LEISHMANIA HERTIGI SP. N., FROM THE TROPICAL PORCUPINE, COENDOU ROTHSCHILDI THOMAS

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ABSTRACT: A skin-inhabiting species of Leishmania found in Panama in the tropical porcupine, Coendou rothschildi, is considered new and described as Leishmania hertigi sp. n. L. hertigi is differentiated from other species of Leishmania found in mammals mainly by the nature and course of the infection in its natural host. It is apparently harmless to its host, in which it produces a long-lasting infection, showing a well-established host-parasite relationship. There is a complete absence of any gross skin alteration due to the infection, although the parasite frequently is found in skin over the whole body. L. hertigi seems to be host-specific.

In the search for reservoir hosts of buman cutaneous leishmaniasis in Panama a new approach was initiated at the Gorgas Memorial Laboratory in March 1965. Starting from the hypothesis that natural leishmanial infections among animals might occur with no gross skin alterations, wild-caught mammals were investigated using skin smears and the recently described biopsy-skin-culture technique (Herrer et al., 1966). The latter technique greatly simplified the detection of parasites in the skin and proved to be very useful in these studies.

Early in the investigation, a tropical porcupine, Coendon rothschildi Thomas, was found lightly infected. Subsequent studies showed a high prevalence rate.

Morphological and other studies indicate that this is a new species which is described in this paper and named *Leishmania hertigi*.

MATERIALS AND METHODS

Porcupines (C. rothschildi) were obtained alive from different localities throughout the central part of the Republic of Panama. Skin from several parts of the body was cultured by the biopsy-skin-culture technique. Skin cultures were made periodically from porcupines maintained in captivity to study the course of the infection. Cultures were also made from skin, liver, and spleen at autopsy. Skin smears were prepared only in certain cases, and skin samples were preserved for sections from animals with positive skin smears.

Senekjie's culture medium was used throughout this study, and all cultures were incubated at 19 to 22 C. The parasite could be maintained for about

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a month before transfer to a new culture. Promastigate (leptomonal) flagellates used for the morphological description were from 2 strains maintained for several months after isolation and were taken from the culture slant 6 to 8 days after a transfer. Drawings were made with the aid of a camera lucida and 80 flagellates were measured for the description.

Leishmania hertigi sp. n. (Fias. 1-18)

Morphological description (all measurements in microns)

Cultural forms

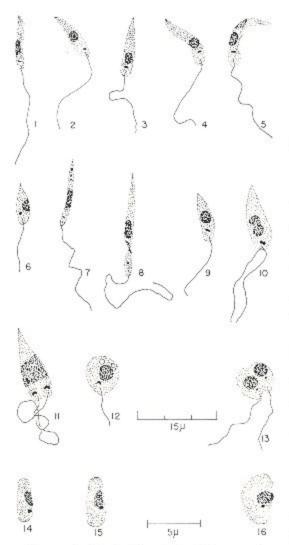
Promastigote body length, 7.5 to 20.5; body width, 1.3 to 4.1; free flagellum, 9.1 to 35.5 (in rare slender forms). Posterior end to middle of nucleus, 3.4 to 14.5; middle of nucleus to anterior end, 3.6 to 9.1; kinctoplast to anterior end, 1.4 to 3.6; diameter of nucleus (only rounded and/or ovoid forms considered), 1.4 to 3.6.

Anterior end somewhat rounded (Figs. 2-5, 9). Body width frequently tapering rather abruptly (Figs. 2-4, 9), similar to some promastigotes of L. adleri (Heisch, 1958), some of these showing a bananalike appearance (Fig. 4). Slender forms larger than ordinary promastigotes and with a long flagellum (Figs. 7-8). Nucleus rounded, somewhat elongated in anterior-posterior direction in slender forms (Fig. 7), located about middle of body or slightly anterior in most specimens. Kinetoplast medium-sized and rounded, elongated or kidneyshaped, near anterior end in slender promastigotes (Figs. 7-8), closer to nucleus in broad flagellates (Fig. 6). Rosettes uncommon. Both elongated and rounded forms found in binary fission (Figs. 10-11, 13). Oval or rounded forms occasionally with a short flagellum, some with vacuoles in the cytoplasm (Fig. 12).

Amastigote or tissue form

Found mainly in the upper dermis (Figs. 17-18) where, except for some vacuoles in the cytoplasm of host cells, there is no cell or tissue reaction to its

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FIGURES 1-16. Leishmania hertigi sp. n. from the tropical porcupine, Goendou rothschildi, drawn from Giemsa-stained smears. 1-13. Common forms found in cultures. 14-16. Amastigote forms, or L-D bodies, from the skin of its natural host.

presence. In skin smears the parasite is conspicuously clongated (Figs. 14–15), with one end slightly broader than the other. Kinetoplast frequently rounded and located close to the nucleus (Figs. 15–16). Among 30 L-D bodies found in smears from 3 different porcupines, which were used for biometric purposes, only 2 ovoid forms were found, one of which is presented in Figure 16. Body length varies from 3.5 to 4.8 μ and the body width, from 1.2 to 2.5 μ . Nucleus is usually ovoid and most of the time adjacent to the cell wall.

