The vectorial competence of *Phlebotomus sergenti* is specific for *Leishmania tropica* and is controlled by species-specific, lipophosphoglycan-mediated midgut attachment

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SUMMARY

The vectorial competence of *Phlebotomus sergenti* for 3 Old World species of *Leishmania*, *L. tropica*, *L. major* and *L. donovani*, was investigated *in vivo* and by *in vitro* midgut binding assays using living promastigotes and purified lipophosphoglycan (LPG). *P. sergenti* consistently showed a high specificity for *L. tropica* strains, which were able to develop mature, potentially transmissible infections. The loss of infection with *L. major* and *L. donovani* correlated with the excretion of the digested bloodmeal. These strains were able to produce sustained infections in the midguts of their appropriate vectors, *P. papatasi* and *P. argentipes*, respectively. In *in vitro* binding assays, a significantly higher number of *L. tropica* procyclic promastigotes attached to the midgut lining of *P. sergenti*, compared to those of *L. major* and *L. donovani* ($P < 0{\cdot}05$). The prediction that the species specificity of midgut attachment is controlled by polymorphic structures on the parasite LPG was supported by the finding that *P. sergenti* midguts were intensely stained following incubation with purified phosphoglycan (PG) from *L. tropica* compared with PGs from *L. major* or *L. donovani*. The results provide further evidence that LPG structural polymorphisms are driven by the species diversity of molecules present on the sandfly midgut that function as parasite attachment sites.

Key words: *Phlebotomus sergenti*, *Leishmania tropica*, lipophosphoglycan, vector specificity.

INTRODUCTION

Leishmania produce a spectrum of diseases, the clinical forms of which are determined by the species of transmitted parasite. Important Old World *Leishmania* species include *L. major* and *L. tropica*, which produce localized cutaneous ulcers, and *L. donovani*, which causes visceral leishmaniasis, or kala-azar. The distribution of *Leishmania* species is determined by the availability of competent vectors. Based on epidemiological observations, there appears to be a close evolutionary fit between the *Leishmania* species and the sandfly species that transmits it in nature (Killick-Kendrick, 1985). Most of the evidence suggesting species-specific vector competence has been obtained from studies involving *P. papatasi*. Despite its wide distribution in regions endemic for transmission of other species of *Leishmania*, there is no evidence that *P. papatasi* is involved in the natural transmission of any species other than *L. major*. This specificity has been reproduced in the laboratory; *P. papatasi*, fed on either experimental lesions or through a membrane, will support the full growth and development of *L. major*, but not of any other *Leishmania* species (Adler & Theodor, 1927; Adler, Theodor & Witenberg, 1938; Heyneman, 1963; Pimenta *et al*. 1994). With respect to *L. tropica*, which also has a wide distribution within the Old World, *P. sergenti* is the only proven vector in all foci studied to date (al-Zahrani *et al*. 1988; Killick-Kendrick, 1990; Guilvard *et al*. 1991), with the exception of Kenya, where *P. guggisbergi* has been implicated (Lawyer *et al*. 1991). Furthermore, *P. sergenti* has not been found infected with any species other than *L. tropica*. In the only study to examine the vector competency of *P. sergenti* in the laboratory, *L. tropica* produced heavy anterior infections in 95% of flies on days 8 and 9 post-infection (Killick-Kendrick, Killick-Kendrick & Tang, 1995).

Comparison of the development of *Leishmania* parasites in permissive and non-permissive vectors suggests several barriers that might explain the specificity of vector competence indicated in nature. Potential barriers include the digestive enzymes that are released into the midgut that can inhibit parasite growth and development (Schlein & Romano, 1986; Borovsky & Schlein, 1987; Schlein, Schnur & Jacobson, 1990; Dillon & Lane, 1993; Pimenta *et al*.

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1997; Schlein & Jacobson, 1998); the peritrophic membrane that can prevent the migration of the parasite to different parts of the gut (Schlein, Jacobson & Shlomai, 1991; Schlein & Jacobson, 1994); the excretion of the midgut contents following bloodmeal digestion that can result in the passage of parasites out of the gut (Pimenta *et al*. 1994; Sacks *et al*. 2000); and changes in the anatomy of the anterior gut that might interfere with the egestion of parasites from the fly (Killick-Kendrick & Molyneux, 1981; Schlein, Jacobson & Messer, 1992).

The ability of the parasite to maintain infection in the gut following bloodmeal excretion has been shown to be related to its ability to attach to the epithelial cells lining the midgut via the major surface glycoconjugate of *Leishmania* promastigotes, termed lipophosphoglycan (LPG) (Pimenta *et al*. 1992, 1994; Sacks *et al*. 1995, 2000; Butcher *et al*. 1996). LPG consists of a GPI-anchored polysaccharide made up of an oligosaccharide core linked to a polymer of phosphorylated disaccharide repeats that display a high degree of inter-species polymorphisms (reviewed by Turco & Descoteaux, 1992; McConville & Ferguson, 1993). The backbone repeats of Old World LPGs express the conserved structure 6Galβ1-4Manα1-PO_4, which can either be unsubstituted (*L. donovani*, Sudan) (Turco *et al*. 1987), partially substituted with glucose side-chains (Indian *L. donovani*) (Mahoney *et al*. 1999), or completely substituted with side-chain sugars that terminate primarily in galactose (*L. major*) (McConville *et al*. 1990) or in glucose and arabinose (*L. tropica*) (McConville *et al*. 1995). The evidence that the polymorphic nature of LPG accounts, at least in part, for the specificity of vector competence was demonstrated in *L. major–P. papatasi* interactions, for which side-chains terminating in galactose residues were shown to be required for midgut binding (Pimenta *et al*. 1992, 1994).

