Double Development Cycle of *Trypanosoma Cruzi* in the Opossum

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Opossums are important wild reservoirs of *Trypanosoma cruzi*, and by their frequent entry into houses can form a link between sylvatic and domestic cycles of Chagas disease transmission. The finding of a developmental cycle of *T. cruzi* in the anal glands of opossums therefore has epidemiological as well as biological significance.

T. cruzi was found to grow abundantly as epimastigotes and to transform into trypomastigotes identical to metacyclic forms, in the lumen of the anal glands of a high proportion of laboratory reared opossums (*Didelphis marsupialis*) that had been subcutaneously inoculated with faeces of infected triatomine bugs. No intracellular amastigotes were found in the various layers of the gland wall, not even in the thick striated muscular layer. The developmental phases in the lumen of the anal glands appear to mimic the parasite's developmental cycle in the intestinal tract of the insect vectors (Figs 1 and 2). At the same time, a classic intracellular cycle, with trypomastigotes in the bloodstream, was shown by patent parasitaemia, positive haemoculture and xenodiagnosis, and by finding amastigotes in myofibres of several muscles. All the opossums were positive in indirect fluorescent antibody tests. Parasites taken directly from the anal glands were cultivated in axenic media, infected triatomine bugs, mice and opossums, and were typed to the same schizodeme and zymodeme of those derived from the blood and those of the inoculated strain. No doubt, the population that developed in the lumen of the anal glands was part of the population inoculated and circulating in the tissues[1–5].

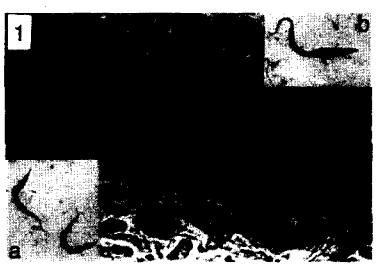

Fig. 1. Giemsa-stained preparations of the contents of the anal gland of one opossum infected with T. cruzi, *showing a mass of parasites, chiefly epimastigotes.* Insets: *smear of same material showing (a) two trypomastigotes, and (b) one epimastigote. (a) and (b) from Ref. 4.*

The anal glands of opossums appear to be involved in a defence reaction; they are present in both sexes and produce material with an offensive odour which is ejected when the opossum is attacked or irritated. The glands are paired and communicate through a narrow duct with the lumen of the rectum very near the anal opening (Fig. 3). Their contents are rich in neutral lipids and hyaluronic acid[6] and usually seem to be bacteriologically sterile, despite the rich rectal flora (C.A. Solari, unpublished).

T. cruzi epimastigotes in the glands are full of lipid inclusions[1]. There is no attachment to the epithelial cells of the gland wall, as has been described for these parasites in the rectum of their insect vectors[7,8].

Epidemiological Implications

The family Didelphidae is one of the most widely distributed mammalian groups in the Western Hemisphere[9]. All three species, *Didelphis azarae* (= *albiventris*), *D. marsupialis* and *D. virginiana* have been found naturally infected with *T. cruzi*. Due to their ample distribution, which spreads beyond the limits of the endemic areas of human trypanosomiasis, and to their high rates of natural infection, these marsupials are one of the most important wild reservoirs of Chagas disease. They are also incriminated as links between sylvatic and domestic transmission cycles, because of their omnivorous foraging habits, that frequently bring them into contact with human dwellings in rural and suburban areas[10,11].

Transmission of Chagas disease is normally maintained by domestic species of blood-sucking triatomine bugs. However, limited epidemics of acute human cases (with several deaths) have been reported outside the endemic areas and in the absence of domestic species of Triatominae. Investigation of two of these epidemics led to the conclusion that infections had been simultaneous and by the oral route, but the source remained obscure. In both cases opossums were suspected of being involved, either directly by contaminating food with their urine, or indirectly, through a sylvatic triatomine bug flying into the house and contaminating food with its faeces, (*T. cruzi* strains isolated from the human patients and from opossums captured in the neighbourhood belonged to the same zymodeme[12–15]). Our discovery of the cycle of *T. cruzi* in the anal glands of opossums supports the idea of direct involvement of this animal, without necessarily involving the insect vectors. In the laboratory we could infect mice by feeding them on a mixture of breadcrumbs moistened with milk and the material squeezed from the glands of an infected opossum; the mixture was infective for at least 24 h[16].

