Promastigote secretory gel from natural and unnatural sand fly vectors exacerbate *Leishmania major* and *Leishmania tropica* cutaneous leishmaniasis in mice

E. Giraud1,*, M. Svobodová2, I. Müller3, P. Volf2 and M. E. Rogers4

1Faculty of Infectious Tropical Diseases, Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, UK; 2Department of Parasitology, Faculty of Science, Charles University in Prague, Prague, Czech Republic; 3Faculty of Medicine, Division of Infectious Diseases, Department of Medicine, Section of Immunology at St Mary’s, Imperial College London, London, UK and 4Faculty of Infectious Tropical Diseases, Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK

**Abstract**

*Leishmania* rely heavily on glycans to complete their digenetic life cycle in both mammalian and phlebotomine sand fly hosts. *Leishmania* promastigotes secrete a proteophosphoglycan-rich gel (Promastigote Secretory Gel, PSG) that is regurgitated during transmission and can exacerbate infection in the skin. Here we explored the role of PSG from natural *Leishmania*-sand fly vector combinations by obtaining PSG from *Leishmania (L.) major*-infected *Phlebotomus (P.) papatasii* and *P. duboscqi* and *L. tropica*-infected *P. arabicus*. We found that, in addition to the vector’s saliva, the PSG from *L. major* and *L. tropica* potently exacerbated cutaneous infection in BALB/c mice, improved the probability of developing a patent cutaneous lesion, parasite growth and the evolution of the lesion. Of note, the presence of PSG in the inoculum more than halved the prepatent period of cutaneous *L. tropica* infection from an average of 32 weeks to 13 weeks. In addition, *L. major* and *L. tropica* PSG extracted from the permissive experimental vector, *Lutzomyia (Lu.) longipalpis*, also exacerbated infections in mice. These results reinforce and extend the hypothesis that PSG is an important and evolutionarily conserved component of *Leishmania* infection that can be used to facilitate experimental infection for drug and vaccine screening.

**Introduction**

*Leishmania* are parasitic protozoa, which are transmitted by the bite of a female phlebotomine sand fly. Depending on the species of parasite, *Leishmania* can cause a spectrum of diseases, ranging from painful and disfiguring skin lesions (cutaneous leishmaniasis, CL, and mucocutaneous leishmaniasis) to fatal visceral infection of the spleen and liver (visceral leishmaniasis). In the mammalian host, *Leishmania* parasites reside within professional phagocytic cells of the monocyte-macrophage lineage as amastigote forms. When they are taken up by a sand fly they multiply and undergo a series of transformations as various flagellated promastigote forms; culminating in the differentiation to a mammal-infective stage, the metacyclic promastigote (reviewed in Bates, 1997).

It is becoming increasingly clear that transmission by sand fly bite has considerable bearing on the infection and is vastly more efficient than needle-based infection of parasites alone (reviewed by Serafim et al., 2017). From a sand fly bite metacyclic promastigotes are delivered into the skin of a host in the context of a unique inflammatory environment (Belkaid et al., 1998; Kamhawi et al., 2000). This is generated by the complex combination of parasite dose (Kimblin et al., 2008), dose composition (i.e. proportion of metacyclic and non-metacyclic promastigotes) (Giraud et al., 2019), damage caused by the bite (Giraud et al., 2018), sand fly-associated bacteria (Dey et al., 2018; Giraud et al., 2019) and the introduction of the vector saliva and other parasite-released molecules (Titus and Ribeiro, 1988; Belkaid et al., 1998; Rogers et al., 2004; Vollova et al., 2008; Atayde et al., 2015).