The unique structure of *L. tropica* LPG predicts that it might similarly account for the apparent species restriction of *P. sergenti* vector competence. In the present study the vector competence of *P. sergenti* for *L. tropica*, *L. major* and *L. donovani*, is examined in detail. The *in vivo* outcomes are compared with midgut binding assays using living promastigotes and purified LPGs. The results are the first to confirm the remarkable specificity of *P. sergenti* for *L. tropica*, and they provide strong evidence that differences in LPG-mediated midgut attachment explain the specificity observed.

MATERIALS AND METHODS

Leishmania *parasites*

The following strains of *Leishmania* were used in this study. Two cutaneous isolates of *L. tropica* from Afghanistan (MHOM/AF/87/RP) and (MHOM/AF/88/KK27), described previously (Lira *et al*. 1998), and a cutaneous isolate of *L. tropica* from a focus in the district of Bani Kinana, North Jordan (Kamhawi, Abdel-Hafez & Arbagi, 1995) (MHOM/JO/94/MA37), identified by isoenzyme analysis (Dr M. Gramiccia, Istituto Superiore di Sanita, Rome, Italy); the NIH Friedlin strain of *L. major*, clone V1 (MHOM/IL/80/FN); and the *L. donovani* Mongi strain (MHOM/IN/83/Mongi), isolated from a patient with kala-azar in India. Amastigotes were purified from macerated mouse footpad lesions (*L. major* and *L. tropica*) or from hamster spleens (*L. donovani*) as previously described (Saraiva *et al*. 1983). Amastigotes were stored frozen at −70 °C until use. All promastigotes were grown in medium 199 supplemented with 20% (v/v) heat-inactivated fetal calf serum, penicillin (100 U/ml) streptomycin (50 μg/ml), and 12·5 mM L-glutamine (all from ABI, Columbia, MD), 40 mM Hepes, pH 7·4, 0·1 mM adenine, and 0·0005% haemin. Procyclic promastigotes were harvested in the logarithmic phase (1–2 days) and washed with Hanks balanced salt solution containing 1 mM $MgCl_2$ and 0·15 mM $CaCl_2$ (HBSS2+).

Sandfly infections

For the purpose of this study, a colony of *Phlebotomus sergenti* was established at the Department of Entomology, Walter Reed Army Institute of Research (WRAIR), from a field collection carried out in September 1997, from a focus of *L. tropica* in the district of Bani Kinana, North Jordan (Kamhawi *et al*. 1995). *P. papatasi* and *P. argentipes* colonies were also maintained at WRAIR and originated from Israel and India respectively. Two to five-day-old female sandflies were membrane fed on a bloodmeal containing 2 million amastigotes per ml of heparinized mouse blood. Bloodfed females were separated the day after, maintained at 26 °C, and given water and 50% sucrose solution. At each timepoint, 8–10 specimens were examined for their bloodmeal status, then dissected in phosphate-buffered saline (PBS). Individual midguts were placed in microfuge tubes containing 50 μl of PBS, and macerated using a Teflon coated micro-tissue grinder. The number of promastigotes per midgut was counted using a haemocytometer.

In vitro *binding of promastigotes or phosphoglycan to sandfly midguts*

Two to five-day-old female sandflies, maintained only on water plus 50% sucrose, were dissected in PBS. The crop, malpighian tubules and hindgut of each fly were removed leaving only the midgut. The posterior end was slit open longitudinally towards the thoracic midgut. The opened midguts of 10–15 specimens were collectively incubated in a concave well of a microscope chamber slide, in 50 μl of PBS

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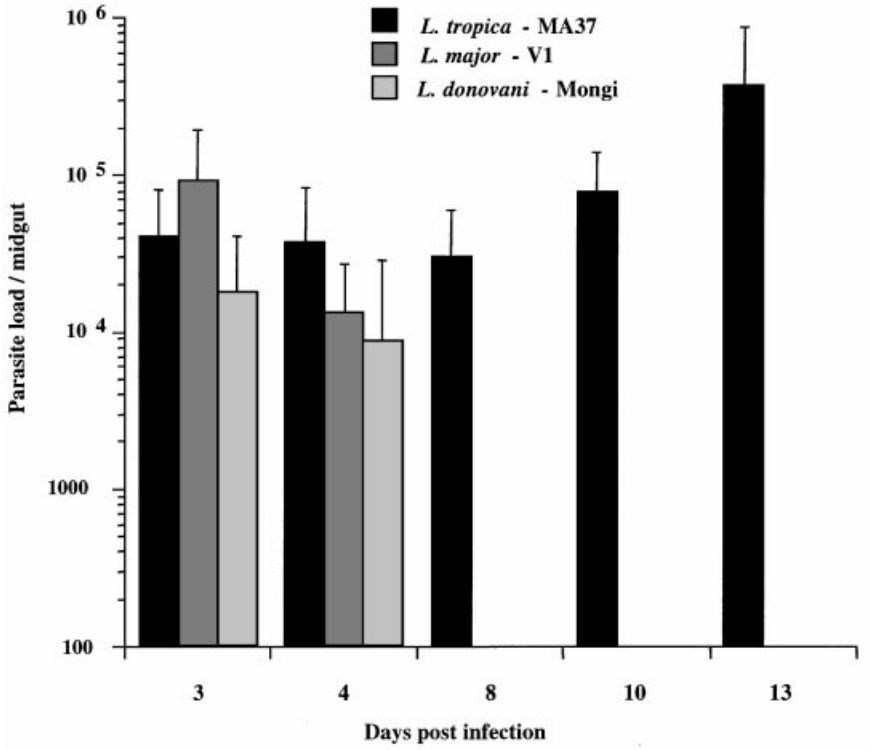

Fig. 1. *In vivo* development of *Leishmania tropica* (MA37), *L. major* (V1) and *L. donovani* (Mongi) in *Phlebotomus sergenti* membrane fed on bloodmeals containing 2 million amastigotes per ml. Histograms represent the mean ± 1 S.D. of 8–10 midguts examined per time-point.

