doi: 10.1111/j.1365-3083.2007.01964.x

Role of Sand Fly Saliva in Human and Experimental Leishmaniasis: Current Insights

B. B. Andrade*, C. I. de Oliveira*,†, C. I. Brodskyn*,†, A. Barral*,† & M. Barral-Netto*,†

*Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, and Universidade Federal da Bahia, Bahia, Brazil; and †Instituto de Investigação em Imunologia, Instituto do Milênio, Salvador, Brazil

Received 10 April 2007; Accepted in revised form 6 May 2007

Correspondence to: Dr Manoel Barral-Netto, Centro de Pesquisas Gonçalo Moniz (FIOCRUZ), Rua Waldemar Falcão, 121, Candeal, CEP: 40295-001 Salvador, Bahia, Brazil. E-mail: mbarral@cpqgm.fiocruz.br.

Abstract

Leishmaniases are wide spread diseases transmitted to their vertebrate host by infected sand fly. The saliva from these arthropods contains a vast repertoire of pharmacologically active molecules that hampers the host's haemostatic, inflammatory and immune responses. The early interactions between *Leishmania* and the host's immune response are closely linked to disease evolution or protection against the protozoan, and the ectoparasite saliva contributes directly to these interactions. Current studies have depicted these features, and these relations are being widely explored. There are concrete indications that the host response against sand fly saliva influences disease outcome in leishmaniasis. Additionally, there are demonstrations that immunization with whole sand fly saliva, or its components, leads to protection against leishmaniasis in different host species. The combination of these evidences opens up optimistic perspectives for improving vaccine development against *Leishmania* infection.

Introduction

*Leishmania*ses are a major public health problem in vast areas of the World, with a huge impact on the economy of developing countries. These infirmities are transmitted by female sand flies during blood repast, needed for egg maturation. For blood meal obtainment, sand flies locate blood by introducing their mouthparts into the skin, tearing tissues, lacerating capillaries and creating haemorrhagic pools upon which they feed [1]. During this process, the salivary gland content is injected together with *Leishmania* promastigotes into the host's skin [2]. Vector's saliva is a potent pharmacologically active fluid that directly affects the haemostatic, inflammatory and immune responses of vertebrate hosts. These actions are indispensable for a successful blood meal. The modifications on host physiology caused by pharmacologically active molecules may favour the transmission of *Leishmania* parasites that colonize the digestive tract of the sand fly. Nevertheless, sand flies are not regarded simply as tools for pathogen delivery. Advances in biomedical research focused on the role of their saliva in the transmission of leishmaniasis have shown the presence of a co-evolutionary relationship between these vectors and the pathogen they transmit. Recently, the role of sand fly salivary components in the establishment of *Leishmania* infection has became clearer, opening new perspectives for disease control. In this review, we will shortly discuss about available scientific evidence on the involvement of the sand fly saliva in human leishmaniasis. This review also aims to explore the animal models current available to study the biological problem.

The complexity of salivary components repertoire

Attempting to probe and feed, sand flies must first circumvent the host's haemostatic system, composed of blood-coagulation cascade, vasoconstriction, fibrinolysis and platelet aggregation. Besides haemostasis, sand flies must also evade the host's innate and acquired immune responses. To overcome these obstacles, sand flies evolved within their salivary secretion an array of potent pharmacological components, such as anticoagulants [3], anti-platelet [4], vasodilators [5] and, importantly, immunomodulator and anti-inflammatory molecules [6]. These mediators with redundant and synergic activities incite a favourable microenvironment for adequate blood-feeding and may also be important for parasite establishment (see Fig. 1 for review). Thus, characterization of salivary components has become necessary for a better understanding of disease pathogenesis and for the development of new strategies to block pathogen transmission. High-throughput analyses have characterized and isolated a number of molecules responsible for these effects on host