In the sand fly, *Leishmania* secrete high molecular weight proteophosphoglycans (filamentous proteophosphoglycan, fPPG; secreted acid phosphatase and a number of poorly defined lower molecular weight proteophosphoglycans (Montgomery et al., 2002), which condense in the limited volume of the sand fly anterior midgut to form a gel-like obstruction, termed the Promastigote Secretory Gel (PSG) (Ilg et al., 1996; Stierhof et al., 1999; Rogers et al., 2002, 2004). PSG, has been noted for many natural and experimental *Leishmania*-sand fly combinations (reviewed by Rogers, 2012) and the blocked fly phenotype has been hypothesized to contribute to the transmission of leishmaniasis by encouraging the regurgitation of parasites (Shortt and Swaminath, 1928). The role of PSG was first demonstrated in 2004 using the experimental, yet highly transmissible, combination of *Leishmania mexicana* and *Lutzomyia longipalpis* (Rogers et al., 2004). Blocked sand flies were shown to regurgitate an average of 1000 parasites and a proportion of the PSG during transmission. Co-inoculation of this dose of *L. mexicana* metacyclic promastigotes with PSG promoted the survival and
growth of amastigotes, which strongly aggravated skin infections in mice (Rogers et al., 2004, 2009). Using this sand fly in a natural parasite-vector combination, PSG was also shown to exacerbate cutaneous and visceral infection of *Leishmania infantum* in mice (Rogers et al., 2010).

As it appears that PSG can manipulate both the sand fly and mammalian hosts for efficient transmission, we aimed to see if this was relevant to other natural models of leishmaniasis. Therefore, we recovered, characterized and tested the infection-modulating properties of PSG from a variety of *Leishmania*-sand fly combinations responsible for CL in the field.

**Materials and methods**

**Infection of sand flies**

*Leishmania major* strain FV39 clone 5 (RHO/SU/S9/P) and *Leishmania tropica* from Sanlurfa, Turkey (MHOM/1999/TR/SU23) were originally isolated from human cutaneous lesions and have been routinely passaged through mice. Promastigotes of these parasites were cultured as previously described (Rogers et al., 2002), resulting in an enriched population of metacyclic promastigotes as determined by morphological analysis of Giemsa-stained parasites. Briefly, 1 × 10^6* BALB/c lesion amastigotes per ml were transformed into promastigotes in M199 culture medium (Hank’s modified with L-glutamine) supplemented with 10% heat-inactivated foetal calf serum (FCS, v/v), 1 × Basal Medium Eagle vitamins (v/v), 1% penicillin-streptomycin (v/v), 400 mEq sodium bicarbonate (all Sigma UK), pH 7.2, at 26 °C for 3 days. Following this, promastigotes were sub-passaged to 5 × 10^5 ml in fresh M199 promastigote medium and allowed to undergo metacyclogenesis by 10–13 days post-culture. The cultures were assessed daily to select those with the highest proportion of metacyclic promastigotes. Using this culture method typically yielded 80–85% metacyclic promastigotes. Five-day-old female sand flies were infected with amastigotes through an artificial membrane feeding system at 2 × 10^6 amastigotes per ml in fresh rabbit blood and maintained in a 12 h light:dark cycle at 28 °C, 80% relative humidity with free access to 12.5% sucrose.

**Preparation of vector saliva and parasite PSG**

Sand fly saliva was obtained from 6–8 day-old female uninfected flies by piercing individual salivary glands in ice-cold PBS, followed by centrifugation at 3000 rpm for 10 min to remove any salivary gland epithelia or debris (Rogers et al., 2004). Pools of 10 flies were processed at a time to prevent degradation of the saliva and a minimum of 60 flies processed to supply saliva for the co-infections. For *L. mexicana* and *L. infantum* we have observed that PSG accumulates within the *L. longipalpis* midgut from day 7 onwards (Rogers and Bates, 2007). Therefore, PSG plugs were isolated from the midguts of day 10–13 infected flies in PBS to ensure that all parasites had reached maturity i.e. completed metacyclogenesis. PSG was obtained by dissecting the gut along its length and removing the PSG plug manually using 30 gauge insulin syringe needles. Each PSG plug was transferred to a tube in 5 µL of the dissection medium. Parasites were removed by centrifuging twice at 3000 rpm for 10 min followed by two spins at 7,000 rpm for 10 min. The average yield of PSG for each sand fly-Leishmania combination used is presented in Table 1. Saliva and PSG were filter-sterilized (0.2 µm pore diameter) and stored in aliquots at −40 °C until use. The protein content of PSG and saliva was determined using the BCA method and standardized to 1 µg per injection. As a negative control for the Western Blot, the luminal content of 30 age-matched, bloodfed uninfected flies was sampled by dissecting each gut in the same way as an infected fly then sucking 5 µL of dissection medium near the incision. These were pooled and processed as above.

