LEISHMANIA MEXICANA COMPLEX: HUMAN INFECTIONS IN THE REPUBLIC OF PANAMA

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Abstract. Parasites of the genus Leishmania responsible for human cutaneous leishmaniasis in the New World form 2 major taxonomic divisions: the Leishmania braziliensis and the L. mexicana complexes. We report the isolation and characterization of the L. mexicana complex among humans in the Republic of Panama. Characterization was based on parasite morphology, pathogenesis in infected golden hamsters, cellulose acetate isoenzyme electrophoretic mobilities, and membrane-specific monoclonal antibodies using the radioimmune binding assay technique.

Human cutaneous leishmaniasis was first reported in the Republic of Panama in 1910 by Darling. Research on the ecology of the disease was initiated in 1947 at Gorgas Memorial Laboratory (GML) where 5 sand fly species and 8 sylvatic hosts were incriminated in the epidemiology of the parasite Leishmania panamensis, a member of the L. braziliensis complex which, until recently, was thought to be the only agent responsible for human cutaneous leishmaniasis in Panama.

The isolation of L. aristides from 3 species of rodents and a single marsupial, Marmosa robinsoni, in Sasaardi, San Blas Territory in 1968 documented the presence of a member of the L. mexicana complex. To date, this parasite has never been isolated from humans. We report 2 cases of human cutaneous leishmaniasis due to L. mexicana complex in Panama.

MATERIALS AND METHODS

During 1985–1986, 839 cases of cutaneous leishmaniasis were seen at the GML clinic. Diagnostic procedures included the Montenegro skin test, Giemsa-stained smears, and culture of tissue sample. The methodology for skin tissue cultures has been described.

Three hundred isolates from the 839 patients seen, mainly from the provinces of Panama, Colon, and Darien, have been screened by isoenzyme analysis using cellulose acetate electrophoresis (CAE). Preparation of extracts and conditions for antigens used in the technique have been described. Nine enzymes systems were used in the CAE testing: ALAT E.C.2.6.1.2, (alanine amino transferase), ASAT E.C.2.6.1.1, (aspartate amino-transferase), PGM E.C.2.7.5.1, (phosphoglucomutase), PGI E.C.5.3.1.9, (phosphoglucoisomerase), MDH E.C.1.1.1.37, (malic enzyme), G-6-PDH E.C.1.1.1.49, (glucose 6-phosphate dehydrogenase), 6-PGDH E.C.1.1.1.44, (6-phosphogluconic dehydrogenase), and MPI E.C.5.3.1.8, (mannose phosphate isomerase). World Health Organization (WHO) strains for L. pifanoi (MHOM/VE/57/L11, L. venezuelensis (MHOM/VE/74/PM-H3), L. garnhami (MHOM/VE/73/JAP78), L. mexicana (MHOM/BR/82/BEL21), L. amazonensis (MHOM/BR/73/M2269), and L. aristides (MORY/PA/68/GML3) were used as L. mexicana complex reference markers.

Only 3 of the isolates, which were recovered from 2 patients, differed in their isoenzyme patterns from those of L. panamensis. These isolates were also characterized by radioimmune binding assay (RIA) at Yale University, New Haven, CT, using monoclonal antibodies (Mabs) against L. mexicana (L11, M379) and L. amazonensis (WR 303, H6, LV72). Promastigotes isolated from both patients were characterized by parasite morphology and pathogenesis in infected golden hamsters (Mesocricetus auratus).

RESULTS

During investigations on leishmaniasis in eastern Panama in April 1985, a 41-year-old Caucasian entomologist spent many hours with the field crew in human-bait collections of phlebotomines. In June of that year, a papule was observed on his lower right leg which later developed into a 17 x 21 mm dry, scaly lesion (Case
No. 1). Giemsa-stained skin scraping of the lesion showed numerous amastigotes larger than those observed in most cases seen at the GML clinic. The Montenegro skin test was positive with an induration 9 mm in diameter. Sodium stibogluconate (Pentostam) was prescribed but the patient received treatment for only 2 days. The treatment was discontinued, at the patient’s insistence, to allow study of the natural course of the infection. The lesion healed spontaneously 11 months after the infection was first observed. No relapse has been observed after 3 years.

Between December 1985 and April 1986, 8 isolates were cultured in Senekjie’s medium and the original isolate was cloned. Nine of the clones and 7 of the other isolates showed identical CAE electrophoretic mobilities for the 9 enzymes systems tested. These mobilities were indistinguishable from those of the WHO reference strains of *L. amazonensis* (MHOM/BR/73/M2269) and *L. garnhami* (MHOM/VE76/JAP78). The original isolate reacted against *L. amazonensis* (WR, 303, H6, LV72) Mabs by radioimmune binding assay.

