THE JOURNEY OF *LEISHMANIA* PARASITES WITHIN THE DIGESTIVE TRACT OF PHLEBOTOMINE SAND FLIES

Shaden Kamhawi.

Laboratory of Parasitic Diseases; NIAID, NIH, Bethesda, MD 20892

INTRODUCTION

Leishmaniasis is a vector-borne disease caused by *Leishmania* parasites and transmitted exclusively by the bite of infected phlebotomine sand flies. In humans, leishmaniasis results in a spectrum of clinical manifestations in which the parasite, sand fly vector and host immune system act in concert to determine the outcome of disease. Sand flies are the driving force in the spread of leishmaniasis and prevalence of a *Leishmania* species is entirely governed by the availability of competent vectors. This lends itself to the question “what is a competent vector?” The vectorial competence of sand flies for a particular *Leishmania* species is complex and multi-factorial. Though extrinsic factors such as geographical distribution of the flies and their feeding preferences can limit the spread of a parasite species, vector competence is mainly determined by factors intrinsic to the fly, some of a general nature and others highly specific, that challenge the successful completion of the *Leishmania* life cycle within the digestive tract of the fly. Apart from the subgenus Vianna, termed peripylarian *Leishmania*, whose development includes a stage in the hindgut, all other species of *Leishmania* that produce disease in mammalian hosts are suprapylarian and confine their development to the midgut and foregut of the sand fly (1). Focusing mainly on suprapylarian *Leishmania*, this chapter reviews the advances we have made in our understanding of the perilous journey *Leishmania* parasites undertake within the digestive tract of sand flies to achieve successful transmission.

Overview of the life cycle of *Leishmania* in the sand fly

*Leishmania* parasites are dimorphic, existing as intracellular amastigotes within the macrophage of the mammalian host, and as flagellated promastigotes in the digestive tract of the sand fly. When a sand fly feeds on infected tissue of a mammalian host, amastigotes are ingested with the bloodmeal and pass directly into the abdominal part of the midgut. The blood stimulates midgut cells to secrete a chitinous protein matrix called the peritrophic membrane. The membrane envelops the bloodmeal within 4 hours (2) and is fully formed within 24 hours after blood ingestion (3). The
peritrophic membrane protects the gut epithelium from the contents of the bloodmeal, and acts as a barrier that regulates the diffusion of digestive enzymes secreted by gut epithelial cells (3). The digestion of the bloodmeal is achieved over 4-5 days after which the undigested remnants are excreted. A successful completion of the life cycle of *Leishmania* parasites in the fly requires that they survive the digestive enzymes of the host; avoid expulsion from the gut; and, at a later stage, migrate anteriorly and break free from the midgut epithelium for transmission to the mammalian host. To accomplish this, the parasites undergo several developmental changes, each adapted to overcome one or more of these barriers. Within 24 hours of ingesting an infected bloodmeal, amastigotes transform into short ovoid promastigotes called procyclics. These forms divide in the abdominal midgut forming rosettes that move sluggishly within the bloodmeal. Around 2-3 days post feeding, procyclics transform into large slender promastigotes termed nectomonads. These forms multiply rapidly and localize at the anterior part of the abdominal midgut. The peritrophic membrane begins to disintegrate 3 days after the bloodmeal due to a chitinase secreted by the sand fly. This disintegration is aided by a parasite-derived chitinase that accelerates the exit of nectomonads into the midgut lumen of the fly (4). Nectomonads attach to the lining of the midgut epithelium, which protects them from getting excreted with the undigested remnants of the bloodmeal, and continue to divide rapidly as they resume their anterior migration towards the thoracic midgut. At around 5-6 days post feeding, the bloodmeal is completely digested and the nectomonads begin their differentiation into two main forms, the haptomonads and metacyclics. Haptomonads are highly specialized forms that appear only in the area of the stomodeal valve. They adhere to the cuticular lining of the valve (5, 6) and to one another forming a plug that appears to consist of parasites embedded in a gelatinous matrix (6-10). The plug most likely blocks the food pathway and interferes with the working of the pharyngeal and cibarial pumps, making it difficult for the fly to engorge. This difficulty in feeding results in increased probing and promotes the transmission of metacyclics (5, 11-13). A study by Schlein et al. (4) demonstrated that a chitinase produced by the parasites damages the chitin based cuticular lining of the stomodeal valve, further disrupting the mechanics of feeding and contributing to enhanced transmission. The differentiating metacyclics are freely motile and accumulate just behind the stomodeal valve, well positioned for egestion from the mouthparts when the fly attempts to take another bloodmeal. Figure 1 illustrates the chronological appearance of the main forms of *Leishmania* developing within the digestive tract of a sand fly.

Molecules central to vector-parasite interactions during the critical stages of the *Leishmania* life cycle in the sand fly, and their relevance to vector competence are discussed below.