**Western blotting**

PSG was separated by SDS PAGE using a 10% acrylamide/bis-acrylamide gel (Sigma) with an extended stacking gel (4% acrylamide/bis-acrylamide) as previously described (Rogers et al., 2004). Gels were transferred to activated nitrocellulose and probed with the LT15 (1:3000) monoclonal antibody against phosphoglycan disaccharide repeats [PO_4−-6Galβ1-4Manz1-](Ilg et al., 1996). Blots were developed using a biotinylated anti-mouse IgG secondary antibody and HRP immunoprecipitation (ABC Elite, VIP, Vector Labs).

**Infection of mice**

Anaesthetized, 20 g, 6–8 week old female BALB/c mice were infected either by the intradermal injection of 1 × 10^7* L. major* into the dorsal surface of the footpad, or 1 × 10^6* L. tropica* metacyclic promastigotes into the shaved rump, in a total volume of 20 µL. Under restraint, Vernier callipers were used to measure thickness of infected and uninfected footpads of mice to calculate lesion swelling (*L. major*), or the average diameter of rump lesions to calculate their area (*L. tropica*). At the end of experiments, mice were euthanized allowing parasite burdens to be determined. Burdens were determined by direct counting of amastigotes from footpad homogenates. All procedures involving animals were approved by a local Animal Welfare Committee and performed in accordance with the United Kingdom Government (Home Office), Czech Ministry of Health and EC regulations.

**Results**

*PSG from L. major, L. tropica and L. mexicana bear similar Gal[β1-4]Man(α1)-PO_4 containing glycans*

The backbone of many O/N-linked *Leishmania* oligosaccharides contains phosphate-linked galactose-mannose disaccharide (Gal-Man-P) repeats (Ilg, 2000). Using Western blotting, the PSG from *L. major* and *L. tropica* extracted from the experimental vector *L. longipalpis*, were probed for Gal[β1-4]Man(α1)-PO_4 repeating epitopes (Fig. 1). The absence of a reaction with the contents of uninfected midguts confirmed the specificity of the antibody for parasite glycans. All infected flies yielded PSGs containing a very high molecular weight smear retained in the 4% stacking gel that is likely to be the fPPG fraction. This gives the PSG much of its structure as a 3D matrix (Stierhoff et al., 1999) and confers the disease-exacerbating properties of PSG from its natural vectors, *Phlebotomus duboscqi*, exacerbates cutaneous infections (Gal-Man-P) repeats (Ilg, 2000). Using Western blotting, the PSG from *L. major* and *L. tropica* extracted from the experimental vector *L. longipalpis*, the amount of fPPG was similar for *L. mexicana* and *L. major*, which were greater than the amount of fPPG produced by *L. tropica*. Notably, this is an experimental model so the precise amounts of fPPG in PSG from natural vectors may vary lipophosphoglycan (LPG) also contains Gal-Man-P repeats, however, based on the expected molecular weight (30–50 kDa for non-metacyclic and 70–90 kDa for metacyclic promastigotes), LPG was not detected in any of the PSG preparations.

