DISSERTATION ABSTRACT

Development of *Leishmania* parasites in vector sandflies (Diptera: Phlebotominae)

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Introduction. The work is dealing with two topics: (1) factors affecting development of *Leishmania* parasites in sandfly vectors and (2) mechanism of *Leishmania* transmission to the vertebrate host. It includes a review on *Leishmania* – sandfly – host interactions, four papers published in scientific journals and one manuscript submitted for publication. (1) Within the sandfly gut, *Leishmania* promastigotes encounter a number of factors which affect their development. They are usually well-adapted to the habitat inside the gut of their natural vectors, but characters of the parasites crucial for the development in the vector have not been elucidated. The main cell-surface molecules of promastigotes, lipophosphoglycan (LPG) and metalloprotease gp63, are known as the most important virulence factors influencing *Leishmania* interactions with the vertebrate host. However, their role in the vector part of *Leishmania* life-cycle remains to be clarified. The idea of the work is based on the fact that there are differences in the courses of sandfly infections with various strains and lines of *L. major*. Therefore, a detailed characterization of individual strains/lines may lead to the recognition of a functional significance of these molecules for the *Leishmania* development in sandflies.(2) *Leishmania* promastigotes are transmitted to the vertebrate host principally by the bite of infected female. However, we found viable promastigotes of *L. major* in urine droplets which infected females discharge during feeding. This finding suggests that contaminative transmission may additionally occur in the sandfly-*Leishmania* system and led us to the study of sandfly diuresis.

Aims of the work. (1) Comparison of four strains of *L. major* and various lines of the LV561 strain in: the development in *P. papatasi* and *P. duboscqi*, infectivity and virulence for BALB/c mice, expression of metacyclic LPG and haemagglutination activity, the amount and enzymatic activity of gp63 and the structure of gp63 genes and gene dosage. (2) Description of basic characteristics of prediuresis in two sandfly species *P. papatasi* and *P. duboscqi* and evaluation of the role of prediuresis for the possibility of contaminative transmission of *Leishmania*.

Summary of results. (1) Five lines of four *L. major* strains were used for experimental infections of *P. papatasi* and *P. duboscqi*. The highest infection rates were found for the more virulent line of strain LV561, while the lowest rates were recorded for strains L119 (low-virulence for mice) and Neal (avirulent for mice). The proportions of the different morphological forms of *Leishmania* seen in gut smears of infected flies varied considerably with the parasite strain/line. Transmission experiments were successful with *P. duboscqi* females infected with the virulent line of LV561. (2) Attenuated line LV561/AV was passaged five times through sandflies or mice and the resulting lines were used for further study: virulence for mice was not regained but resulting lines developed better than LV561/AV in *P. papatasi*. Expression of metacyclic LPG was not increased by passaging, however, a defect in LPG is not likely to be the only reason for the avirulence observed, as the avirulent lines of LV561 still produced about 10 times as many metacyclic promastigotes as the strain L119, which caused delayed lesions in mice. (3) The chromosomal location of the gp63 gene locus was conserved among the strains: gp63 probe hybridized to one chromosome-sized band of about 570 kb in all the strains and lines studied. Hybridization of gp63 probe to genomic DNA cleaved by various restriction enzymes and to Southern blots from PFGE revealed no significant differences among strains/lines in the structure and dosage of gp63 genes, respectively. However, marked differences were observed in amount and proteolytical activity of the gp63 protein: it was very low in attenuated LV561/AV line and slightly increased after five serial passages of this line through sandflies and mice. These parasites developed heavy infections in sandflies, but were avirulent for BALB/c mice. On the other hand, overexpression of gp63 was found in two strains (L119, Neal), which were defective in metacyclic LPG and unable to survive and multiplicate in sandflies, although they caused lesions in some of mice. The possibility that overexpression of gp63 may overcome the LPG defect is discussed. The results do not confirm a notable role of the surface protease in the vector part of *Leishmania* life-cycle while they support a significance of this molecule as a virulence factor. (4) Sandfly females, while feeding on the host, excrete urine to concentrate proteins of the bloodmeal and restore weight and water balance. This process was observed in 100% of *P. papatasi* and 85% of *P. duboscqi* females studied. In both species the prediuresis generally started 1-2 minutes after the commencement of feeding, the first one or two droplets were opaque whitish while the remaining ones were clear. Erythrocytes were found sporadically in first droplets of some females. The study revealed prediuresis in *P. papatasi* and *P. duboscqi* as a regular physiological process which may have consequences in transmission of infective diseases. (5) Promastigotes of *Leishmania major* were frequently detected in the urine droplets discharged by infected *P. papatasi* and *P. duboscqi* females during feeding. Parasites were present in the urine of 37.5% *P. papatasi* and 16.1 *% P. duboscqi* females, even in those with low intensity gut infections. Free-swimming forms predominated in excreted droplets. Viability of excreted parasites was proved by cultivation on blood agar, and the presence of metacyclic forms in urine droplets was confirmed by specific fluorescence assay with 3F12 antibodies. The possible role and significance of the discharge of promastigotes in sandfly urine are discussed.