LEISHMANIA DEVELOPMENT IN SAND FLIES AND MAIN ASPECTS OF THIS PARASITE-VECTOR INTERACTION

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Leishmaniasis are important vector-borne disease with a wide range of clinical symptoms in humans and domestic animals. Leishmania species infecting humans are transmitted by phlebotomine sand flies belonging to genera Phlebotomus and Lutzomyia. Here we summarize main aspects of Leishmania development in sand flies. In the vector, Leishmania development is confined to the digestive tract, mainly midgut, where parasites encounter various mechanical or biochemical barriers. The first one is mounted by a proteolytic attack by digestive proteases secreted following a bloodmeal. A second barrier is presented by peritrophic matrix surrounding the bloodmeal. At the end of bloodmeal digestion, when peritrophic matrix breaks, Leishmania must attach to midgut epithelium to prevent excretion with the remnants of the bloodmeal. In matured infections parasites migrate towards the thoracic midgut, destroy the stomodeal valve and produce promastigote secretory gel which blocks the midgut lumen. Blocked sand flies have problem to take a bloodmeal, bite repeatedly, increasing the chance of Leishmania transmission. Finally, parasites are injected into the vertebrate host together with PSG and sand fly saliva. Metacyclic promastigotes deposited into the host skin are swallowed by the phagocytic cells, mainly macrophages, and inside them transform to non-flagellated amastigotes.

KEY WORDS: Phlebotomus, Leishmania, parasite-vector interaction, sand fly, midgut.

INTRODUCTION

Leishmaniasis are a group of important human and veterinary diseases endemic in 88 countries, putting estimated 350 million people at risk and afflicting 12 million people per year. The causative agents of the disease are parasites of genus Leishmania, dixenous flagellates belonging to family Trypanosomatidae. Human infections are worldwide-spread and caused by more than 20 different species of Leishmania (Alvar et al., 2012).

Leishmaniasis cause a wide range of clinical symptoms. Some Leishmania species, like L. major, cause primary skin infections (cutaneous leishmaniasis) which may heal even without treatment. However, in several other Leishmania species the infection can spread and produce secondary lesions in the skin (diffuse cutaneous leishmaniasis), in the mucosa (muco-cutaneous leishmaniasis) and invade inner organs, mainly the spleen, liver and bone marrow (visceral leishmaniasis). Visceral leishmaniasis is a deadly disease occurring mainly in Indian subcontinent and East Africa, where the causative agent is Leishmania donovani. However, VL caused by another species, L. infantum, occurs also all around the Mediterranean basin and in Latin America.

Importantly, leishmaniasis are vector-borne diseases transmitted by phlebotomine sand flies (Diptera: Phlebotominae). During their life cycle, Leishmania parasites alternate between two major forms: flagellated promastigotes developing inside the digestive tract of sand flies and amastigotes, rounded forms without flagellum which develop inside phagocytic cells of vertebrate hosts. In sand fly, Leishmania development is confined to the digestive tract, mainly to the midgut.

Sand flies are tiny and hairy insects grouped in the order Diptera, suborder Nematocera. They seldom exceed 3 mm in body length. They are holometabolous insects and their life cycle includes egg, larva (four instars), pupa and adult. Larvae are terrestrial and develop in environment (soil, rodent burrows etc.) rich in organic content. Both males and females feed on sugar solutions, like nectar. Only females are hematophagous and require a blood meal as a source of proteins for oocyte development. The adult sand flies are active in the evening and during the night. Their flight range is typically very short, seldom exceeding hundreds of meters.
In Mediterranean countries their activity peaks during summer and they have one or two generation per year (Killick-Kendrick, 1999).

Two sand fly genera are involved in transmission of human leishmaniasis. While sand flies of genus Phlebotomus transmit the disease within the Old World, species of genus Lutzomyia serve as the vectors in the New World. In addition to Leishmania parasites, sand fly females also transmit other human pathogens, like bacteria (Bartonella) and viruses (mainly Bunyaviridae), which further increase their medical significance (Maroli et al., 2013).