**L. major PSG from its natural vectors, *Phlebotomus (Phlebotomus) papatasi* and *Phlebotomus (Phlebotomus) dubosuci* exacerbates cutaneous infections in vivo**

Inclusion of sterile *L. major* PSG from the natural vectors, *P. papatasi* or *P. dubosuci* promoted *L. major* infection in the skin of BALB/c mice (Fig. 2). The presence of *L. major* PSG during
infection resulted in more mice developing a lesion earlier than the controls [10 vs 25 days for infections with L. major PSG from P. papatasi (Fig. 2A and C) and 14 vs 28 days for infections with L. major PSG from P. duboscqi (Fig. 2B and D)]. These infections grew faster than the controls, resulting in significantly larger lesions, which persisted (Fig. 2C and D). The final parasite burdens for these lesions showed that PSG benefited L. major growth, resulting in an average 30-fold higher number amastigotes per lesion compared to saline with metacyclics alone [10-fold for lesions with L. major PSG from P. papatasi (Fig. 2E), and 50-fold for L. major lesions co-inoculated with PSG from P. duboscqi (Fig. 2F)]. Similarly, the saliva from these two natural sand fly vectors significantly exacerbated infection with L. major, resulting in an average 45- to 60-fold higher amastigote burden at the end of the experiment.

**L. tropica PSG from its natural vector, Phlebotomus (Adlerius) arabicus, exacerbates cutaneous infection in vivo**

The infections with L. tropica significantly benefited from the presence of PSG, recovered from its natural vector, P. arabicus (Fig. 3). Even at a high dose of metacyclic promastigotes (1 × 10⁶), infection of mice with L. tropica in the absence of vector components took 30–33 weeks to generate cutaneous lesions (Fig. 3A and B). The presence of PSG cut this time by 60%, allowing the detection of lesions on the rumps of BALB/c mice by 13 weeks post-infection. The addition of PSG or saliva resulted in lesions that were chronic and non-healing, compared to the controls (Fig. 3B). Lesions from L. tropica-PSG co-infections grew significantly faster than the controls or those co-inoculated with P. arabicus saliva, resulting in an average 22-fold higher final parasite burdens compared to the controls. By comparison, co-infection with P. arabicus saliva resulted in an average 16-fold higher amastigote burden at the end of the experiment (Fig. 3B and C). Similarly, the proportion of mice, which developed lesions were equally high in the PSG and saliva co-infection groups (PSG: 83%, saliva: 83%, control: 50%) (Fig. 3A).

**L. major and L. tropica PSG from the experimental vector, Lu. longipalpis, exacerbates cutaneous infection in vivo**

The PSG extracted from the experimental vector, Lu. longipalpis promoted L. major and L. tropica infection in BALB/c mice (Fig. 4). Similar to the infections using PSG from their natural vectors, the inclusion of Lu. longipalpis-derived PSG in the inoculum contracted the time to the first appearance of cutaneous lesions by 25 days and 21 weeks for L. major and L. tropica, respectively, compared to 35 days and 33 weeks for infections with metacyclic promastigotes alone (Fig. 4A and B). The resultant lesions also displayed accelerated growth (Fig. 4C and D) and higher final parasite burdens (Fig. 4E and F; L. major: 11-fold; L. tropica: 35-fold more amastigotes recovered from lesions), similar to those observed from the co-inoculation of PSG from their natural vectors. Saliva from Lu. longipalpis promoted cutaneous infection and pathology for L. major resulting in a 43-fold higher amastigote burden at the end of the experiment. For L. tropica, however, Lu. longipalpis saliva appeared to promote lesion size but had little influence over the growth of these parasites in skin (Fig. 4E and F). Collectively, these results show that PSG from Lu. longipalpis can exacerbate L. major and L. tropica to a similar extent as the material collected from their natural vectors.

