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Research brief

Leishmania mexicana: promastigotes migrate through osmotic gradients

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Abstract

During the insect phase of the parasite lifecycle, *Leishmania* promastigotes move from the midgut to the anterior regions of the alimentary tract of their sandfly vector. Chemotaxis of *Leishmania* promastigotes towards sugars has been reported, and the putative presence of sugar gradient in the insect foregut has been suggested to play a role in promastigote development in the insect. We have further investigated the potential of *Leishmania mexicana* promastigotes to respond to chemical stimulii. We find that promastigotes move towards concentrations of all substances tested and that this taxis requires the presence of an osmotic gradient. Our results indicate that behaviour that has previously been interpreted as chemotaxis is better understood as osmotaxis. The implications of this observation are discussed in the context of promastigote development.

Index Descriptors and Abbreviations: Leishmania mexicana; promastigotes; taxis; osmotaxis; osmotic gradient; promastigote development; WIS, Washing and Incubation Solution

1. Introduction

Leishmania promastigotes are motile cells, swimming with the aid of their single flagellum. The importance of motility in promastigote development is not understood, although the generation of infectious metacyclic promastigotes involves the transition of parasites from the midgut to the mouthparts of the sandfly. Motile organisms typically respond to specific environmental stimuli by directed movement, a phenomenonen called taxis. *Leishmania* promastigotes may undergo taxis during their development in the insect vector.

Sandflies feed on plant-derived fluids that are composed principally of sugars. Sugar meals are initialy sequestered in the crop and subsequently released into the gut under the control of the stomodeal valve that delimits the cuticle-lined foregut from the midgut, lined with epithelial cells (Tang and Ward, 1998). Female sandflies augment their diet at the onset of oogenesis with blood that is delivered directly into the midgut. If the bloodmeal includes *Leishmania*-infected macrophages, amastigotes are released, transforming into promastigotes that multiply and colonize anterior regions of the insect midgut (reviewed (Killick-Kendrick, 1978; Schlein, 1986)). Metacyclic promastigotes, that are infectious to mammals (Sacks and Perkins, 1984), accumulate in the region of the stomodeal valve (Rogers et al., 2002), from where they are well positioned for transmission to a mammal when bloodfeeding next occurs. Movement of promastigotes to the front of the sandfly midgut is thus critical for efficient transmission.

The sandfly alimentary tract is divided into a number of specialized regions along its length, presenting a variety of chemical and structural features that might be exploited by promastigotes for orientation. Killick-Kendrick (Killick-Kendrick, 1978) first speculated that sugar concentration gradients might exist along the sandfly alimentary tract and that this could provide the stimulus for anterior migration of promastigotes during development. This suggestion was supported by results of the first attempts to measure chemotaxis in *Leish-*

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mania promastigotes (Bray, 1983), although these experiments were cumbersome to perform and revealed only moderate movement towards certain sugars. More recently Oliveira et al. (Oliveira et al., 2000), using an adaptation of a simple chemotaxis assay that has been widely exploited to study chemotaxis in prokaryotes (Adler, 1973), observed movement of *Leishmania* promastigotes towards concentration of all sugars that were tested (Oliveira et al., 2000). These results were interpreted as evidence of sugar chemotaxis, although the absence of a control substance that was not attractive to parasites left open the possibility that promastigotes were moving towards an osmotic gradient rather than, or in addition to, a specific chemotaxis.