Case No. 2, a 17-year-old mestizo involved in hunting and agriculture in the northern and eastern part of the country, noted skin lesions 2 months prior to visiting the GML clinic in October 1986. He was exposed to sand fly bites during hunting in the sylvatic areas of Alcalde Diaz in Panama Province and during agricultural activities in Darien Province. The patient had 2 ulcerative lesions on his right forearm (17 × 19 mm and 17 × 23 mm). There was no evidence of local lymphatic dissemination. The Montenegro skin test was positive with an induration of 12 mm. He was treated with oral ketoconazole 600 mg daily for 28 days. At the end of treatment, November 1986, the ulcers were 16 × 13 mm and 20 × 20 mm. An evaluation in December 1986 showed complete healing of both ulcers. In January 1987 he developed a reactivation with the appearance of a new papular lesion surrounding the area of the scars. He was treated im with sodium stibogluconate (Pentostam), 850 mg every other day for 20 days. He did not return until August 1987, by which time both lesions had healed. No reactivation has been observed for 2 years. Two isolates were made, 1 in October and 1 in November 1986. Isoenzyme analysis on these isolates revealed profiles that did not co-migrate with any of the known species within the *L. mexicana* complex used as markers by WHO. At least 3 enzymes, MPI, PGI, and ME, separated them from *L. amazonensis* and *L. mexicana* (Fig. 1).

Eight clones each were made of the 2 original isolates by streaking a loop of diluted, 8-day-old culture onto plated solid blood agar media, which was then sealed and incubated at 22°C for 8 days in a humidified chamber. Individual colonies were placed in culture tubes by means of a bacteriological needle. This was followed by massive growth. Colonies were tested by CAE. All clones from each original isolate showed identical isoenzyme profiles, but the clones from each isolate differed from those of the other, as well as from the original isolates.

Clones from the October isolate co-migrated with the WHO reference strain for *L. amazonensis* (MHOM/BR/73/M2269), while clones from the November isolate co-migrate with the WHO reference strain for *L. mexicana* (MHOM/BZ/82/BEL21). Characterization of clones using Mabs against *L. mexicana* (L11, M379) and *L. amazonensis* (WR 303, H6, LV 72) corroborated the CAE results.

Isolates from both patients inoculated in noses of golden hamsters (*M. auratus*) produced the large tumors characteristic of *L. mexicana* infection at the site of inoculation within 45 days. Stained smears from the tumors showed abundant intracellular amastigotes. Metastatic tumors in the paws developed within 7 months.

**DISCUSSION**

The Republic of Panama forms the geographical link between Central and South America, sharing their fauna and zoonoses. The region is undergoing marked ecological changes. Settlers from arid regions of the central provinces, where cutaneous leishmaniasis is no longer endemic, have been moving into forested areas seeking free land for agriculture and cattle raising. Studies by GML staff in 18 communities along the Inter-American Highway in eastern Panama showed a leishmaniasis prevalence rate of 4–78% (A. M. de Vasquez, personal communication).

*L. panamensis*, which has been reported from most Central American countries as well as from Colombia and Ecuador, was considered to be the only causative agent of human leishmaniasis in Panama. In 1977, a group of U.S. Army personnel stationed at Fort Sherman near the Caribbean entrance to the Panama Canal, acquired leishmaniasis after conducting jungle exercises
in the area. Several isolates from these soldiers were characterized in our laboratory by CAE as *L. amazonensis* (data not shown).

*L. amazonensis* has been reported from Bolivia, Brazil, Peru, Venezuela, Ecuador, and French Guiana and probably occurs in the Amazon Region in Colombia. In Brazil, 30% of the patients found to be infected with *L. amazonensis* developed diffuse cutaneous leishmaniasis, while the remainder developed lesions that healed spontaneously. *L. mexicana* has been reported from Mexico, Guatemala, and Belize.

Case No. 2 probably represents a dual infection by *L. amazonensis* and *L. mexicana*, both of the *L. mexicana* complex. It is not known whether the samples were collected from the same ulcer. Characterization of the parasites was not possible until cloning. Isoenzyme profiles on original, uncloned isolates could not be interpreted. Similar results have been obtained by other investigators when co-cultivating *L. amazonensis* and *L. mexicana*.

The rarity of human cutaneous leishmaniasis due to *L. amazonensis* and *L. mexicana* in Panama may be partially attributed to the rodentophilic feeding habits of *Lutzomyia olmeca bicolor*, the implicated vector of *L. aridus*, and potential vector of the *L. mexicana* complex in this country.

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