**Discussion**

This study demonstrates for the first time the infection-enhancing effect of PSG of two related CL Leishmania species from natural and unnatural phlebotomine sand fly vectors. L. major is the causative agent of zoonotic cutaneous leishmaniasis, endemic in the Middle East and Northern Africa (Maroli et al., 2013). Its natural vectors include P. papatasi and

---

**Table 1. The average amount of PSG recovered from sand flies used in this study**

| Sand fly species | Yield of PSG recovered from late-stage sand fly infections (average ± s.d. μg/fly total protein) |
|-----------------|---------------------------------------------------------------------------------------------------|
| P. papatasi     | 0.32 ± 0.13 | nd: incompatible |
| P. duboscqi     | 0.502 ± 0.11 | nd: incompatible |
| P. arabicus     | nd: incompatible | 0.36 ± 0.22 |
| Lu. longipalpis | 0.56 ± 0.29 | 0.38 ± 0.25 |

P. papatasi and P. duboscqi were infected with L. major and PSG extracted from day 12–13 p.i. P. arabicus was infected with L. tropica and PSG was extracted 12–13 p.i. Lu. longipalpis was infected with L. major and L. tropica and the PSG extracted 8 days p.i. For reference, Lu. longipalpis infected with L. mexicana yielded an average 0.88 ± 0.14 μg protein per infected fly at 8–9 days p.i. nd, not done.
P. dubosci (Dvorak et al., 2018). Cutaneous infections in non-healing strains of mice, such as BALB/c, typically result in strong Th2 polarization, driven largely by IL-4, allowing uncontrolled parasite growth (Noben-Trauth et al., 1996; Belkaid et al., 1998). Vector saliva benefits L. major infection in naïve hosts by biasing the local immune environment towards a Th2 phenotype, through increased expression of IL-4 and IL-10 (Belkaid et al., 1998). This immunomodulation promotes the initial infection and allows L. major to persist in skin, even after lesion resolution. The persistence of low numbers of L. major can establish concomitant immunity to reinfection and establish the host as a long-term reservoir of infection (Kimblin et al., 2008).

L. tropica overlaps in much of its range with L. major and is the causative agent of anthroponotic CL, endemic in the Middle East, Mediterranean basin, Central Asia, and East Africa. The primary vector of L. tropica is Phlebotomus (Paraphlebotomus) sergenti (Kamhawi et al., 2000; Mykova et al., 2007; Volf and Mykova, 2007), although other sand fly species have been shown to transmit, including P. arabicus in northern Israel (Jacobson et al., 2003; Svobodova et al., 2006a, 2006b) and Ethiopia (Gebre-Michael et al., 2004). L. tropica is responsible for cutaneous lesions which are typically more chronic, longer lasting, require longer to heal and are often more difficult to treat compared to L. major, resulting in considerable scarring (Klaus and Frankenburg, 1999). In mice models, the persistence of L. tropica is also Th2-dependent, requiring manipulation of IL-10 and TGF-β signalling (Anderson et al., 2008; Kobets et al., 2012). Similar to L. major, chronic lesions of L. tropica in mice harbour sufficient numbers of parasites to be transmitted to their natural vector (Anderson et al., 2008).

Components of the infectious sand fly bite greatly influence the course of leishmaniasis in mammalian hosts (Serafim et al., 2014). This effect appears to be under the control of insulin growth factor 1 path- way, as part of the wound healing process, resulting in the enhanced survival of Leishmania and their growth inside macrophages (Giraud et al., 2018). Using this parasite-vector model, we have also shown that the PSG-blockage impacts on the blood-feeding ability of the sand fly and promotes transmission by manipulating the sand fly feeding behaviour (Rogers and Bates, 2007). It is likely that both L. major and L. tropica would benefit
from the ability of PSG to promote M2 macrophage activation, as polyamines are essential for their intracellular growth in mice (Green et al., 1990; Badirzadeh et al., 2017). Moreover, arginase 1 activity is closely associated with acute lesions of *L. major* and *L. tropica* in humans (Mortazavi et al., 2016).