We established a similar assay to that previously reported (Oliveira et al., 2000) to study taxis of *Leishmania mexicana mexicana* promastigotes (WHO reference strain M379). Important features of the assay and our modifications to the original method are described. Glass capillary tubes (75 mm long, 1 mm internal diameter) were loaded with a 1% agarose solution, prepared with WIS buffer (Oliveira et al., 2000) and putative attractant solute, such that exactly 1 cm at the end of each tube remained unoccupied by agarose gel. Promastigotes were grown to late-log phase in HOMEM medium, supplemented with 10% heat-inactivated fetal calf serum, at 25 °C, washed with WIS, resuspended in 1 ml WIS (supplemented with solute where appropriate) at a density of 2.5×10^7/ml, and placed in a small "bijou" tube. The open end of each capillary tube was filled with buffer of the same composition as that used to suspend promastigotes and capillary tubes were suspended vertically in the bijou tube, so that the open end of each capillary was immersed in the promastigote suspension. Capillary tubes were incubated at room temperature for 1 h with tape covering the open end of the bijou tube, to minimize evaporation. Tubes were carefully removed from the cell suspension and buffer (approximately 8 μl) was collected from the open end of each tube using a fine pipette and mixed with 8 μl of 1% formaldehyde to fix the cells. The density of the promastigotes in each sample was determined using an improved Neubauer cell-counting chamber and the mean density and standard deviation calculated for both controls and tests.

This simple taxis assay directly measures the number of promastigotes that move, over a period of 1 h, from a buffered cell suspension into the open end of a capillary tube that contains an agarose gel matrix containing a source of the putative chemoattractant. Diffusion of this substance from the capillary tube establishes a concentration gradient within, and diffusing from, the open end of the capillary tube (Oliveira et al., 2000). Estimation of the cell density within the open portion of the capillary tube permits a measurement of the attractive capacity of the substance under test. Each condition was replicated in six similar capillary tubes, and substantial variability was recorded between these replicates. This is to be anticipated as a result of the manipulations that occur during the course of the assay, and has been noted by previous workers (Bray, 1983; Oliveira et al., 2000). Interexperiment numerical variability was eliminated by expressing the mean movement (together with standard deviation) towards a specific solute relative to movement in the absence of the test substance (attraction coefficient). An attraction coefficient of 1 thus indicates no specific taxis towards the test solute. Under conditions where taxis was observed, the attraction coefficient was between 10 and 20 (Fig. 1).

We wished further to investigate taxis by *Leishmania* promastigotes, with the aim of identifying the substrate specificity of the previously reported chemotaxis response to sugars (Oliveira et al., 2000). We tested some of the hexoses that were previously found to be attractive to promastigotes and then applied the taxis assay to other compounds. In preliminary experiments (data not shown), we measured taxis towards a range of solutes, including pentose and hexose sugars, disaccharides, amino acids, and inorganic salts. Although substantial variability was observed between replicate samples, in all cases significantly more promastigotes were recovered from capillary tubes that contained a diffusible solute than from tubes that contained buffered agarose gel alone. Indeed, we were unable to identify a solute that

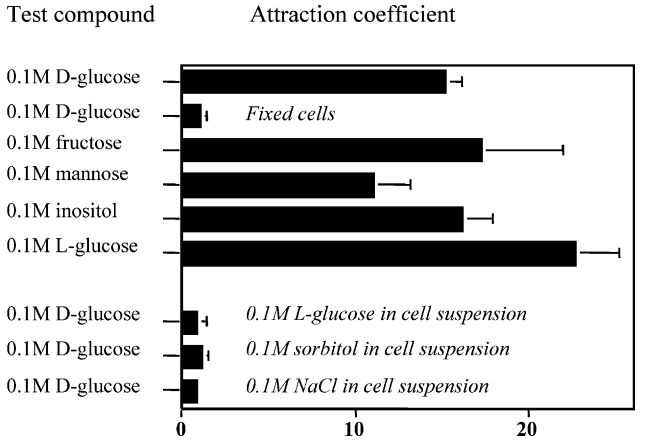

Fig. 1. Movement of promastigotes in the presence or absence of solute gradients. *Test compounds* (0.1 M) were included in the WIS-buffered agarose gel that partially filled each capillary tube. *Attraction coefficient.* The mean number of cells moving into capillary tubes containing the indicated test compound is expressed relative to the mean number of cells moving toward capillary tubes lacking the test compound. Error bars indicate the standard deviation within the attraction coefficient ($n = 6$). All experiments were replicated two or more times with similar results and representative examples are shown. *Fixed cells.* Promastigotes were preincubated for 10 min in 1% formaldehyde, to fix the cells prior to initiation of the taxis assay. *0.1 M solute in cell suspension.* Cell suspensions supplemented with various solutes to equalize the osmotic difference between test compound and cell suspension.