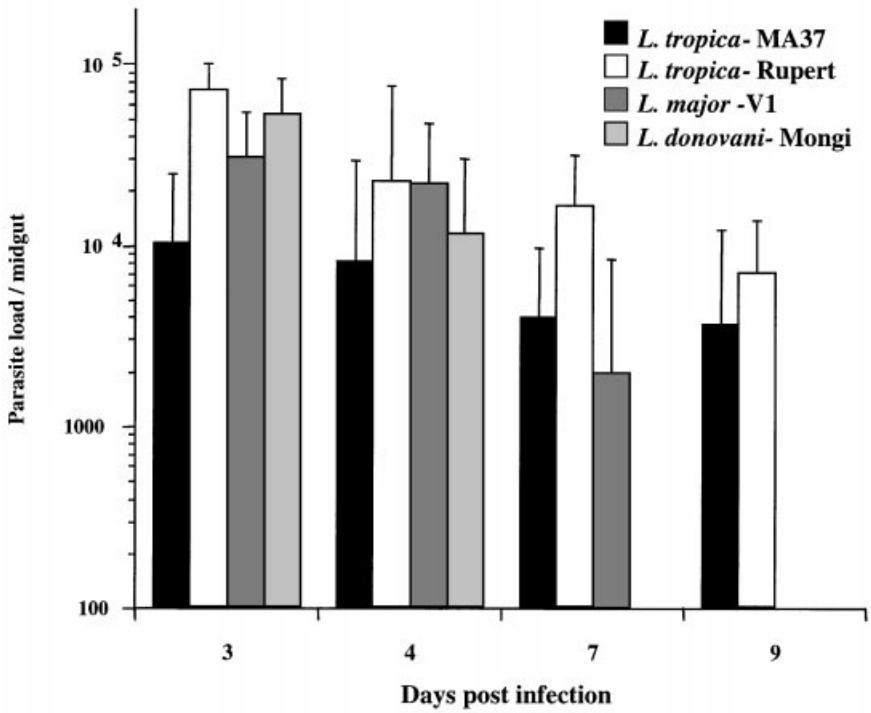

Fig. 2. *In vivo* development of 2 strains of *Leishmania tropica* (MA37 and RP), *L. major* and *L. donovani* in *Phlebotomus sergenti* membrane fed on bloodmeals containing 2 million amastigotes per ml. Histograms represent the mean ± 1 S.D. of 8–10 midguts examined per time-point.

containing 1 million logarithmic phase (1–2 day culture) promastigotes. The optimum incubation period for midguts of *P. sergenti* was found to be 10 min, after which time the midgut lining began to detach. Following a 10 min incubation period, individual midguts were washed in several drops of PBS, placed in a microfuge tube with 30 μl of PBS, macerated and counted as previously mentioned.

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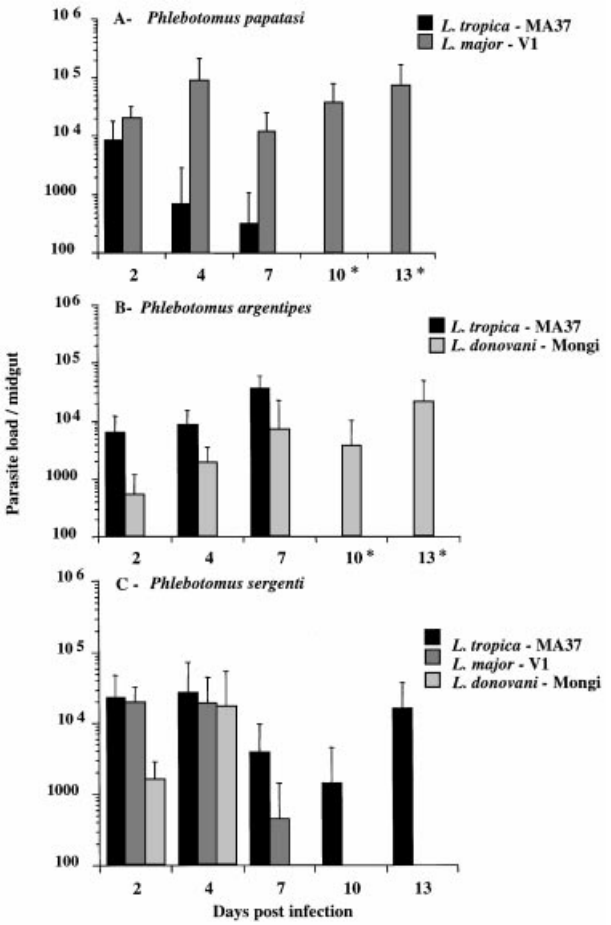

Fig. 3. *In vivo* development of *Leishmania tropica* (MA37) and *L. major* in *Phlebotomus papatasi* (A); *L. tropica* (MA37) and *L. donovani* in *P. argentipes* (B); and *L. tropica* (MA37), *L. major* and *L. donovani* in *P. sergenti* (C). Histograms represent the mean ± 1 S.D. of 8–10 specimens examined per time-point. * Flies infected with *L. tropica* were not available at these time-points.