Host: Coendou rothschildi Thomas, tropical porcupine.

Type locality: Quebrada Bonita, central Panama.

Prevalence: 83 (88%) of 94 specimens infected. Infection rate similar in both sexes. Older specimens more frequently infected.

Site of infection: Upper dermis, especially on

dorsal side. Also spleen and liver.

Longevity of infection: For at least 30 months and probably for lifetime. No observed hosts lost the infection.

Pathogenesis: None observed. No lesions present on skin or viscera from which the parasite was cultured.

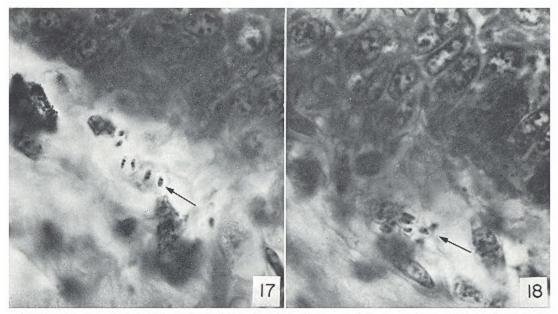
Susceptibility of laboratory animals

The golden hamster, Mesocricetus auratus, the cotton rat, Sigmodon hispidus, the jerboa, Jaculus jaculus, and the domestic guinea pig, Cavia porcellus, have been experimentally inoculated with cultures of L. hertigi. Ten different strains, both original isolates and subcultures up to the 20th transfer, were used. Most of the animals were inoculated intradermally on the nose, with large numbers of flagellates.

For some weeks rare L-D bodies were observed in skin smears from the site of the inoculation, especially in hamsters, although they showed no gross skin alterations. The parasite could be recovered up to about 1 year in exceptional cases in hamsters, but only by cultural methods. In all eases parasites were recovered only from the site of inoculation.

Immunological difference between L. hertigi and L. braziliensis s. lat.

Hamsters inoculated with L. hertigi and challenged later with virulent strains of L. braziliensis were not protected against the latter species of Leishmania. Challenge inoculations were performed at different intervals from 1 to 5 months after the original inoculation with L. hertigi and the results were assessed according to the size and persistence of swelling (due to the challenge inoculation) at the site of inoculation, as well as to the intensity of the parasitism. Cultural forms were always used as inoculum, the number of flagellates inoculated being about the same in most of the cases both for L. hertigi (original inoculation) and L. braziliensis (challenge inoculation). There was no indication that L. hertigi conferred protection against the challenge inoculation with L. braziliensis, which probably indicates absence of immunological relationships between the two species.



Figures 17–18. L-D bodies of L, hertigi in the skin of its natural host. Sections fixed in 10% formal-dehyde and stained with Giemsa's stain; about \times 1,000. 17. Six characteristic, elongated L-D bodies close to the lower limit of the epidermis. The rounded kinetoplast is visible. A vacuole partially surrounding two L-D bodies also shown. 18. A group of seven L-D bodies; here again the elongate nature of the parasite is evident.

DISCUSSION

Only Leishmania species that have been found parasitizing mammals are compared in this discussion.

- 1. The most striking aspect of L. hertigi is the nature and the course of the infection in its natural host, the tropical porcupine, C. rothschildi. It seems that L. hertigi is harmless to its host, although the parasite is widely distributed throughout the skin and frequently reaches the internal organs. The infection appears to persist for the life of the animal. There is no case among other species of Leishmania so far found infecting mammals that shows these characteristics,
- 2. L. hertigi appears to be host-specific in wild animals. Although during the last 5 years about 2,300 forest mammals were processed searching for natural leishmanial infections, L. hertigi was found only in the porcupine, C. rothschildi. This is in contrast to L. braziliensis and L. mexicana, which are found in a variety of animals belonging to five different orders.

Moreover, in several species of rodents experimentally inoculated with cultures of *L.* hertigi, no case comparable to the natural infection of the porcupine was observed. In all inoculated animals the parasite was seen transitorily in skin smears and only at the site of inoculation. By cultural methods the inoculated parasite was sometimes recovered for months, but again only from the site of inoculation. The consistently negative results of cultures made from other parts of the skin and viscera indicate that the parasite does not become disseminated from the site of inoculation in the experimental hosts used.

- Immunological differences seem to exist between L. hertigi and L. braziliensis, since hamsters inoculated with cultures of the porcupine leishmania acquired no protection to a challenge inoculation with virulent strains of L. braziliensis.
- 4. Although morphology by itself is not an accepted criterion for the differentiation of species of *Leishmania* it should be emphasized that the amastigote of *L. hertigi*, in skin smears, is conspicuously elongated.

The above characteristics indicate that L. hertigi is readily differentiated from other species of the genus Leishmania. This new species is dedicated to my old friend and colleague, Dr. Marshall Hertig, in recognition of his many contributions to the study of leishmaniasis.

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