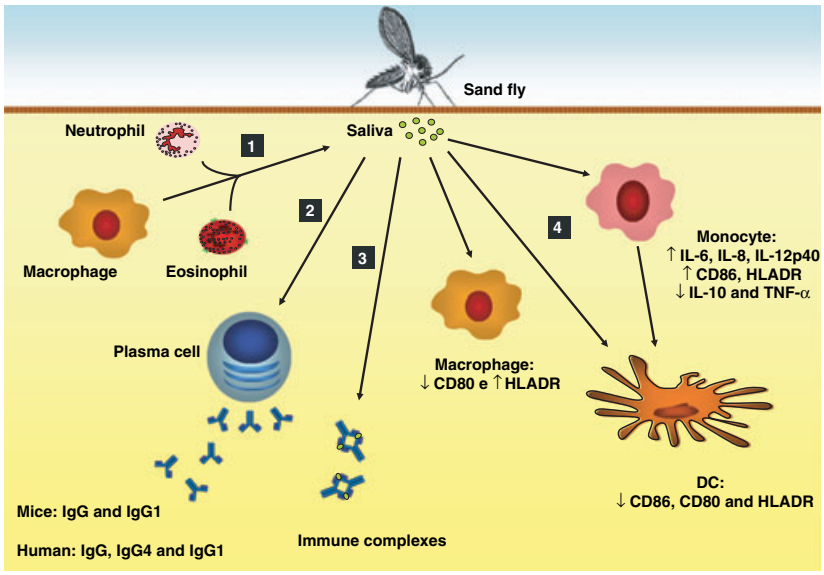

Figure 1 Effects of *Lutzomyia longipalpis* saliva on vertebrate host. When the sand fly saliva is injected into host skin, it induces an inflammatory cell infiltration (1) [26], and antibody production (2) [9, 10, 20]. In this scenario, immune complexes are formed [20] at early phases of exposure. Moreover, sand fly saliva also modulates co-stimulatory molecules and cytokine release by antigen presenting cells (4) [11].

homoeostasis and explored the possibility to neutralize them [7, 8]. Some of these molecules and their actions are illustrated in other papers [6, 9, 10].

Role of sand fly saliva in human leishmaniasis

Studies in endemic areas of leishmaniasis conducted by our group suggest that natural exposure to non-infected sand fly bites can influence the epidemiology of this disease [11, 12]. Previous epidemiological observations suggested that travellers from non-endemic areas, whom were not previously exposed to sand flies or *Leishmania*, displayed a higher risk of presenting severe clinical forms of leishmaniasis. More recently, immunological studies developed in an area endemic for visceral leishmaniasis (VL) evidenced the presence of IgG antibodies against saliva from *Lutzomyia (Lu.) longipalpis*, the vector of VL, in resident children [11]. Furthermore, there was a simultaneous appearance of an anti-saliva humoral response and an anti-*L. chagasi* cell-mediated immunity [12] favouring the hypothesis that induction of immune response against sand fly saliva can facilitate the generation of a protective response against human leishmaniasis. Thus, we can infer that anti-sand fly saliva antibodies can serve as an important epidemiological marker of vector exposure in endemic areas and even as a surrogate marker of protection. Therefore, individuals residing in VL endemic areas that display a positive delayed-type hypersensitivity (DTH) skin test to *Leishmania* antigens also develop anti-saliva IgG and may be protected against leishmaniasis. On the other hand, other individuals from the same area that present low levels of specific humoral response against vector saliva and a negative DTH skin test are not protected.

In an attempt to characterize the immunological patterns following sand fly saliva exposure, we have utilized an *in vivo* bite model in which human subjects from a non-endemic area of leishmaniasis were exposed to the laboratory-reared *Lu. longipalpis.* Immunological analysis focused on the host anti-saliva antibody production kinetics revealed high levels of anti-saliva IgG1, IgG4 and IgE antibodies (V. Vinhas, M. Barral-Netto, unpublished results). This investigation also evidenced two major patterns of clinical and serological responses to sand fly saliva. Volunteers who developed intense skin reactions with indurated nodules and a DTH-like response after exposure to bites displayed higher IgG/IgE ratio than those who evolved mild erythematous reactions. Thus, it is possible that host response to sand fly saliva may present some degree of variation regarding genetic variations that could influence indirectly *Leishmania* establishment.