For *in vitro* binding of phosphoglycan (PG), LPG was organically extracted from other lipids and glycosylated products and solubilized in solvent E(H_2O/ethanol/ethylether/pyridine/NH_4OH; 15:15:5:1:0·017) as previously described (Orlandi & Turco, 1987). The solvent E extract was dried by N_2 evaporation, resuspended in 0·1 M acetic acid/0·1 M NaCl, and applied to a column of phenyl-Sepharose (2 ml), equilibrated in the same buffer. LPG was eluted using solvent E (Orlandi & Turco, 1987). Purified LPG was treated with phospholipase C from *B. cereus* for 16 h at 37 °C. The released PG was separated from the cleaved lipid by hydrophobic chromatography by elution from a phenyl-Sepharose column (2 ml). The PG was concentrated and dialysed against PBS. For binding of purified PG to midguts *in vitro*, opened, dissected midguts were fixed with 2% formaldehyde in PBS at 4 °C for 20 min. After several washes in PBS they were incubated for 45 min with PG (10 μg/ml) in a total volume of 50 μl. After several washes the guts were incubated in a 1:200 dilution of ascites containing the anti-LPG monoclonal antibody 45D3, followed by incubation with fluorescein anti-mouse IgG. Stained guts were examined microscopically under ultraviolet and bright-field exposures. Controls were incubated with the primary and secondary antibodies alone.

Statistical analysis

The 2-tailed Student's *t*-test was used to examine the significance in the differential *in vitro* binding of *Leishmania* species to the opened midguts of *P. sergenti*.

RESULTS

Specificity of P. sergenti *for* L. tropica *in vivo*

In an initial experiment, 3 Old World *Leishmania* species, *L. major* (V1), *L. tropica* (MA37) and *L. donovani* (Mongi) were used to infect *P. sergenti* via a membrane feed on heparinized mouse blood seeded with 2 million amastigotes/ml. The mean parasite load per midgut (MPL) was calculated for 8–10 specimens at each time-point. At days 3 and 4 following the ingestion of blood, the MPL was high for all species, ranging from 20000 to 100000 promastigotes (Fig. 1). No significant difference in the intensity of infection at these early time-points was observed between strains. All *L. major* and *L. donovani*-infected flies were negative for parasites by day 8. In contrast, the Jordanian *L. tropica* strain maintained late infections in the fly (days 8–13), at which time-points metacyclic promastigotes, based on morphology, represented 28–70% of the parasite forms observed. In a repeat experiment, an *L. tropica* strain from Afghanistan was included to investigate whether the susceptibility of *P. sergenti* (originating from Jordan) to infection with *L. tropica* extended to an *L. tropica* substrain isolated from an endemic focus distinct from that of the sandfly (Fig. 2). A similar pattern of early promastigote growth (days 3–4) was again observed for each of the strains, and *L. donovani* and *L. major* again failed to persist beyond days 7 and 9, respectively. Both the Jordanian and Afghanistan *L. tropica* strains maintained heavy midgut infections up to day 9, at which time each strain had generated a high proportion of metacyclic forms located in the anterior midgut.

In a third experiment, the susceptibility of *P. sergenti* for the 3 species of *Leishmania* was compared with the infection outcomes in *P. papatasi* and *P. argentipes*, which are the respective natural vectors of *L. major* and *L. donovani*. In *P. papatasi*, a reciprocal pattern of species-specific vector compatibility was observed. *L. major* maintained high loads of midgut promastigotes following bloodmeal excretion (days 4–7) and achieved a MPL of 37000 and 75000 at days 10 and 13 respectively (Fig. 3A). In contrast,

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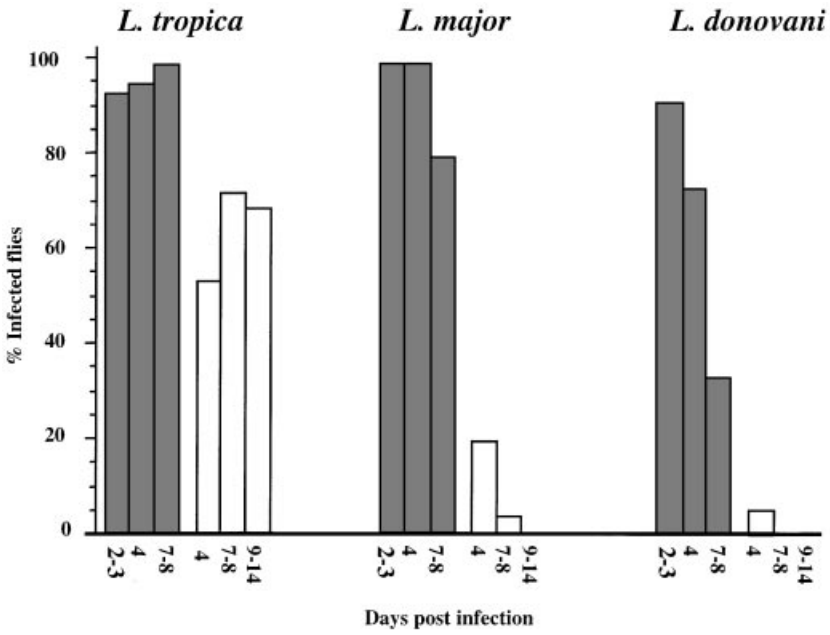

Fig. 4. The status of infections at different time-points compared in *Phlebotomus sergenti* midguts containing complete or partial bloodmeals versus those without evidence of blood. Data are pooled from the 3 experiments shown in Figs 1–3. Midguts containing blood are shown in dark grey and those without blood in white.

