# Use of Monoclonal Antibodies for the Identification of *Leishmania* spp. Isolated from Humans and Wild Rodents in the State of Campeche, Mexico

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*The genus* Leishmania *includes 30 described species which infect a wide variety of mammalian hosts. The precise identification of leishmanial parasites at the species level is very important in order to determine whether an organism, causing the disease in a given area, is of the same biotype as that found in suspected mammalian reservoirs. The objectives of the present study were (1) to identify leishmanial parasites isolated from humans and wild rodents from the State of Campeche, an endemic focus of localized cutaneous leishmaniasis (LCL) in southern Mexico, using an indirect immunofluorescent assay (IFA) with monoclonal antibodies (Mabs); and (2) to determine if the parasites of the two types of hosts were of the same biotype. All the wild rodents (six* Ototylomys phyllotis, *eight* Oryzomys melanotis, *five* Peromyscus yucatanicus *and two* Sigmodon hispidus*) and 96% (24/25) of the human isolates were identified as* Leishmania (L.) mexicana *confirming that this specific LCL focus is a wild zoonosis. The presence of one human isolate of* L. (Viannia) braziliensis *in the State of Campeche, confirmed the importance of an accurate taxonomic identification at species level.*

Key words: *Leishmania* (*Leishmania) mexicana* - *Leishmania* (*Viannia*) *braziliensis* - monoclonal antibodies - human - rodents - Mexico

Recent studies in epidemiology and molecular characterization of the New World leishmaniases have revealed that the genus *Leishmania* Ross, 1903 (Protozoa: Trypanosomatidae) is far more complex than originally thought (Grimaldi & Tesh 1993, Cupolillo et al. 1994, Pérez-Mutul et al. 1994, Shaw 1994, Lainson 1997). The genus comprises 30 species which infect a wide variety of mammalian hosts (wild or domestic) and vectors (Grimaldi et al. 1991, Rebollar-Téllez et al. 1995, 1996a,b). Each of the New World species of *Leishmania* has unique ecological and geographical distributions (Grimaldi et al. 1989, Grimaldi & Tesh 1993). From an epidemiological point of view and disease-control stand-point, it is very important to know whether an organism, causing the disease in a given area, is of the same biotype as that found in suspected mammalian reservoirs (WHO 1990).

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Localized cutaneous leishmaniasis (LCL) in the peninsula of Yucatán, Mexico, known as the "Chiclero's Ulcer", was described by Seidelin in 1912. Thereafter, additional cases of the disease were reported and the forest was considered an endemic focus of LCL (Beltrán & Bustamante 1942, Biagi et al. 1957, Andrade-Narváez et al. 1990, Ramírez-Fraire 1992, Chablé-Santos 1994, Rebollar-Téllez 1995). *Leishmania* (*L.*) *mexicana* Biagi, 1953 *emend*. Garnham, 1962 was considered the main agent based on the clinical and epidemiological features of the disease (Biagi 1953b, Lainson & Strangways-Dixon 1963, Albertos-Alpuche et al. 1996), and on the biological characteristics of the parasite in laboratory animals (Biagi 1953a, Biagi & Velasco 1967).

Seventy-five leishmanial isolates from humans cases of LCL from the State of Campeche have been characterized by isoenzyme markers. Seventy (93.3%) were identified as *L.* (*L.*) *mexicana* and five (6.7%) were *L.* (*Viannia*) *braziliensis* Viannia, 1911 *emend.* Matta, 1916 (Cárdenas-Marrufo 1993, Canto-Lara et al. 1998). Isolates from two wild rodents (one *Oryzomys melanotis* Thomas, 1893, and one *Sigmodon hispidus* Say and Ord, 1825) have been characterized as *L.* (*L.*) *mexicana* with monoclonal antibodies (Mabs) using an indirect immunofluorescent assay (IFA) (Chablé-Santos et

al. 1995, Ojeda-Farfán 1996), but the parasites from other infected species of rodents have yet to be characterized. The objectives of the present study were (1) to identify leishmanial parasites isolated from humans and four species of wild rodents from the State of Campeche, Mexico, using IFA with Mabs; and (2) to determine if the parasites of both types of hosts were of the same biotype.

## MATERIALS AND METHODS

*Study area* - The study area was the forest of the State of Campeche, Mexico. This area is an endemic focus of LCL probably due to its ecological conditions. A medium-height tropical forest covers about 59% of Campeche, the humidity reaches 80%, the annual rainfall is around 1400 mm, and the average temperature is 27ºC (Flores & Espejel Carvajal 1994). The rodents were trapped at 8 km southeast of La Libertad, a small village located 24 km east of Escarcega (previously described in Ramírez-Fraire 1992, Chablé-Santos 1994, Rebollar-Tellez 1995, Chablé-Santos et al. 1995).

*Isolation of parasites* - From September 1993 to May 1995, isolates of *Leishmania* were obtained from patients of LCL who became infected in the State of Campeche. Parasites were also obtained from small terrestrial wild rodents which were caught in collapsible Sherman traps from February 1993 to March 1994 (Chablé-Santos 1994, Chablé-Santos et al. 1995) and kept in captivity in the animal-care facilities of the Centre of Regional Research, University of Yucatán, Mérida, Yucatán, Mexico.