To assess whether individuals exposed to sand flies were able to recognize salivary proteins, we performed Western blot analysis using serum from residents of endemic areas or volunteers from a non-endemic area of VL. The volunteers from a non-endemic area displayed no previous history of clinical leishmaniasis and, at the time of initial investigations, were negative for *Lu. longipalpis* saliva exposure as measured by an absence of antibodies against SGS. Total IgG from both groups of volunteers was able to detect several salivary proteins with molecular weights close to 45, 44, 43, 35, 27 and 16 kDa (Fig. 2). These data point out the molecules from *Lu. longipalpis* saliva that are most strongly immunogenic

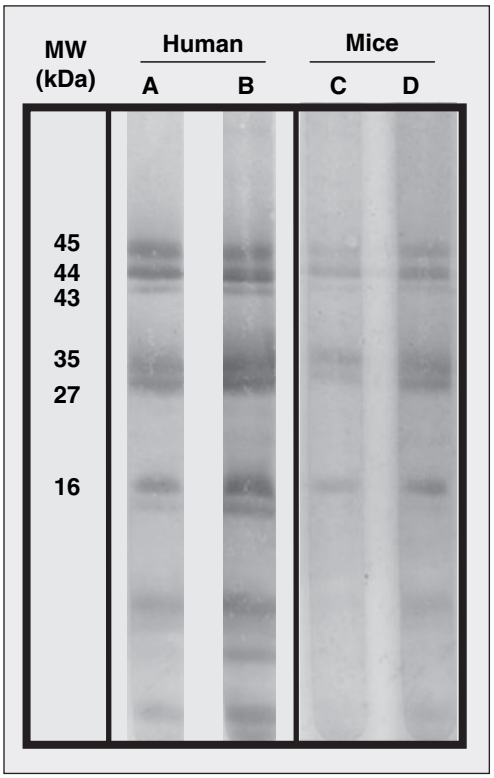

Figure 2 Serum from human and mice pre-exposed to sand fly bites recognize salivary proteins identified by Western blot. Volunteers from a non-endemic area for leishmaniasis, when experimentally exposed to *Lu. longipalpis* bites (A) displayed a protein band recognition pattern similar to children from an endemic area for leishmaniasis (B). Related bands are also identified by serum samples from BALB/*c* (C) and C57BL/6 (D) mice pre-exposed to *Lu. longipalpis* bites.

to man and that, as such, could serve as possible targets for future approaches in anti-*Leishmania* vaccine development. Nevertheless, a note of caution is needed for their use as vaccine candidates, as we have no information on the type of response these proteins elicit when used as a single product, nor on their capacity to favour a protective anti-*Leishmania* reaction. Although tempting, it remains to be demonstrated that protection against *Leishmania* infections is conferred by pre-exposure to sand fly bites in endemic areas for leishmaniasis.

Sand fly saliva also seems to exert a direct effect on human antigen presenting cells. *Lu. longipalpis* salivary gland sonicate (SGS) inhibited IL-10 and TNF-α production but induced IL-6, IL-8 and IL-12p40 production by LPS-stimulated monocytes and dendritic cells [13]. Besides cytokine production, sand fly saliva also interfered with the expression of co-stimulatory molecules in macrophages (reduced CD80 and increased HLA-DR expression) and in monocytes (increased CD80 and HLA-DR expression). During dendritic cell differentiation induced by CD40L, a slight reduction in CD80, CD86, HLA-DR and CD1a expression was also observed [13]. Moreover, these saliva effects on human immune cells were abrogated by *in vitro* incubation with anti-SGS antibodies [13], suggesting the importance of a specific humoral response for the neutralization of sand fly saliva deleterious effects on human immune system.