Compared to *L. major*, *L. tropica* is a poorly studied parasite because of a lack of a reliable model of infection. In rats, *L. tropica* does not produce lesions despite surviving in the skin and being able to infect naïve sand flies for more than a year post-infection (Svobodová et al., 2003). In mice, although lesions can be generated, they are notoriously difficult and slow to establish. Lira et al., showed that experimental infections were possible in mice, requiring large doses (10^6) of highly enriched metacyclic promastigotes (Lira et al., 1998). We extend their findings by revealing that both vector saliva and PSG from infected vectors can substantially accelerate their model of cutaneous *L. tropica* infection in mice, thereby highlighting the probable role of vector-derived products for the establishment of *L. tropica* infection and improving the current model of infection. In the future, it would be worth testing the exacerbatory role of PSG on *L. tropica* infection in mice.

**Fig. 3.** *L. tropica* infection in mice is exacerbated by PSG and saliva from a natural vector. *L. tropica* PSG was obtained from infected *P. arabicus* sand flies and saliva collected from uninfected flies. One million *L. tropica* metacyclic promastigotes were injected i.d. into the rumps of BALB/c mice without (open circles) or with 1 µg sterile PSG (closed squares) or 1 µg sterile saliva (open triangles). (A) Proportion of mice presenting with cutaneous lesions over the course of study. (B) Rump lesion evolution showing total lesion size. (C) Final parasite burdens of lesions from homogenates. Each point represents individual mice. Infections performed in duplicate and representative data is shown, *n* = 6 mice per group. *: *P* < 0.05 by Mann Whitney unpaired two-tailed *t*-test.

**Fig. 4.** *L. major* and *L. tropica* infections in mice are exacerbated by PSG and saliva from the experimental vector, *Lu. Longipalpis* (*L. major*: A, C and E; *L. tropica*: B, D and F). *L. tropica* and *L. major* PSG was obtained from infected *Lu. longipalpis* sand flies and saliva collected from uninfected flies. One thousand *L. major* metacyclic promastigotes or one million *L. tropica* metacyclic promastigotes were injected i.d. into the footpads or rumps of BALB/c mice without (open circles) or with 1 µg sterile PSG (closed squares) or with 1 µg sterile saliva (open triangles). (A and B) Proportion of mice presenting with cutaneous lesions over the course of study. (A and B) Proportion of mice presenting with cutaneous lesions over the course of study. (B and D) Lesion evolution showing average lesion thickness ± S.E.M or total lesion size. (E and F) Final parasite burdens of lesions from tissue homogenates. Each point represents individual mice and bars represent the average value per group. Representative data is shown of duplicate experiments, *n* = 6 mice per group. *: *P* < 0.05; **: *P* < 0.005 by Mann Whitney unpaired two-tailed *t*-test.
infection in the ear dermis, as this site of infection has been shown to produce reliable infections with lower numbers ($10^3$–10$^5$) of parasites (Anderson et al., 2008). In this study, it is striking that the growth of L. tropica in BALB/c mice appears to be much less vigorous than L. major but both species can benefit substantially from either the vector saliva or PSG. How this general mechanism of parasite survival contributes to L. tropica pathogenesis will require careful further study but is likely to be part of a complex of regulatory mechanisms, which subtly control both the Th1 and Th2 arms of the adaptive immune response.

PSG from natural Leishmania-sand fly combinations significantly contributes to the progression of the infection in skin of the mammalian host. From these findings, we can now add the parasite PSG to the list of vector-derived products that exacerbate L. major infection and significantly improve the currently intractable model of L. tropica infection (Kobets et al., 2012). Taken collectively, this reinforces the hypothesis that PSG is a common component of sand fly transmission and a virulence factor to Leishmania sand fly vector combinations found in nature. This has implications for interpreting the pathogenesis of Leishmania infection and the choice of an appropriate challenge for anti-leishmanial drug and vaccine screening.

Acknowledgements. We thank Patricia Ayenuro and Shaida Begum for assistance maintaining the Lu. longipalpis sand fly colony in London and Anna Dostálová and Jitka Myšková for maintaining colonies of P. papatasi, P. duboscqi and P. arabcis sand flies in Prague. These results were presented as part of the 2018 British Society for Parasitology Autumn Symposium on Parasite Glycobiology.

