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INFECTION OF A MAMMAL BY MONOGENETIC INSECT TRYPANOSOMATIDS (KINETOPLASTIDA, TRYPANOSOMATIDAE)

ANA M. JANSEN, JOÃO C. CARREIRA & MARIA P. DEANE

Instituto Oswaldo Cruz, Departamento de Protozoologia, Caixa Postal 926, 20010 Rio de Janeiro, RJ, Brasil

Monogenetic insect trypanosomatids of the genera Crithidia, Leptomonas *and* Herpetomonas, *multiplied as in axenic cultures, for many months, in the lumen of the scent glands of the opossum* Didelphis marsupialis. *Specific antibodies were detected in the serum of the animals but there was no evidence of invasion of their tissues by the parasites.*

Key words: monogenetic trypanosomatids – *Crithidia* – *Leptomonas* – *Herpetomonas* – opossum – *Didelphis marsupialis* – scent glands

As previously reported (Deane et al., 1984, 1986; Deane & Jansen, 1986; Lenzi et al., 1984), trypanosomes of two different subgenera, *Trypanosoma (Schizotrypanum) cruzi* and *T. (Megatrypanum) freitasi*, were found multiplying as epimastigotes and differentiating into metacyclic trypomastigotes in the lumen of the scent glands of experimentally or naturally infected opossums of the species *Didelphis marsupialis*. In this site the flagellates reproduce the cycle that occurs in the intestinal tract of insect vectors, while the phases corresponding to the cycle in the vertebrate are found in the opossum tissues. These findings prompted us to investigate if the glands could support growth of other trypanosomatids that develop in insects. We wished also to know if antigenic material could pass from the glands into general circulation and stimulate immunological response in the absence of a general infection.

MATERIAL AND METHODS

Parasites – Representatives of three genera of monogenetic trypanosomatids were used: *Crithidia deanei* Carvalho, 1973 (ATCC 30255), *Leptomonas samueli* Carvalho, 1973, *Herpetomonas samuelpessoai* (Roitman et al., 1976) (ATCC 30252) and *Leptomonas* sp. The organisms were kindly supplied in cultures, by Agrellos Filho, of the Microbiology Institute, Federal University of Rio de Janeiro.

Opossums – These were adult specimens reared in the laboratory from pouch young of a captured *D. marsupialis* female.

Inoculations – Cultures in liquid (LIT) medium were washed twice in PBS, the last sediment resuspended in a small volume and the flagellates counted in a Neubauer camera.

The animals were anesthetized with Ketalar (Parke Davis) and injected in each of the two glands as follows: after manual expression to reduce the volume of the intraglandular contents, a small incision in the skin permits removal of subcutaneous fat tissue and exposure of the gland surface; with a 1 ml/0.01 syringe and a 27,5 G needle, 20-30 μl of the PBS suspension containing $10^6 - 10^7$ flagellates are injected, and the procedure is repeated for the other gland. With some experience and careful manipulation it is possible to inject directly in the lumen of the glands and to avoid reflux of the material when the needle is withdrawn. Under adequate aseptical conditions secondary infections are exceptional.

Examinations – Just before inoculations the animals were bled from the femural vein for hemoculture and serology and the material obtained by expression of the glands was examined in fresh preparations. Starting 12-15 days post-inoculation the same examinations were made periodically.

Serology was by an indirect immunofluorescence test (IFAT) using a "sandwich" method developed in our laboratory (Jansen, et al., 1985) and antigens prepared from culture forms of the trypanosomatids included in the experiment; controls were sera of non-inoculated opossums. Triple N with a LIT overlay was used

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for the hemocultures which were examined fortnightly in fresh preparations, up to 60 days.

RESULTS

The glands were positive from the first to the last examination made when the animals were killed: 6 months post-inoculation for *H. samuelpessoai, C. deanei* and *L. samueli*, and 10 months for *Leptomonas* sp. Amidst the coarse glandular material with much cellular débris, the parasites were quite abundant and motile. In Giemsa-stained smears, morphology was as usual in cultures but the flagellates had cytoplasmic inclusions probably of lipids, as it has been described for *T. cruzi* in identical localization (Deane et al., 1984; Lenzi et al., 1984). Many dividing forms and rosettes were common for all the flagellates and opisthomastigotes were seen among the herpetomonas.

The hemocultures, a total of 6-8 in duplicate tubes for each opossum, were always negative.

IFAT was negative on the day of inoculation and started to give consistently positive results within a month with titers that went from 1:40 up to 1:640, more frequently of 1:160 and that are equivalent to those we have detected in *T. cruzi* infected opossums.

The animals were in apparent good health throughout.

COMMENTS

Conversion of IFAT after inoculation is an indication that antigenic material can pass from the glands into general circulation and stimulate the synthesis of specific antibodies. The fact that hemocultures were always negative suggests that the humoral response was against soluble antigens. However we intend a more detailed investigation of these aspects that could help us to understand the actual barriers that prevent a monogenetic trypanosomatid to settle in mammalian tissues.

Working with a *Crithidia* sp. isolated from a phytophagous bug, McGhee (1957) found the flagellate multiplying abundantly in chick embryos, was able to isolate the protozoon from their liver and to make serial transfers to other chick embryos. He concluded that the possibility of a crithidia infecting a vertebrate host had been demonstrated and that the fact could "shed some light on the little understood subject of evolution of parasites".

We have now demonstrated that monogenetic insect trypanosomatids can infect a mammalian host. Our own speculations on the significance of these findings are the subject of another paper (Deane & Jansen, 1988).

RESUMO

Infecção de um mamífero por tripanosomatídeos monogenéticos de insetos (Kinetoplastida, Trypanosomatidae) – Tripanosomatídeos monogenéticos de insetos, dos gêneros *Crithidia, Leptomonas* e *Herpetomonas* foram inoculados no lumen das glândulas de cheiro do gambá e aí se multiplicaram como em culturas axênicas durante muitos meses. No soro dos animais foram detectados anticorpos específicos mas não houve evidência de invasão dos tecidos pelos parasitas.

Palavras-chave: tripanosomatídeos monogenéticos – *Crithidia* – *Leptomonas* – *Herpetomonas* – gambá – *Didelphis marsupialis* – glândulas de cheiro

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