Role of sand fly saliva in experimental models of leishmaniasis

Most of the knowledge concerning resistance and susceptibility to *Leishmania* infection was built based on experimental models. Titus and Ribeiro [2] first demonstrated that saliva from *Lu. longipalpis* enhanced *L. major* infection when the parasite was co-inoculated with the sand fly SGS in mice. In addition to enhancing lesion size, sand fly saliva also markedly raised the parasite burden within the lesions. Similar findings were further reported with other *Leishmania* species [14–17]. Maxadilan, a 6.5 kDa peptide from *Lu. longipalpis* saliva, is a potent salivary vasodilator and it also displays important immunomodulatory properties [5]. This peptide exacerbated lesion size in *L. major* infected mice and parasite burden within the lesions to the same degree as sand fly SGS [18]. Moreover, despite the absence of Maxadilan, *Phlebotomus (P.) papatasi* SGS co-inoculated with *L. major* into mice footpads [19] or the ear dermis [20] induced a dramatic lesion exacerbation, probably mediated by IL-4 production [19].

In murine models of Leishmaniasis, resistance and protection are associated with the expression of IFN-γ and IL-12 driving a $CD4^+$ Th1 response, while susceptibility is linked to production of IL-4 and the development of a $CD4^+$ Th2 response [21]. *Lu. longipalpis* saliva seems to drive, by an unknown mechanism, the host immune response to a Th2 type, less effective in terms of parasite clearance. Despite their immunomodulatory features, sand fly salivary components seem to display an immunogenic activity, eliciting antibody production and cell mediated immunity by the host. Nevertheless, the specific role of antibodies against sand fly saliva in the outcome of *Leishmania* infection is not fully described. Experimental studies, together with clinical data, suggest a potential transmission blocking mechanism. Similarly to clinical studies observations, high levels of anti-saliva IgG are produced by mice exposed to sand fly bites or inoculated with SGS [22]. BALB/*c* mice repeatedly exposed to *Lu. longipalpis* bites produced high levels of anti-saliva antibodies, with a predominance of IgG1 subclass [20]. Total serum IgG from these animals recognized predominantly bands of 45, 44 and a 16 kDa, displaying some degree of similarity between murine and human anti-saliva humoral responses (Fig. 2). These proteins were also the major targets of human antibody response in an endemic area [11, 12]. As these proteins are widely recognized, they are natural candidates to be used as markers

of exposure to *Lu. longipalpis* bites. Besides antibody production, an intense cellular infiltrate comprised by neutrophils, eosinophils and macrophages at the site of sand fly bites was noted [20]. Additionally, the injection of immune serum previously incubated with saliva induced an early infiltration with neutrophils and macrophages, suggesting the participation of immune complexes in triggering inflammation [22]. Moreover, pre-exposure to *P. papatasi* SGS [20] or immunization with SP15, a protein present in *P. papatasi* saliva [10], blocked the establishment of *L. major* infection in mice. Later it was shown that such protection could also be obtained upon exposure of mice to the bites of uninfected sand flies [23]. Compared with naïve mice, mice pre-exposed to the bites of uninfected flies showed reduction in lesion pathology, in parasite load, and also in their ability to transmit *Leishmania* to uninfected flies [23]. In these cases, the protection conferred by immunization with SP15 or by pre-exposure sand fly bites was also associated with the development of a strong DTH response [23]. However, the host humoral response may also influence the vector feeding behaviour as *P. argentipes* sand flies, when fed on hamsters previously exposed to bites, displayed inadequate blood repast and increased mortality rates, which were proportional to anti-saliva antibody titres present therein [24].