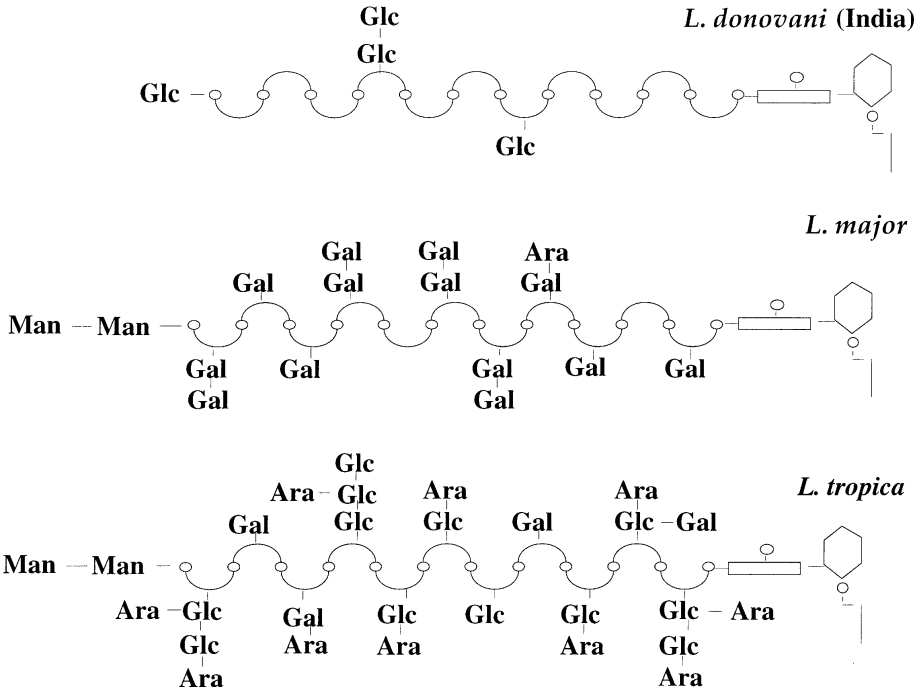

Fig. 5. Schematic representation of the procyclic LPGs of *Leishmania donovani* (India), *L. major* and *L. tropica* showing the PO_4-Gal-Man backbone repeats and the increasing complexity in side-chain substitutions.

L. tropica survived poorly in *P. papatasi* following bloodmeal excretion; the MPL dropped from 8500 on day 2 to 300 by day 7. The complete development of the Indian *L. donovani* strain was observed in *P. argentipes*, maintaining high midgut infections out to day 13 (Fig. 3B). Contrary to its development in *P. papatasi*, *L. tropica* survived well in *P. argentipes*, even in flies that had passed their bloodmeals on days 4–7. The exquisite specificity of *P. sergenti* for *L. tropica* was reproduced (Fig. 3C). These parallel studies in 3 vector species rule out any possibility that the failure of *L. major* or *L. donovani* to develop fully in *P. sergenti* was due to any generalized defect in the growth or differentiation of these parasite strains.

The loss of infection with *L. major* or *L. donovani* in *P. sergenti* followed closely with the loss of the digested bloodmeal in these flies. In data pooled from the 3 experiments described above, when the comparison of infection status at each time-point

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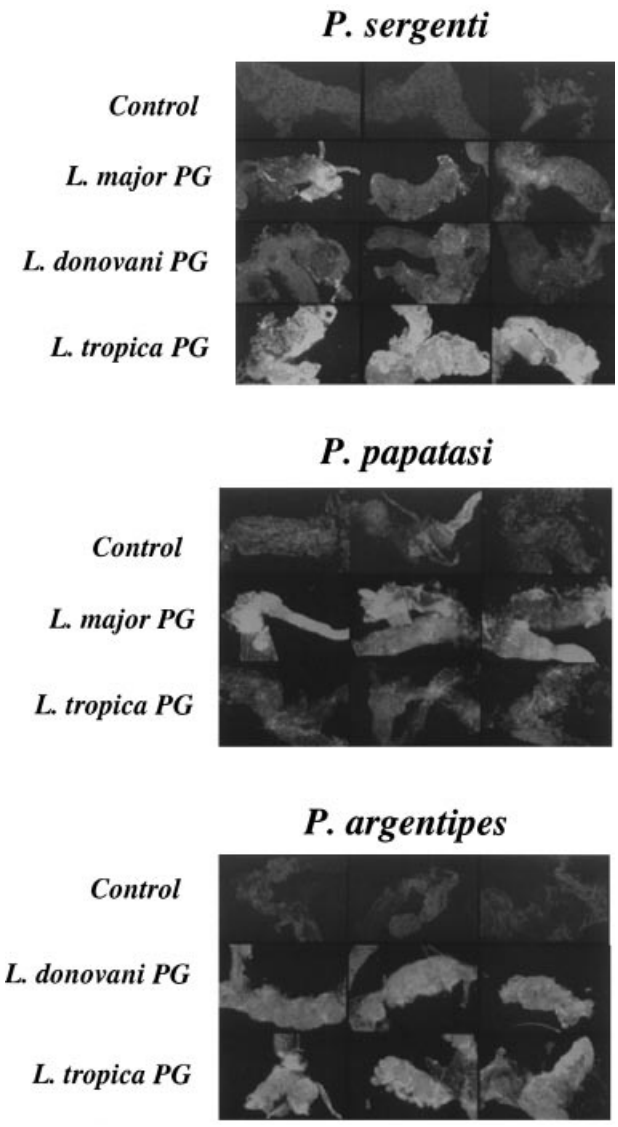

Fig. 6. Fluorescent staining of individual *Phlebotomus sergenti*, *P. papatasi* and *P. argentipes* midguts, incubated with PG (10 μg/ml) purified from procyclic promastigotes of *Leishmania tropica*, *L. major* and *L. donovani*. Controls were incubated with primary and secondary antibodies alone.

