LEISHMANIA AND SAND FLIES

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Renewed interest in the relationships between *Leishmania* and their insect hosts was a development from the establishment, during the 1970s, of closed laboratory colonies of sand flies. Successful colonies of certain species had been established earlier but their maintenance proved to be time consuming and laborious. In the 1970s, it was found that *Lutzomyia longipalpis* is more amenable to laboratory colonization; one eminent leishmaniac went so far as to compare this species of sand fly with *Aedes aegypti*, a mosquito that thrives in laboratory conditions. This is certainly an over-evaluation of the capabilities of *Lu. longipalpis* to adapt to conditions that can be provided in insectaries. One can still easily lose what had previously been a flourishing laboratory colony. From experience in Belo Horizonte with various strains of *Lu. longipalpis* (*unpublished results*), inbreeding can lead to the emergence of a lethal gene that kills off males while they are still in the immature phases of development.

Recent studies on the interactions between *Leishmania* and sand flies have mainly used *Lu. longipalpis* as the experimental insect host. *Lu. longipalpis* is a proved natural host for the insect phase in the life cycle of *Le. chagasi* and laboratory observations on this host-parasite combination probably reflects real processes that occur in foci of American visceral leishmaniasis. In the laboratory, *Lu. longipalpis* serves as insect host for many other species of *Leishmania*, including strains from the Old World. When *Lu. longipalpis* is used as a laboratory model for any leishmanial parasite other than *Le. chagasi*, interactions between the parasites and their hosts must be view more critically. The same is true of other laboratory models that have been established. A meticulous study of the development of a species of *Leishmania* in *Lu. abonnenci* has recently been published. There is little or no field evidence that *Lu. abonnenci* blood feeds on man in nature. But sound field evidence exists that females of this species, together with females of closely related sand flies, are the natural insect hosts of species of *Endotrypanum*, intra-erythrocytic parasites in the blood-stream of sloths.

The basic developmental cycles of *Leishmania* in susceptible species of sand flies have been known for 60-70 years and appear to conform with the developmental cycle of the parasites in *in vitro* cultures. Concepts of *Leishmania*/sand fly relationships mainly derived from observations made in the Old World. A blodd feeding female phlebotomine ingests amastigotes which transfórm into promastigotes, which multiply in the abdominal portion of the stomach. Subsequently, promastigotes migrate through the thoracic portion of the stomach and aggregate at the stomodeal invagination, to which they attach themselves by the tips of the flagellum. There was some evidence of multiplication of amastigotes in the insect's stomach before transformation into promastigotes. There was also evidence of the presence of large and small promastigotes in the mid gut. It was suggested that only the small promastigotes migrate into the fore gut and, therefore, constitute an infective form. This idea dates from the late 1920s and early 1930s.

Studies in Panama during the 1960s showed that species of *Leishmania* now placed in the subgenus *Vianaia* have a different developmental cycle. The use of the electron microscope revealed the complexity of the developmental cycle of American species of *Leishmania* in Neotropical sand flies and the diversity of forms occurring in the digestive tract of the insect host. These studies revealed the presence, in the sand fly gut, of amastigotes, promastigotes and parama When *Lu. longipalpis* is infected with *Le. amazonensis*, there are two types of promastigotes : haptomonads and nectomonads. These various terms, introduced in the 1970s to define the morphological diversity of *Leishmania* within Neotropical sand flies, are now so widely used that no further explanation is necessary. It would seem that species of the subgenera *Leishmania* sensu stricta and *Vianaia* have adapted themselves, differently, to morphological and physiological differences that exist along the length of the digestive tract of sand flies.