did not elicit a taxis response. Fig. 1 shows the results of taxis assays with D-fructose, D-mannose, sorbitol, myo-inositol, and both D-glucose and L-glucose. L-glucose is not a substrate for *Leishmania* hexose transporters (Burchmore and Hart, 1995), is not metabolized by *Leishmania* promastigotes (Schaefer and Mukkada, 1976) and may be presumed not to be abundant in the diet of the sandfly host. However, L-glucose elicited a similar taxis response to D-glucose, which is a major energy source for promastigotes in vitro (Hart and Coombs, 1982) and a major component of the sandfly diet. Since L-glucose is not likely to be encountered by the organisms this raised the possibility that *Leishmania* promastigotes exhibit osmotaxis, a phenomenon not previously described in any eukaryote.

In order to exclude the possibility that promastigotes were passively carried up concentration gradients, we performed taxis assays using promastigotes that had been fixed with formaldehyde. Fixed cells did not accumulate in capillary tubes containing D-glucose and similar numbers of fixed cells were recovered from tubes containing no solute source, indicating that promastigote motility is involved in the migration measured in this assay.

We then tested the movement of promastigotes towards D-glucose in the absence of an osmotic gradient by preparing cell suspensions in 100 mM L-glucose, sorbitol, or NaCl and assaying cell movement from these suspensions into capillaries containing 100 mM D-glucose. Under these conditions, where the solute levels were equimolar, no accumulation of promastigotes could be measured within capillary tubes. This result indicates that promastigotes are moving up an osmotic gradient.

The habitat of *Leishmania* promastigotes, within the alimentary tract of the sandfly vector is poorly characterized. Sandflies feed on a variety of plant sources as well as aphid honeydew (Young et al., 1980 and references therein). This diet is rich in both simple and complex sugars and a solute gradient may be established down the insect gut because water is extracted from the crop contents before they are gradually released into the midgut. Osmotaxis by promastigotes might thus explain how the parasites migrate into the anterior region of the insect gut. However, such gradients may not be stable because feeding is sporadic, mixing of gut contents may be significant and secretion, digestion and absorption will confound the maintenance of a steep downhill solute gradient from mouthparts to midgut. A sugar meal is required before transmission of *Leishmania* from sandfly to human (Wallach et al., 1982) but this could be because material released from the crop provides the sole nutrient source for parasites in the cuticle-lined foregut (Richards, 1975). A separate possibility is that osmotaxis represents an adaptation by which the parasite can avoid osmotic stress. *Leishmania* promastigotes react to osmotic changes in the environment, responding to osmotic stress by metabolism and secretion of alanine and other amino acids (Blum, 1993). Osmotaxis has been described in *E. coli* (Li et al., 1988) but, to our knowledge, this is the first report of osmotaxis in a eukaryotic cell.

This work calls into question the interpretation of previous studies (Bray, 1983; Oliveira et al., 2000) which concluded that movement toward sugar sources was evidence of sugar chemotaxis. The relatively large sugar concentrations used in this study and by previous workers are biologically relevant because sugars are abundant in the sandfly diet. The migration of *Leishmania* promastigotes from the insect midgut, where an infection is established, to the insect mouthparts, where infectious metacyclic promastigotes are found, is critical to the parasite life cycle. Promastigote motility may play a role in this migration and chemical stimuli seem likely to provide an orientation stimulus. Attractive substances might have their source in the insect mouthparts (saliva, ingested food, etc.) or alternatively might act as repellents produced in the midgut (digestive enzymes, products of digestion, or released parasite factors). Many of these substances will be present at only low concentrations that will give rise to negligible osmotic gradients. The taxis assay described here might be exploited in the identification of substances that elicit chemotaxis at concentrations that may not trigger osmotaxis.

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