Financial support. This work was supported by the Biotechnology and Biological Sciences Research Council (M.E.R., David Phillips Fellowship, BB/H022406/1); the Wellcome Trust (M.E.R., grant number GR078223MA) and the Czech Ministry of Education (ERA funds [CZ.02.1.01/0.0/0.0/16_019/0000759]) to P.V.

Ethical standards. All animal and sand fly infections in the UK were carried out in accordance with the UK Animal Scientific Procedure Act (ASPA) 1986, which transposes European Directive 2010/63/EU into UK national law. Animal studies are approved by the UK home office in granting Project licence 70/8427 under the Animal Scientific Procedure Act and all protocols had undergone appropriate local ethical review procedures by the Animal Welfare and Ethical Review Board (AWERB) of LSHTM.

References

Anderson CF, Lira R, Kamhawi S, Belkaïd Y, Wynn TA and Sacks D (2008) IL-10 and TGF-beta control the establishment of persistent and transmissible infections produced by Leishmania tropica in C57Bl/6 mice. Journal of Immunology 180, 4090–4097.

Atayde TD, Aslan H, Townsend S, Hassani K, Kamhawi S and Olivier M (2015) Exosome secretion by the parasitic protozoan Leishmania within the sand fly midgut. Cell Reports 13, 957–967.

Badirzadeh A, Taheri T, Taslimi Y, Abdossamadi Z, Heidari-Kharaji M, Carvalho J, Nasereddin A, El Fari M, Shaloom U, Volf P, Votypka J, Dedet JP, Pralong F, Schonian G, Schurz LF, Jaffe CL and Warburg A (2003) Outbreak of cutaneous leishmaniasis in northern Israel. Journal of Infectious Diseases 188, 1065–1073.

Kamhawi S, Belkaïd Y, Modì G, Roton E and Sacks DL (2000) Protection against cutaneous leishmaniasis resulting from bites of uninfected sandflies. Science 290, 1351–1354.

Kimblin N, Peters N, Debrabant A, Secundo N, Egen J, Lawyer P, Fay MP, Kamhawi S and Sacks D (2008) Quantification of the infectious dose of Leishmania major transmitted to the skin by single sand flies. Proceedings of the National Academy of Science USA 105, 10125–10130.

Klaus S and Frankenburg S (1999) Cutaneous leishmaniasis in the Middle East. Clinical Dermatology 17, 137–141.

Kobets T, Havelková H, Grekov I, Volkova V, Vojitšiková J, Slápnicková M, Kurej I, Souhrbí Y, Svobodová M, Demant P and Lipoldová M (2012) Genetics of host response to Leishmania tropica in mice – different control of skin pathology, chemokine reaction, and invasion into spleen and liver. PLoS Neglected Tropical Diseases 6, e1667.

Lira R, Méndez S, Carrera L, Jaffe G, Neva F and Sacks D (1998) Leishmania tropica: the identification and purification of metacyclic promastigotes and use in establishing mouse and hamster models of cutaneous and visceral disease. Experimental Parasitology 89, 331–342.

Maroli M, Feliciani MD, Bichaud L, Charrel RN and Gradoni L (2013) Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern. Medical and Veterinary Entomology 27, 123–147.

Montgomery J, Curtis J and Handman E (2002) Genetic and structural heterogeneity of protozoophyphoglycans in Leishmania. Molecular and Biochemical Parasitology 121, 75–85.

Mortazavi H, Sadeghipour P, Taslimi Y, Habibzadeh S, Zali F, Zabedifard F, Rahmati K, Kamky K, Ghandi N, Zamanian A, Reza Tohidlinik H, Muller I, Kropf I and Rafati S (2016) Comparing acute and chronic human cutaneous leishmaniasis caused by Leishmania major and Leishmania tropica focusing on arginase activity. Journal of the European Academy of Dermatology and Venereology 30, 2118–2121.

Myszkova J, Svobodova M, Beverley SM and Volf P (2007) A lipophosphoglycan-independent development of Leishmania in permissive sand flies. Microbes and Infection 9, 317–324.