Here we briefly summarize main aspects of Leishmania development in phlebotomine sand flies with emphasis to own studies on parasite-vector interaction.

**Leishmania Development Within the Sand Fly Midgut**

Sand fly midgut is composed of a single layered epithelium with a brush border of microvilli lining the lumen. In contrast, the foregut (including the stomodeal valve) and the hindgut (including the pyloric triangle) are lined by chitin. In subgenus Viannia and in reptile pathogens of genus Sauroleishmania, haptomonads attach also to chitin lining of the pylorus region, but members of subgenus Leishmania develop exclusively in the midgut and foregut (Lainson et al., 1977).

Population of Leishmania promastigotes in sand fly gut includes several morphological forms which differ in cell shape, flagellum length, motility, surface molecules and other biochemical properties. During the early stage infection, amastigotes ingested along with a bloodmeal into abdominal midgut transform first into procyclic promastigotes, these replicate and in few days transform to long nectomonads. At this stage of infection, barriers to Leishmania development may include proteolytic enzymes, immune molecules and the peritrophic matrix surrounding the ingested blood meal (reviewed by Dostalova and Volf, 2012).

In the midgut of unfed sand flies there is little baseline protease activity but the ingestion of bloodmeal induces secretion of digestive enzymes which can be attributed mainly to serine proteases, namely trypsin- and chymotrypsin-like enzymes (Dillon and Lane, 1993, Telleria et al., 2010). Leishmania are protected against proteolytic damage by surface glycoconjugates called phosphoglycans, in natural parasite-vector pair these molecules enable promastigotes to thrive in environment full digestive enzymes (Secundino et al., 2010, Svarovska et al., 2010). However, the role of effector molecules with potential to kill the Leishmania awaits further investigation. Alternatively, the killing effect may results from a combined action of midgut trypsins in concert with other, as yet unidentified, factors present in the midgut lysate. Immune-related sand fly molecules, like P. duboscqi defensin, when activated, may adversely impact the development of Leishmania in the midgut (Boulanger et al., 2004).

The peritrophic matrix (PM, previously known as peritrophic membrane) represents the main mechanical barrier to Leishmania development in the midgut. It is an acellular layer composed of chitin, proteins, and glycoproteins. In most insects encloses ingested blood meal, protects the midgut epithelium against pathogens and abrasion and compartmentalizes digestion between endo- and ectoperitrophic spaces (Lehane, 1997). In hematophagous insects the PM also performs a central role in heme detoxification (Pascoa et al., 2002). The role of the PM in vector competence seems to be dual. According to some authors (Pimenta et al., 1997), in the very early stage of infection the PM protect parasites transforming from amastigotes to promastigote stages, i.e., when they are vulnerable to proteolytic damage. Later, however, PM creates a physical barrier that prevents escape of parasites from the endoperitrophic space which may result in their defecation with blood meal remnants (reviewed by Bates, 2008).

The structure of PM is complex and rearranges during the course of blood digestion. Within several hours post blood meal a thin PM composed mainly of chitin fibrils covers the whole surface of the blood bolus. At later stages the PM gets thicker and matures; proteins and glycoproteins are incorporated in its structure and PM darkens due to heme incrustations. Few days later, the PM structure appears wrinkled and then starts to break down (Walters et al., 1993, Sadowa and Volf, 2009). It was a matter of debate whether the process of disintegration of the PM is caused only by sand fly own chitinases or is accelerated by Leishmania derived chitinase. In a natural vector – parasite pair L. major – P. duboscqi we clearly demonstrated that parasite chitinase has no role in disintegration of the PM: Leishmania “wait” until the PM is broken and migrate through its posterior opening. We concluded that the parasites taking advantage of the sand fly chitinolytic activity...
within the midgut is the main mechanism for their escape and *Leishmania* chitinase is not required for escape of long nectomonads from the peritrophic matrix-encased blood meal into the midgut lumen (SADLOVA and VOLF, 2009). However, it is important to note that the rate of formation and disintegration of the PM in blood-feeding Diptera is highly species-specific (LEHANE, 1997). As our preliminary experiments comparing PM persistence in various sand flies revealed striking differences between species, the further research on PM is needed. Anyway, this structure should be considered as an important factor contributing to the vector competence of sand flies.

