adenosine, AMP, and protein phosphatase activity in sandfly saliva. *Am. J. Trop. Med. Hyg. 62:145.*