A major illustration of the sand fly saliva ability to induce cell mediated immune response is the development of a DTH-like reaction, characterized by cellular infiltration at the bite site [25]. The DTH-like reaction induced by sand fly saliva could primarily represent a survival strategy, as inflamed sites display larger blood flow than the normal skin, which may facilitate arthropod blood feeding [25]. Furthermore, cellular recruitment elicited by sand fly saliva may be an important event for establishment of *Leishmania*, favouring parasite growth inside host immune cells. In this sense, experimental studies brought new insights in chemotaxis stimulated by exposure to sand fly saliva. Saliva from *P. duboscqi* attracts vertebrate monocytes *in vitro* [26] and saliva from *P. papatasi* not only attracts macrophages but also enhances infection of these cells by *L. donovani*, resulting in increased parasite loads [27]. Interestingly, *Lu. longipalpis* saliva rapidly induces CCL2/MCP-1 expression and macrophage recruitment to the inoculation site in the air pouch model of inflammation in BALB/*c* mice [28]. The presence of macrophages in a microenvironment which also contains salivary immunomodulatory factors may favour *Leishmania* infection if such host cells are not adequately activated. As expected, the effect of *Lu. longipalpis* saliva on macrophage recruitment is abrogated when SGS is pre-incubated with anti-SGS antibodies, obtained from mice previously exposed to sand fly bites [28]. Injection of *Lu. longipalpis* SGS and *L. major* into the mouse peritoneal cavity leads to an inflammatory reaction characterized by neutrophils, eosinophils and T $CD4^{+}CD45RB^{low}$ (effector or memory cells) migration, with considerably more IL-10 production than IFN-g [29]. This reinforces the idea that sand fly saliva preferentially induces a cytokine inhibition and deviates an adequate anti-*Leishmania* immune response. Another relevant aspect concerning an anti-saliva DTH reaction development is the protection against *Leishmania* observed in animals that display this skin reaction after repeated exposures to sand fly bites. Thus, although the development of an anti-saliva DTH reaction facilitates the vector's blood meal, it could also render a disadvantageous microenvironment for parasite establishment by inducing early important events that may influence host-immune response against Leishmania.

Most investigations regarding the effects of sand fly saliva on *Leishmania* infection have been conducted with *Lu. longipalpis*, the natural vector of *L. chagasi*, and *P. papatasi*, the natural vector of *L. major*. *L. braziliensis*, however, is known to cause highly severe disease in human beings but has an insidious growth in mice. Taking advantage of a reliable murine model for intradermal infection with *L. braziliensis* developed by us [30], we engaged in evaluating the effects of immunization with *Lu. intermedia* saliva using this model, this sand fly being the natural vector of *L. braziliensis*. We observed that mice immunized with *Lu. intermedia* SGS followed by a challenge infection with *L. braziliensis* + *Lu. intermedia* SGS are not protected against infection (Fig. 3), differently from previous reports employing *P. papatasi* saliva and *L. major* [20, 23]. In fact, although lesion development is not significantly higher in *Lu. intermedia* saliva-immunized mice, disease persistence is longer as evidenced by the prolonged period before lesion healing. In control mice, on the contrary, lesions peaked at

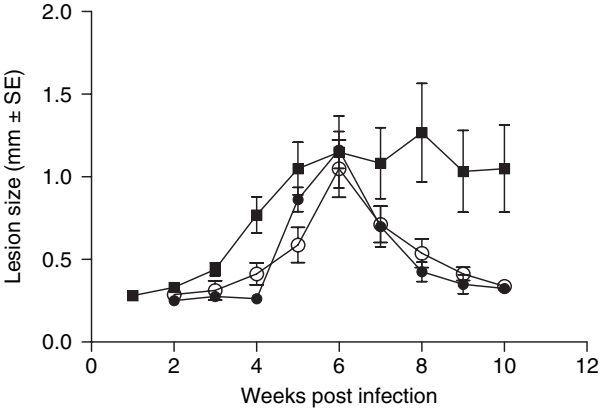

Figure 3 Lesion development in SGS immunized mice following infection with *L. braziliensis* + SGS. BALB/*c* mice (3–5 per group) were inoculated three times in the right ear with *Lu. intermedia* SGS (■), *Lu. longipalpis* SGS (•) or PBS (○) and were challenged in the left ear with *L. braziliensis* plus *Lu. intermedia* SGS. The course of lesion development was monitored weekly and bars represent the mean and standard errors of the mean from three independent experiments.