Noben-Trauth N, Kropf I and Müller I (1996) Susceptibility to Leishmania major infection in interleukin-4-deficient mice. Science 271, 987–990.

Rath M, Müller I, Kropf P, Closs EL and Munder M (2014) Metabolism via arginine or nitric oxide syntheses: two competing arginine pathways in macrophages. Frontiers in Immunology 532, 10.

Rogers ME (2012) The role of Leishmania proteophosphoglycans in sand fly transmission and infection of the mammalian host. Frontiers in Microbiology 223, 13.
Rogers ME and Bates PA (2007) Leishmania manipulation of sand fly feeding behavior results in enhanced transmission. PLoS Pathogens 3, e91.

Rogers ME, Chance ML. and Bates PA (2002) The role of promastigote secretory gel in the origin and transmission of the infective stage of Leishmania mexicana by the sandfly Lutzomyia longipalpis. Parasitology 124, 495–507.

Rogers ME, Ilg T, Nikolaev AV, Ferguson MA and Bates PA. (2004) Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. Nature 430, 463–467.

Rogers M, Kropf P, Choi BS, Dillon R, Podinovskaia M, Bates P and Müller I (2004) Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. Nature 430, 463–467.

Rogers ME, Corware K, Müller I and Bates PA. (2010) Leishmania infantum proteophosphoglycans regurgitated by its natural sand fly vector, Lutzomyia longipalpis, promote parasite establishment in mouse skin and skin-distant tissues. Microbes and Infection 12, 875–879.

Serafim TD, Dey R, Nakhasi HL, Valunzuela JG and Kamhawi S (2017) Unique features of vector-transmitted leishmaniasis and their relevance to disease transmission and control. In Wikel SK, Aksoy S and Dimopoulos G (eds), Arthropod Vector: Controller of Disease Transmission, Volume 2. Vector Saliva-Host-Pathogen Interactions. United States of America: Academic Press, pp. 91–114.

Shortt HE and Swaminath CS (1938) The method of feeding of Phlebotomus argentipes with relation to its bearing on the transmission of kala-azar. Indian Journal of Medical Research 15, 827–836.

Stierhof YD, Bates PA, Jacobson RL, Rogers ME, Schlein Y, Handman E and Ilg T (1999) Filamentous proteophosphoglycan secreted by Leishmania promastigotes forms gel-like three-dimensional networks that obstruct the digestive tract of infected sandfly vectors. European Journal of Cellular Biology 78, 675–689.

Svobodová M, Votýpka J, Nicolas L and Volf P (2003) Leishmania tropica in the black rat (Rattus rattus): persistence and transmission from asymptomatic host to sand fly vector Phlebotomus sergenti. Microbes and Infection 5, 361–364.

Svobodová M, Volf P and Votýpka J (2006a) Experimental transmission of Leishmania tropica to hyraxes (Procavia capensis) by the bite of Phlebotomus arabicus. Microbes and Infection 8, 1691–1694.

Svobodova M, Votycka J, Peckova J, Dvorak V, Nasredden A, Baneth G, Sziern J, Kravchenko V, Orr A, Meir D, Schur LF, Volf P and Warburg A (2006b) Distinct transmission cycles of Leishmania tropica in 2 adjacent foci, Northern Israel. Emerging Infectious Diseases 12, 1860–1868.

Titus RG and Ribeiro JM (1988) Salivary gland lysates from the sand fly Lutzomyia longipalpis enhance Leishmania infectivity. Science 239, 1306–1308.

Volf P and Myškova J (2007) Sand flies and Leishmania: specific versus permissive vectors. Trends in Parasitology 23, 91–92.

Volfova V, Hostomska J, Cerny M, Votycka J and Volf P (2008) Hyaluronidase of bloodsucking insects and its enhancing effect on Leishmania infection in mice. PLoS Neglected Tropical Diseases 2, e294.