was confined to those flies that still contained blood, then the specificity phenotype was no longer apparent. The pattern of blood digestion in *P. sergenti* was not altered by infection with different *Leishmania* species. On day 4, a significant proportion of the flies in each group began to pass their digested bloodmeals, ranging from 10 to 70 % in the 3 experiments. Of the flies that retained blood on day 4, the percentage of flies that were infected remained high in each of the groups (76–100 %) (Fig. 4). When the day 4 flies that had passed their bloodmeals were compared, the percentage of *L. tropica*-infected flies that remained infected declined to 54 %, while the loss in the *L. major* and *L. donovani*-infected flies was severe, down to 20 and 5 %, respectively. By days 7–8, the majority of flies had lost their blood and only a few flies from all groups (14–27 %) showed a remnant of the bloodmeal. Of the flies that still contained blood on days 7–8, a significant proportion of the *L. major* and *L. donovani*-infected groups continued to be positive for parasites even at this late time-point (78 and 36 %, respectively). Some of the flies in these groups contained only a remnant of blood, and many of these flies were negative for parasites. Of the flies that had no evidence of blood, there were virtually no flies positive for *L. major* or *L. donovani*, whereas the flies positive for *L. tropica* remained high (72 %). None of the flies dissected on days 9 and beyond contained blood, and accordingly only the *L. tropica*-infected flies remained positive for parasites at these late time-points.

In vitro *binding of* Leishmania *promastigotes to* P. sergenti *midguts*

Considering results from 2 pooled experiments, the mean number of parasites bound per *P. sergenti* midgut was 2683 ± 2614 for *L. tropica* MA37 and 1367 ± 2316 for *L. tropica* KK27 compared with only 177 ± 310 for *L. major* and 86 ± 164 for *L. donovani*. The binding of MA37 and KK27 was significantly higher than that of *L. major* ($P < 0{\cdot}005$; $P = 0{\cdot}05$ respectively) and *L. donovani* ($P < 0{\cdot}005$; $P = 0{\cdot}05$ respectively).

In vitro *binding of* Leishmania *PG to sandfly midguts*

The species specificity of promastigote binding to *P. sergenti* midguts was investigated in the context of the structure and function of LPG. The characterized structures of *L. donovani* (India), *L. major*, and *L. tropica* LPGs are depicted in Fig. 5. The intact phosphoglycan chain, linked to the core oligosaccharide structure, was purified from each of these strains and used in direct binding assays to midguts prepared from each vector species. *P. sergenti* midguts incubated with *L. tropica* PG were intensely stained throughout the abdominal and thoracic regions of the gut (Fig. 6). Midguts incubated with *L. major* and *L. donovani* PG were stained at above background levels in every region of the gut, though in each case the staining was substantially less intense relative to midguts incubated with *L. tropica* PG. As positive controls for the binding of *L. major* and *L. donovani* PGs to the midguts of their appropriate vectors, *P. papatasi* midguts stained brightly with *L. major* PG but poorly with *L. tropica*, and *P. argentipes* midguts stained brightly with both *L. donovani* and *L. tropica* PG, confirming prior observations (Pimenta *et al.* 1994). Thus the pattern of PG binding to the midguts of different vector species is consistent with the pattern of promastigote binding, and in each case the binding patterns predict which sandfly will permit the full development of the parasite during infection.

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DISCUSSION

L. tropica is responsible for anthroponotic cutaneous leishmaniasis, and it has more recently been associated with systemic forms of leishmanial disease. The vector of urban anthroponotic cutaneous leishmaniasis is believed to be *P. sergenti* based on its distribution and on the fact that *L. tropica* has been identified in infected flies recovered from endemic foci. Although *P. sergenti* is also present in areas endemic for diseases due to other species of *Leishmania*, including *L. major*, *L. infantum* and *L. donovani*, there is to date no evidence that it is involved in their transmission, and the question of species-specific vector competence has been raised. The demonstration that *P. sergenti* is permissive to *L. tropica* in the laboratory was reported early on by Adler (1938), although the species of *Leishmania* used in these studies could not be validated; and more recently by Killick-Kendrick *et al*. (1995), using a limited number of flies. Here we report a series of studies involving a large number of flies and 3 different species of *Leishmania*, the results of which confirm that *P. sergenti* supports the full development of *L. tropica*, and reveal for the first time that this vector is refractory to other Old World *Leishmania* species.

The strong specificity observed for the *L. tropica–P. sergenti* pair supports previous accounts relating the structure of *Leishmania* LPG to vectorial competence (Pimenta *et al*. 1994; Sacks *et al*. 1994; Butcher *et al*. 1996). In 3 separate experiments in which parasite development was monitored *in vivo*, *L. tropica* survived well beyond the digestion of the bloodmeal and developed potentially transmissible infections, defined by the presence of large numbers of metacyclic promastigotes in the anterior midgut. The flies supported the full development both of a sympatric strain of *L. tropica* from Jordan, as well as a strain from Afghanistan. In contrast, *L. major* and *L. donovani* failed to establish late infections in *P. sergenti*. The early transformation and growth of these strains in *P. sergenti* did not appear to be impaired, indicating that they were not overly sensitive to the digestive enzymes present in the bloodfed midgut. There was, however, a strong correlation between the loss of blood and the loss of promastigotes, suggesting that their inability to persist in the *P. sergenti* midgut is related to their failure to remain anchored to the gut wall via specific attachment sites. This was supported by the *in vitro* midgut binding assays, in which only *L. tropica* promastigotes bound in significant numbers. That this specificity was mediated by the species-specific oligosaccharide structures present on LPG was indicated by the strong staining of *P. sergenti* midguts following their incubation with purified PG from *L. tropica* relative to the staining observed with *L. major* or *L. donovani* PGs. It was not possible to use the PGs to specifically inhibit the binding of promastigotes because the *P. sergenti* midguts tended to degenerate rapidly following dissection, with a sloughing off of epithelial cells following 10–15 min incubation in PBS.