6 weeks post-infection and were completely healed by 10 weeks post-infection. Using this model, we also evaluated the effect that immunization with *Lu. longipalpis* saliva immunization had on this type of challenge. Interestingly, mice immunized with *Lu. longipalpis* saliva showed a course of disease similar to that found in control mice (Fig. 3), indicating that immunization with *Lu. longipaplpis* saliva does not lead to protection following challenge with *L. braziliensis* + *Lu. intermedia* saliva. In this case, however, we did not observe any disease exacerbation. It should be noted that both *Lu. intermedia* saliva (T. de Moura, C.I. de Oliveira, unpublished results) and *Lu. longipalpis* saliva [15] have the ability to exacerbate *L. braziliensis* infection. However, as reported here, none can cause protection in immunized mice when these are challenged with *L. braziliensis* + *Lu. intermedia* saliva, the parasite/sand fly system that people living in endemic areas are exposed to. It remains to be determined whether immunization with *Lu. longipalpis* saliva is able to protect against challenge with *L. braziliensis* + *Lu. longipalpis* SGS.

Concluding remarks

Sand flies use its saliva to manipulate host homoeostasis favouring the blood meal. These disturbances in host physiology by active pharmacological molecules may favour the delivery of *Leishmania* parasites, as the key for the success of *Leishmania* parasitism is the capacity of evade host immune responses. Analyses of these salivary components revealed a significant biochemical and pharmacological diversity. Over the last 10 years, the isolation of specific molecules through experimental techniques has contributed to a better understanding of pathogen–vector–host interactions. Although many aspects have been described, important issues remain to be understood. Variation in salivary content has been described within the same sand fly species in different geographical regions. An expanded effort for studying salivary content of species from different parts of the World will certainly increases the chances of finding common molecules that could function as markers or as vaccines candidate. The appearance of novel molecules on sand fly saliva can reveal strategies in avoiding host-defence mechanism. This natural diversity of substances can serve in therapeutics and biomedical research but caution is necessary as salivary products have diverse behaviour in distinct models of inflammation or immune response. Understanding such variation, as well as testing the same molecule in several models, is important for unravelling differences in their composition and molecular interactions with potential practical applications. There is also a need to expand our understanding of host protective mechanisms as a successful immune response widely depends on the first attempt of host's innate response to contain infection.

Sand fly saliva components could also be used as a combined vaccine with *Leishmania* antigens to enhance protection. Moreover, as sand flies feed on many other animals besides humans, immunological approaches that target specific saliva components may enable protection against leishmaniasis in different vertebrate species, as in the case of canine VL. Some effects of single salivary proteins on vertebrate host-immune system have already been demonstrated. However, present results indicate that different molecules seem to mediate protection in diverse species, suggesting heterogeneity of sand fly saliva influence in different hosts. Furthermore, in endemic areas, there is the possibility that ongoing immune responses against other vectors can interfere with host immune responses against sand fly saliva, and indirectly against *Leishmania* masking some results described. Despite optimistic suggestions from these studies, we must be more cautious in data interpretation to better describe the real sand fly saliva effect on leishmaniasis. Some issues about future challenges regarding sand fly saliva are summarized in Table 1.

The ultimate purpose of research that examines pathogens transmitted by arthropods is to develop an effective vaccine. However, it has proven very difficult to efficiently develop long lasting host sterile immunity and vaccines against vector-borne pathogens. These organisms often present very complex life cycles and vaccines that target more than one facet of parasite's life cycle, such as the pathogen itself, vector salivary proteins and vector–pathogen interactions, may prove to be more effective.