The next important step in parasite life cycle is the establishment within the midgut. As the blood digestion proceeds, parasites need to bind to the midgut epithelium to avoid being excreted with the blood meal remnants. Following the escape from the endoperitrophic space, the parasites attach to the midgut, inserting their flagella between microvilli (reviewed by BATES, 2008). It has been postulated that this binding is the main determinant of parasite-vector specificity (PIMENTA et al., 1994). *Leishmania* gut binding is strictly stage-dependent (SACKS and PERKINS, 1985), is a property of those forms found in the middle phase of development (nectomonad and leptomonal forms), but is absent in the early blood meal and final stages (procyclic and metacyclic forms) (WILSON et al., 2010).

Based upon experimental tests of their ability to support development various *Leishmania* species, sand flies have been classified into two categories. Some sand fly species, namely *P. papatasi, P. duboscqi* and *P. sergenti*, display remarkable specificity for Leishmania they transmit. There appears to be a close evolutionary fit between *P. papatasi* and *P. duboscqi* with *L. major* and *P. sergenti* with *L. tropica*, as other *Leishmania* species survive poorly in these sand fly hosts. These sand fly species are called “specific” or “restrictive vectors”. In contrast, other Phlebotomus and Lutzomyia species tested to date support development of multiple *Leishmania* species and are thus called “permissive vectors” (reviewed by VOLF and MYSKOVA, 2007).

In specific vector *P. papatasi*, group of David Sacks showed that attachment is controlled by species-specific receptors for lipophosphoglycan (LPG) the main surface glycoconjugate on parasite surface. This specific receptor for terminal galactose on *L. major* LPG was identified as galectin (KAMHAWI et al., 2004). Studies using LPG-deficient parasites confirmed the crucial role of LPG in the attachment of *L. major* in the midgut of *P. papatasi* and *P. duboscqi* (SVAROVSKA et al., 2010). On the other hand, in permissive vectors the attachment does not require LPG; parasites deficient in LPG were able to survive well in four permissive vectors *P. arabicus, P. argenteipes, P. perniciosus* and *L. longipalpis* (MYSKOVA et al., 2007, SVAROVSKA et al., 2010).

Research on various sand fly species revealed an interesting correlation between permissivity and presence of N-acetyl-D-galactosamine (NacGal) - displaying glycoconjugates in the midgut of sand flies. These glycoconjugates were present in *Lutzomyia longipalpis, Phlebotomus baleensis, P. perniciosus, P. argenteipes* and *P. arabicus*, all known as permissive vectors. On the other hand, they were not present in *P. papatasi* and *P. sergenti*, two species known as specific vectors of *Leishmania major* and *L. tropica*, respectively. We proposed that these NacGal-containing glycoconjugates may serve as ligands for *Leishmania* attachment in permissive vectors; they are present on microvillar surface of the midgut, the right place for attachment of promastigotes, and they also bind to *Leishmania* surface in vitro (MYSKOVA et al., 2007). This new binding modality implies involvement of a parasite lectin-like receptor, similar to those described by SVOBODOVA et al. (1997).

Differences in glycosylation of sand fly midgut may have important consequences for vector competence of sand flies. Permissive vectors should be considered as potential vectors of various *Leishmania* species. In favourable conditions they may even spread *Leishmania* into new areas and establish new foci of leishmaniases. Probably the most important example is the establishment of *L. infantum* (= *L. chagasi*) in Latin America. In the Old World around Mediterranean Sea, *Leishmania infantum* is transmitted by *P. perniciosus* and other sand fly species belonging to subgenus Larroussius. However, when imported into the New World by conquistadores and their dogs, it was able to develop in *Lutzomyia longipalpis*, another highly permissive sand fly with midgut covered by NAcGal-containing glycoconjugates (VOLF and MYSKOVA, 2007).