These data extend to an additional proven vector species a remarkable selectivity for the *Leishmania* species that it transmits in nature. As with the *P. papatasi–L. major* interactions described previously, the LPG that controls the specificity of *P. sergenti–L. tropica* interactions is characterized by a highly branched and species-specific structure. Although the backbone repeat units of both *L. tropica* and *L. major* LPGs are fully substituted, the side-chain sugars are distinct, with *L. tropica* synthesizing an abundance of side-chain sugars terminating in arabinose and glucose, and with *L. major* side-chains terminating in galactose. The reciprocal outcomes that these sugars are thought to control were demonstrated in parallel infection and midgut binding studies using *P. papatasi*. The full development of *L. major* promastigotes in these flies and the binding of *L. major* PG was confirmed, as was the loss of infections involving *L. tropica* and the relatively poor staining following incubation with *L. tropica* PG. These results provide strong additional support for the hypothesis that midgut attachment is a sufficiently critical event for the development of transmissible infections that the sandfly molecules controlling it, which presumably vary between vector species, provide strong selection pressure for LPG structural diversity. While we know nothing about the midgut receptors that are involved in *P. sergenti–L. tropica* interactions, preliminary information regarding a protein from *P. papatasi* midguts that binds to *L. major* LPG has recently been reported (Dillon & Lane, 1999).

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REFERENCES

ADLER, S. (1938). Factors determining the behaviour of *Leishmania* sp. in sandflies. *Harefuah* **14**, 1–6.

ADLER, S. & THEODOR, O. (1927). The behaviour of cultures of *Leishmania* sp. in *Phlebotomus papatasii*. *Annals of Tropical Medicine and Parasitology* **21**, 111–132.

ADLER, S., THEODOR, O. & WITENBERG, G. (1938). Investigations on Mediterranean kala-azar. 1. A study of leishmaniasis in Canea. *Proceedings of the Royal Society of London*, *B* **125**, 491–516.

AL-ZAHRANI, M. A., PETERS, W., EVANS, D. A., CHIN, C., SMITH, V. & LANE, R. P. (1988). *Phlebotomus sergenti*, a vector of *Leishmania tropica* in Saudi Arabia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 416.

BOROVSKY, D. & SCHLEIN, Y. (1987). Trypsin and chymotrypsin-like enzymes of the sandfly *Phlebotomus*

papatasi infected with *Leishmania* and their possible role in vector competence. *Medical and Veterinary Entomology* **1**, 235–242.

BUTCHER, B. A., TURCO, S. J., HILTY, B. A., PIMENTA, P. F., PANUNZIO, M. & SACKS, D. L. (1996). Deficiency in beta1,3-galactosyltransferase of a *Leishmania major* lipophosphoglycan mutant adversely influences the *Leishmania*-sand fly interaction. *Journal of Biological Chemistry* **271**, 20573–20579.

DILLON, R. J. & LANE, R. P. (1993). Influence of *Leishmania* infection on blood-meal digestion in the sandflies *Phlebotomus papatasi* and *P. langeroni*. *Parasitology Research* **79**, 492–496.

DILLON, R. J. & LANE, R. P. (1999). Detection of *Leishmania* lipophosphoglycan binding proteins in the gut of the sandfly vector. *Parasitology* **118**, 27–32.

GUILVARD, E., RIOUX, J. A., GALLEGO, M., PRATLONG, F., MAHJOUR, J., MARTINEZ-ORTEGA, E., DEREURE, J., SADDIKI, A. & MARTINI, A. (1991). [*Leishmania tropica* in Morocco. III – The vector of *Phlebotomus sergenti*. Apropos of 89 isolates.] *Annales de Parasitologie Humaine et Comparée* **66**, 96–99.

HEYNEMAN, D. (1963). Leishmaniasis in the Sudan Republic. 12. Comparison of experimental *Leishmania donovani* infections in *Phlebotomus papatasi* (Diptera: Psychodidae) with natural infections found in man baited *P. orientalis* captured in a kala-azar endemic region of Sudan. *American Journal of Tropical Medicine and Hygiene* **12**, 725–740.

KAMHAWI, S., ABDEL-HAFEZ, S. K. & ARBAGI, A. (1995). A new focus of cutaneous leishmaniasis caused by *Leishmania tropica* in northern Jordan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 255–257.

KILLICK-KENDRICK, R. (1985). Some epidemiological consequences of the evolutionary fit between Leishmaniae and their phlebotomine vectors. *Bulletin de la Société de Pathologie Exotique* **78**, 747–755.

KILLICK-KENDRICK, R. (1990). Phlebotomine vectors of the leishmaniases: a review. *Medical and Veterinary Entomology* **4**, 1–24.

KILLICK-KENDRICK, R., KILLICK-KENDRICK, M. & TANG, Y. (1995). Anthroponotic cutaneous leishmaniasis in Kabul, Afghanistan: the high susceptibility of *Phlebotomus sergenti* to *Leishmania tropica*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 477.

KILLICK-KENDRICK, R. & MOLYNEUX, D. H. (1981). Transmission of leishmaniasis by the bite of phlebotomine sandflies: possible mechanisms. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 152–154.

LAWYER, P. G., MEBRAHTU, Y. B., NGUMBI, P. M., MWANYUMBA, P., MBUGUA, J., KIILU, G., KIPKOECH, D., NZOVU, J. & ANJILI, C. O. (1991). *Phlebotomus guggisbergi* (Diptera: Psychodidae), a vector of *Leishmania tropica* in Kenya. *American Journal of Tropical Medicine and Hygiene* **44**, 290–298.