Table 1 Prospects regarding sand fly saliva.

Isolation of novel salivary molecules by high-throughput genomic and proteomic approaches facilitating the discovery of new molecules for vaccination.
Classification of salivary content from species worldwide targeting common molecules from sibling species for a wide-range vaccine.
Comprehension of protective mechanisms regarding the initial steps of host's response to salivary molecules that can direct to resistance or susceptibility to *Leishmania.*
Assessment of candidate salivary molecules in different models for enlightening differences and/or similarities within components important for pathogen establishment.
Development of vaccines that aim features of pathogens and salivary molecules concurrently.
Evaluation of interference from responses against other vectors in experimental models and endemic areas.

Conflict of interests

There are no commercial or other associations that might pose a conflict of interest.

Acknowledgments

This study was supported by funds from National Institute of Allergy and Infectious Diseases (NIAID) – National Institutes of Health (NIH), Conselho Nacional de Desenvolvimento Científico e Tecnológico- Brazil (CNPq), Fundação de Amparo à Pesquisa do Estado da Bahia-Brazil (FAPESB), and FIOCRUZ. C.I. Oliveira, C. Brodskyn, M. Barral-Netto and A. Barral are investigators from CNPq.

References

1 Ribeiro JM. Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infect Agents Dis* 1995;4:143–52.
2 Titus RG, Ribeiro JM. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. *Science* 1988;239:1306–8.
3 Charlab R, Valenzuela JG, Rowton ED, Ribeiro JM. Toward an understanding of the biochemical and pharmacological complexity of the saliva of a hematophagous sand fly *Lutzomyia longipalpis*. *Proc Natl Acad Sci U S A* 1999;96:15155–60.
4 Ribeiro JM, Katz O, Pannell LK, Waitumbi J, Warburg A. Salivary glands of the sand fly *Phlebotomus papatasi* contain pharmacologically active amounts of adenosine and 5′-AMP. *J Exp Biol* 1999;202:1551–9.
5 Lerner EA, Ribeiro JM, Nelson RJ, Lerner MR. Isolation of maxadilan, a potent vasodilatory peptide from the salivary glands of the sand fly *Lutzomyia longipalpis*. *J Biol Chem* 1991;266:11234–6.
6 Kamhawi S. The biological and immunomodulatory properties of sand fly saliva and its role in the establishment of *Leishmania* infections. *Microbes Infect* 2000;2:1765–73.
7 Anderson JM, Oliveira F, Kamhawi S *et al*. Comparative salivary gland transcriptomics of sandfly vectors of visceral leishmaniasis. *BMC Genomics* 2006;7:52.
8 Oliveira F, Kamhawi S, Seitz AE *et al*. From transcriptome to immunome: identification of DTH inducing proteins from a *Phlebotomus ariasi* salivary gland cDNA library. *Vaccine* 2006;24:374–90.
9 Andrade BB, Teixeira CR, Barral A, Barral-Netto M. Haematophagous arthropod saliva and host defense system: a tale of tear and blood. *An Acad Bras Cienc* 2005;77:665–93.
10 Valenzuela JG, Belkaid Y, Garfield MK *et al*. Toward a defined anti-*Leishmania* vaccine targeting vector antigens: characterization of a protective salivary protein. *J Exp Med* 2001;194:331–42.
11 Barral A, Honda E, Caldas A *et al*. Human immune response to sand fly salivary gland antigens: a useful epidemiological marker? *Am J Trop Med Hyg* 2000;62:740–5.
12 Gomes RB, Brodskyn C, de Oliveira CI *et al*. Seroconversion against *Lutzomyia longipalpis* saliva concurrent with the development of anti-*Leishmania chagasi* delayed-type hypersensitivity. *J Infect Dis* 2002; 186:1530–4.