Once attached to midgut epithelium and established in the midgut, parasites replicate vigorously and then migrate anteriorly. Parasite forms called short nectomonads (= leptomonal) accumulate in large numbers in the thoracic part of the
midgut. They produce promastigote secretory gel (PSG) containing filamentous proteophosphoglycan and creating a gel-like plug. This gel, together with parasite masses, physically obstructs the gut (ROGERS et al., 2002). Another morphological form, called haptomonad, attaches to cuticular lining of the stomodeal valve through an expanded flagellum containing hemidesmosomal structures and also contributes to obstruction of the digestive tract (VOLF et al., 2004). So called “blocked sand flies” have problem to take a bloodmeal, bite repeatedly, increasing the chance of Leishmania transmission (ROGERS and BATES, 2007).

Importantly, in matured infections there are also numerous small, rapid-swimming forms with an elongated flagellum that originate from leptomonads (reviewed by BATES, 2007). These are called metacyclic promastigotes and represent the stage highly infective for vertebrate host (SACKS and PERKINS, 1985). In vitro the metacyclogenesis was induced by several ways, like low pH or nutrient depletion, but very little is known about the signals triggering metacyclogenesis in the sand fly midgut. For L. major metacyclogenesis in P. papatasii the genetic locus encoding HASPs and SHERP, Leishmania-specific proteins of unknown function, was shown to be essential (SADLOVA et al., 2010).

The stomodeal valve, which represents the junction between anterior midgut and foregut, plays an extraordinary role in Leishmania development in transmission. This valve closes the anterior entrance to the midgut and in non-infected sand flies ensures one way flow of the food. It is composed by typical cylindrical cells covered by chitin lining (SCHLEIN et al., 1992, SADLOVA and VOLF, 2009). Below chitin, there are unique filamentous structures called apical filaments (SADLOVA and VOLF, 2009). The attached parasites cause damage to the structure of the stomodeal valve, interfering with its function and facilitating reflux of parasites from the midgut (SCHLEIN et al., 1992). Stomodeal valve of heavily infected sand flies seems to be permanently open, shape of cells is changed, chitin is separated from the apical end of cells and apical filaments are destroyed (VOLF et al., 2004). The destruction is likely due to the action of Leishmania chitinase (ROGERS et al., 2008).

In matured infections, metacyclic promastigotes are concentrated in the anterior part of midgut, close to stomodeal valve, sometimes invading also the pharynx, cibarium and proboscis. Some authors occasionally found Leishmania metacyclics in salivary glands of sand flies (KILLICK-KENDRICK et al., 1996) or in urine discharged by infected females during blood feeding (SADLOVA and VOLF, 1999). However, it is generally accepted that there are two main mechanism of transmission of metacyclic parasites: either small numbers of metacyclics present in the proboscis are deposited into the skin during feeding or parasites masses residing in thoracic midgut behind the stomodeal valve are regurgitated with a backflow of ingested blood (reviewed by BATES, 2008). Therefore, pathological changes of the stomodeal valve, together with obstruction of the thoracic midgut by PSG, are important for parasite transmission. However, in any case of transmission, transformation to metacyclic stages highly infective for the vertebrate host is the important prerequisite for effective transmission.

The cycle is completed when another non-infected female sand fly feeds of the infected host and subsequently spreads parasites to other vertebrates. Leishmania parasites are injected into the vertebrate host together with PSG and sand fly saliva. Metacyclic promastigotes deposited into the host skin are swallowed by the phagocytic cells, mainly macrophages, and inside them transform to non-flagellated amastigotes. Both, PSG and saliva has a crucial role in this event, immunomodulating the host and enhancing the virulence of the parasite, however, these interesting interactions are beyond the scope of this short review.

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RIASSUNTO

rompe la matrice peritrofica, i parassiti di *Leishmania* devono legarsi all’epitelio dell’intestino medio per prevenire l’escrezione con i resti del pasto di sangue. Nelle infezioni che pogrediscono i parassiti, traformati in promastigoti, migrano verso l’intestino toracico dove distruggono la valleia stomodeale e bloccano il lume dell’intestino con un gel noto PSG (Promastigote Secretary Gel). I flebotomi con PSG misto alla saliva del flebotomo. I promastigoti deposi-

**REFERENCES**