Even in endemic areas, infection of man by the oral route is probably more frequent than usually supposed, and according to some authors, should be suspected whenever an acute patient does not show the typical inflammatory reaction at the initial site of infection[12]. Contamination of food or the oral mucosa by triatomine faeces is thought to be the most probable mechanism, but opossums could also be directly involved by contamination of food with anal gland secretions.

Among other mammals, ingestion of infected Triatominae, or meat and viscera of infected animals, is undoubtedly a very important means of acquiring *T. cruzi* infection. This is particularly so for the omnivorous opossums[17]. Many sylvatic, domestic or semi-domestic species of Triatominae have been associated with opossums, either found in opossum nests or with opossum blood in their guts[10], but vector transmission does not easily explain the high rates of infection in opossums in areas where triatomine bugs are scarce[18].

Fig. 2. Epon-embedded semi-thin section showing parasites in the interior of the anal gland amidst secreted material and cells scaled off the inner glandular epithelial layer.

Fig. 3. The anal glands (arrow) in schiopubic region; the ducts (arrow-head) drain to the anus. The glans penis is bifid. (From Ref. 4.)

In our experience with almost 200 closely watched opossums we had no evidence of direct transmission of *T. cruzi* infection, despite the presence of the parasite in the anal glands of a number of them. However, our animals are normally maintained in individual cages, except during lactation and copulation, and it appears that tame specimens seem to forget the defence reaction which culminates with ejection of the anal gland secretion.

Biological Questions

Inside the opossum's anal glands *T. cruzi* obviously finds whatever it needs for nutrition and transformation, and is protected against the immune defences of the host. Could the intra-luminal situation be a sufficient explanation for this protection?

The influence of temperature in triggering transformation of digenetic trypanosomatids is well known. *T. cruzi* strains differ in the thermosensitivity of the amastigote–trypomastigote transformation *in vitro* and, in a study of *T. cruzi* in embryonated chicken eggs, the parasite underwent a double cycle in eggs incubated at 32–34°C, while only the intracellular cycle was found in eggs maintained at 37.5–39°C[19–21].

The average body temperature of opossums is lower than that of eutherian mammals, the rectal average in our specimens being 32.6°C (M.F. Dezonne, unpublished) and varying from 30.2 to 32.9°C for *D. azarae*[22]. So far, only those strains of *T. cruzi* originating from naturally infected opossums have developed in the anal glands of our inoculated animals. It may be that body temperature is one of the factors responsible for the double cycle of *T. cruzi* in the opossum, and this may lead to opossums exerting a selective effect on particular strains of the parasite[23].

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LETTERS

Is *Trypanosoma brucei* an intracellular parasite?

Sir – It has been known for half a century that suramin will only cure infections with *Trypanosoma brucei* if treatment is given in the early stages of the disease. The greater the delay in treatment, the more likely is a relapse of the infection.

Several groups[1–3] have shown that trypanosomes can be found in the tissues of the brain and its appendages. However, the significance of this finding only became apparent when Jennings *et al.*[4] showed that suramin could clear trypanosomes from all tissues of the body except those of the brain, indicating that the relapse of infection would most likely originate from parasites in the brain rather than from other parts of the body. Protection of the trypanosomes from suramin by the 'blood/brain barrier' appeared to many to be a satisfactory explanation of this phenomenon, despite the earlier finding by Keevil[5] that suramin could penetrate this barrier and clear trypanosomes from the cerebrospinal fluid.

Abolarin and colleagues[6] have published electron micrographs of ependymal cells in the choroid plexus which demonstrate the possibility that these specialized cells may harbour the trypanosomes in an intracellular form. The same group, at the 23rd International Congress of Protozoology, demonstrated further 'intracellular trypomastigotes' in the ependymal cells of the choroid plexus (Fig. 1) and also in the same cell type which forms the lining of the ventricles of the brain.

Critics of this work have raised several objections. Firstly they have pointed out that trypomastigotes are such long organisms that it would take about a thousand serial microsections to display a whole individual, and that a few such sections could hardly demonstrate that the whole trypomastigote was inside an ependymal cell – a part of it could just be poking into the host cell and the rest remaining extracellular. Secondly the critics emphasize the state of degeneration of the affected cells and the rarity with which convincing 'intracellular' trypanosomes are seen within them; they argue that if the same amount of time had been spent in looking at other parts of the brain as had been devoted to those that contain ependymal cells, the same appearances of intruding trypomastigotes and degenerating cells might have been found at other sites. Finally it has been shown that apparently intracellular trypomastigotes can also be found in tissues outside the brain, mainly in plasma cells[7]. In evaluating this apparently serious objection one has to remember that the