LIRA, R., MENDEZ, S., CARRERA, L., JAFFE, C., NEVA, F. & 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.

MAHONEY, A. B., SACKS, D. L., SARAIVA, E., MODI, G. & TURCO, S. J. (1999). Intra-species and stage-specific polymorphisms in lipophosphoglycan structure control *Leishmania donovani*–sand fly interactions. *Biochemistry* **38**, 9813–9823.

MCCONVILLE, M. J. & FERGUSON, M. A. (1993). The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochemistry Journal* **294**, 305–324.

MCCONVILLE, M. J., SCHNUR, L. F., JAFFE, C. & SCHNEIDER, P. (1995). Structure of *Leishmania* lipophosphoglycan: inter- and intra-specific polymorphism in Old World species. *Biochemistry Journal* **310**, 807–818.

MCCONVILLE, M. J., THOMAS-OATES, J. E., FERGUSON, M. A. & HOMANS, S. W. (1990). Structure of the lipophosphoglycan from *Leishmania major*. *Journal of Biological Chemistry* **265**, 19611–19623.

ORLANDI, P. A. JR. & TURCO, S. J. (1987). Structure of the lipid moiety of the *Leishmania donovani* lipophosphoglycan. *Journal of Biological Chemistry* **262**, 10384–10391.

PIMENTA, P. F., MODI, G. B., PEREIRA, S. T., SHAHABUDDIN, M. & SACKS, D. L. (1997). A novel role for the peritrophic matrix in protecting *Leishmania* from the hydrolytic activities of the sand fly midgut. *Parasitology* **115**, 359–369.

PIMENTA, P. F., SARAIVA, E. M., ROWTON, E., MODI, G. B., GARRAWAY, L. A., BEVERLEY, S. M., TURCO, S. J. & SACKS, D. L. (1994). Evidence that the vectorial competence of phlebotomine sand flies for different species of *Leishmania* is controlled by structural polymorphisms in the surface lipophosphoglycan. *Proceedings of the National Academy of Sciences, USA* **91**, 9155–9156.

PIMENTA, P. F., TURCO, S. J., MCCONVILLE, M. J., LAWYER, P. G., PERKINS, P. V. & SACKS, D. L. (1992). Stage-specific adhesion of *Leishmania* promastigotes to the sandfly midgut. *Science* **256**, 1812–1815.

SACKS, D. L., MODI, G., ROWTON, E., SPATH, G., EPSTEIN, L., TURCO, S. J. & BEVERLEY, S. M. (2000). The role of phosphoglycans in leishmania-sand fly interactions. *Proceedings of the National Academy of Sciences, USA* **97**, 406–411.

SACKS, D. L., PIMENTA, P. F., MCCONVILLE, M. J., SCHNEIDER, P. & TURCO, S. J. (1995). Stage-specific binding of *Leishmania donovani* to the sand fly vector midgut is regulated by conformational changes in the abundant surface lipophosphoglycan. *Journal of Experimental Medicine* **181**, 685–697.

SACKS, D. L., SARAIVA, E. M., ROWTON, E., TURCO, S. J. & PIMENTA, P. F. (1994). The role of the lipophosphoglycan of *Leishmania* in vector competence. *Parasitology* **108**, S55–S62.

SARAIVA, E. M., PIMENTA, P. F., PEREIRA, M. E. & DE SOUZA, W. (1983). Isolation and purification of amastigotes of *Leishmania mexicana amazonensis* by a gradient of Metrizamide. *Journal of Parasitology* **69**, 627–629.

SCHLEIN, Y. & JACOBSON, R. L. (1994). Haemoglobin inhibits the development of infective promastigotes and chitinase secretion in *Leishmania major* cultures. *Parasitology* **109**, 23–28.

SCHLEIN, Y. & JACOBSON, R. L. (1998). Resistance of *Phlebotomus papatasi* to infection with *Leishmania donovani* is modulated by components of the infective bloodmeal. *Parasitology* **117**, 467–473.

SCHLEIN, Y., JACOBSON, R. L. & MESSER, G. (1992). *Leishmania* infections damage the feeding mechanism of the sandfly vector and implement parasite transmission by bite. *Proceedings of the National Academy of Sciences, USA* **89**, 9944–9948.

SCHLEIN, Y., JACOBSON, R. L. & SHLOMAI, J. (1991). Chitinase secreted by *Leishmania* functions in the sandfly vector. *Proceedings of the Royal Society of London, B* **245**, 121–126.

SCHLEIN, Y. & ROMANO, H. (1986). *Leishmania major* and *L. donovani*: effects on proteolytic enzymes of *Phlebotomus papatasi* (Diptera, Psychodidae). *Experimental Parasitology* **62**, 376–380.

SCHLEIN, Y., SCHNUR, L. F. & JACOBSON, R. L. (1990). Released glycoconjugate of indigenous *Leishmania major* enhances survival of a foreign *L. major* in *Phlebotomus papatasi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 353–355.

TURCO, S. J. & DESCOTEAUX, A. (1992). The lipophosphoglycan of *Leishmania* parasites. *Annual Reviews in Microbiology* **46**, 65–94.

TURCO, S. J., HULL, S. R., ORLANDI, P. A. Jr., SHEPHERD, S. D., HOMANS, S. W., DWEK, R. A. & RADEMACHER, T. W. (1987). Structure of the major carbohydrate fragment of the *Leishmania donovani* lipophosphoglycan. *Biochemistry* **26**, 6233–6238.