13 Costa DJ, Favali C, Clarencio J *et al*. *Lutzomyia longipalpis* salivary gland homogenate impairs cytokine production and costimulatory molecule expression on human monocytes and dendritic cells. *Infect Immun* 2004;72:1298–305.
14 Lima HC, Titus RG. Effects of sand fly vector saliva on development of cutaneous lesions and the immune response to *Leishmania braziliensis* in BALB/c mice. *Infect Immun* 1996;64:5442–5.
15 Samuelson J, Lerner E, Tesh R, Titus R. A mouse model of *Leishmania braziliensis braziliensis* infection produced by coinjection with sand fly saliva. *J Exp Med* 1991;173:49–54.
16 Theodos CM, Ribeiro JM, Titus RG. Analysis of enhancing effect of sand fly saliva on *Leishmania* infection in mice. *Infect Immun* 1991;59:1592–8.
17 Warburg A, Saraiva E, Lanzaro GC, Titus RG, Neva F. Saliva of *Lutzomyia longipalpis* sibling species differs in its composition and capacity to enhance leishmaniasis. *Philos Trans R Soc Lond B Biol Sci* 1994;345:223–30.
18 Morris RV, Shoemaker CB, David JR, Lanzaro GC, Titus RG. Sandfly maxadilan exacerbates infection with *Leishmania major* and vaccinating against it protects against *L. major* infection. *J Immunol* 2001;167:5226–30.
19 Mbow ML, Bleyenberg JA, Hall LR, Titus RG. *Phlebotomus papatasi* sand fly salivary gland lysate down-regulates a Th1, but up-regulates a Th2, response in mice infected with *Leishmania major*. *J Immunol* 1998;161:5571–7.
20 Belkaid Y, Kamhawi S, Modi G *et al*. 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* 1998;188:1941–53.
21 Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat Rev Immunol* 2002;2:845–58.
22 Silva F, Gomes R, Prates D *et al*. Inflammatory cell infiltration and high antibody production in BALB/c mice caused by natural exposure to *Lutzomyia longipalpis* bites. *Am J Trop Med Hyg* 2005;72: 94–8.
23 Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science* 2000;290:1351–4.
24 Ghosh KN, Mukhopadhyay J. The effect of anti-sandfly saliva antibodies on *Phlebotomus argentipes* and *Leishmania donovani*. *Int J Parasitol* 1998;28:275–81.
25 Belkaid Y, Valenzuela JG, Kamhawi S, Rowton E, Sacks DL, Ribeiro JM. Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: An adaptive response induced by the fly? *Proc Natl Acad Sci U S A* 2000;97:6704–9.
26 Anjili CO, Mbati PA, Mwangi RW *et al*. The chemotactic effect of *Phlebotomus duboscqi* (Diptera: Psychodidae) salivary gland lysates to murine monocytes. *Acta Trop* 1995;60:97–100.
27 Zerpa O, Pratlong F, Ulrich M, Convit J. Isolation of *Leishmania infantum*, zymodeme MON-1 from canine and human visceral leishmaniasis on Margarita Island, Venezuela. *Mem Inst Oswaldo Cruz* 2001;96:901–2.
28 Teixeira CR, Teixeira MJ, Gomes RB *et al*. Saliva from *Lutzomyia longipalpis* induces CC chemokine ligand 2/monocyte chemoattractant protein-1 expression and macrophage recruitment. *J Immunol* 2005;175:8346–53.
29 Monteiro MC, Lima HC, Souza AA, Titus RG, Romao PR, de Queiroz Cunha F. Effect of *Lutzomyia longipalpis* salivary gland extracts on leukocyte migration induced by *Leishmania major*. *Am J Trop Med Hyg* 2007;76:88–94.
30 de Moura TR, Novais FO, Oliveira F *et al*. Toward a novel experimental model of infection to study American cutaneous leishmaniasis caused by *Leishmania braziliensis*. *Infect Immun* 2005;73: 5827–34.