The Belém research group has recently published studies, by light microscopsy, of the development of *Le. chagasi* in its natural insect host, *Lu. longipalpis*. These studies revealed that the amastigote phase persists in the sand fly stomach for much longer than hitherto supposed and that amastigotes multiply, within the blood meal enclosed by the peritrophic membrane, before transformation into promastigotes. But, after ingestion by the sand fly, amastigotes change in appearance and, eventually, produce two independent and distinct populations of promastigotes. Some ingested amastigotes retain the size they had in the mammalian host and give rise to small promastigotes that are infective to the next mammalian host. The other line of amastigotes enlarges and produces a population of large promastigotes. The small forms continue to multiply throughout their residence in the insect host, whereas the large form cease division at a certain time after the amastigotes were ingested. On present evidence, the large promastigotes seem to be a "dead end" in the reproductive cycle. In a biological scheme that reveals almost perfect adaptation, in sequence, between the flagellate parasites and their insect hosts, it is difficult to conceive that one of the two parallel populations is eventually consigned to the garbage bin. Those large promastigotes of *Le. chagasi* must surely play some vital rôle in the life cycle of the parasite.

The new information about the development of *Le. chagasi* in *Lu. longipalpis*, especially if it is confirmed in other host-parasite combinations, could put an end to current speculations that the life cycle of *Leishmania* in sand flies culminates in the development of a metacyclic promastigote, comparable to the metacyclic trypomastigote of *Trypanosoma* spp. Evidence for the development of a metacyclic promastigote is based on observations that the infectivity of the gut contents of sand flies increases progressively with the lapse of time after ingestion of amastigotes or the seeding of cultures. The studies on the *Le. chagasi/Lu longipalpis* combination suggests that increasing infectivity can be more simply explained by the numerical increase of small promastigotes derived from small amastigotes. Infectivity of gut contents before the development of sufficient numbers of small promastigotes can be explained by the persistence of multiplying amastigotes in the sand fly stomach.

There is a generally held belief that New World *Leishmania* are not deleterious towards their insect hosts. There is evidence, however, that promastigotes sometimes invade epithelial cells of the mid gut, damaging these cells. Unpublished observations made in Belo Horizonte have shown that there is an increased mortality of *Lu. longipalpis* 3-4 days after ingestion of amastigotes of *Le. amazonensis* and

and those of an un-named species of the *mexicana* complex. On the 3rd-4th days after ingestion of amastigotes, the blood meal has been digested and the parasites have been liberated for the confines of the peritrophic membrane. It is at this stage that promastigotes are inserting the flagellum between the microvilli bordering the epithelial cells of the mid gut, in parasites belonging to the subgenus *Leishmania* s.str. Field studies on *Trypanosoma (Megatrypanum) leonidasdeanei* in one of its insect hosts, *Lu. disneyi*, suggest that the amino acid depletion caused by the multiplying gut parasites disrupts the gonotrophic concordance of the host. A female *Lu. disneyi* infected with *T. leonidasdeanei* needs to take more than one blood meal to produce a batch of eggs. Female sand flies infected with *Leishmania* might also suffer amino acid deficiencies that result in a need to blood feed more frequently. Little attention has been given to the possible change in reproductive physiology of sand flies infected with *Leishmania*.

When promastigotes aggregate at the stomodeal invagination, they interefere with the blood feeding mechanism of an infected sand fly. Infected sand flies have difficulty in obtaining the blood needed for the next batch of eggs. In their frustration to get blood, infected sand flies repeatedly probe mammalian skin and, in each probe, small promastigotes can be inoculated. It thus happens that a single infected sand fly can cause multiple but adjacent lesions on a human host or infect several human hosts. The change in the blood feeding processes of female sand flies infected with *Leishmania* is of fundamental importance in understanding the epidemiology of human leishmaniases.

The bites of most species of Neotropical sand flies are extremely painful, even in individuals not previously exposed to sand fly bites. The mouth parts of the species of phlebotomines that normally blood feed on mammals are fine, delicate structures but they have a formidable armament that is best likened to miniature red-hot needles when inserted into the skin. The inoculative process of transit from the insect to mammalian host must often be assisted when the bitten victim suffers repeated probes and reacts by slapping the offending insect, thus splattering a teeming mass of small promastigotes around the puncture wound.