Parasites were isolated by needle aspirates from the edge of lesions in human cases and from the base of the tail in rodents. Aspirates were inoculated into a tube of Senekjie's modified medium and kept at 22ºC (Weigle et al. 1987). After initial growth in cultures tubes, the parasites multiplied and were mass cultivated for Mabs analyses.

*Mabs identification* - The species of *Leishmania* were identified by IFA using Mabs (as previously described in Chablé-Santos et al. 1995). A panel of species- and complex-specific Mabs of both subgenera *Leishmania* (M3, M7, and M8) and *Viannia* (B4, B12, B16, and B19) were used (Mabs generously donated by Dr D McMahon-Pratt, Yale University, USA and Dr F Modabber TDR/WHO, Switzerland). The following cultures of World Health Organization (WHO) reference strains were used as control: *L.* (*L.*) *mexicana* (MHOM/BZ/82/Bel21), *L.* (*L.*) *amazonensis* Lainson and Shaw, 1972 (MHOM/BR/73/M2269), *L.* (*V.*) *braziliensis* (MHOM/BR/84/LTB300), *L.* (*V.*) *guyanensis* Floch, 1954 (MHOM/BR/75/M4147), *L.* (*V.*) *panamensis* Lainson and Shaw, 1972 (MHOM/PA/71/LS94). Promastigotes were washed three times in phosphate-buffered saline, distributed on multispots slides, air-dried, fixed for 10 min in acetone at 24ºC and stored at -70ºC until used. Optimal dilutions of Mabs and fluorescein isothiocyanate-conjugated immunoglobulin G (IgG) (goat anti-mouse IgG heavy-light chains fraction) were 1:50 and 1:25 respectively.

## RESULTS

Between September 1993 and May 1995, a total of 81 cases of LCL from the State of Campeche were confirmed by at least one of the parasitological analyses employed (smear, biopsy and isolation-culture). From those cases, 25 isolates were successfully cultivated. Twenty-four (96%) reacted with M8 and M7 and were identified as *L.* (*L.*) *mexicana*. Only one (4%), reacting with B12 and B16, was considered as *L.* (*V.*) *braziliensis*.

Between February 1993 and March 1994, 21 strains were isolated from four species of wild rodents: six *Ototylomys phyllotis* Merriam, 1901; eight *O. melanotis*; five *Peromyscus yucatanicus* J.A. Allen and Chapman, 1897; and two *S. hispidus*. As shown in Table, all the isolates from wild rodents reacted with M7 and M8 and thus, were identified as *L.* (*L.*) *mexicana*. No differences in reactivity patterns were found among the different strains of *L.* (*L.*) *mexicana* from humans and from wild rodents.

## DISCUSSION

Because of the complexity of leishmaniases and the huge amount of information collected worldwide, the WHO (1990) emphasized the importance of an accurate taxonomical identification of the species of *Leishmania* as a basis for further comparisons and discussions. Monoclonal antibodies that distinguish New World species of *Leishmania* have been produced and tested (McMahon-Pratt & David 1981, McMahon-Pratt et al. 1985, Grimaldi et al. 1989). The results of the high specificity of Mabs for selected species of *Leishmania* have been confirmed using a large sample of isolates from humans, wild mammals, and sand flies; and in addition Mabs results were confirmed using in parallel isoenzyme electophoresis and other molecular techniques at the genotypic level (Canto-Lara unpublished data, Mimori et al. 1989, Grimaldi et al. 1989). In the present study, 96% (N=25) of the human isolates were identified with Mabs as *L.* (*L.*) *mexicana*. The presence of one human isolate of *L.* (*V.*) *braziliensis* in the LCL focus of the southeast of Mexico, confirmed the importance of an accurate taxonomic identification at the species level. This newly-found autochtonous LCL in the State of Campeche implies that two transmission cycles of LCL co-exist

TABLE

Results of indirect immunofluorescent assay with *Leishmania*-specific monoclonal antibodies on smears of WHO reference strains and wild rodents isolates

| Strain/isolate code | Monoclonal antibody code number | | | | | | | Parasite identification |
|---|---|---|---|---|---|---|---|---|
| | M7 | M8 | M3 | B12 | B4 | B16 | B19 | |
| WHO reference strains | | | | | | | | |
| MHOM/BZ/82/Bel 21 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MHOM/BR/73/M2269 | + | - | + | - | - | - | - | *L(L) amazonensis* |
| MHOM/BR/84/LTB300 | - | - | - | + | - | + | - | *L(V) braziliensis* |
| MHOM/BR/75/M4147 | - | - | - | + | - | - | + | *L(V) guyamensis* |
| MHOM/PA/71/LS94 | - | - | - | + | + | - | - | *L(V) panamensis* |
| Wild rodents isolates. | | | | | | | | |
| MSIG/MX/93/SA2 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MSIG/MX/94/SN5 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MPER/MX/94/P15a | + | + | - | - | - | - | - | *L(L) mexicana* |
| MPER/MX/94/P1a | + | + | - | - | - | - | - | *L(L) mexicana* |
| MPER/MX/94/P3b | + | + | - | - | - | - | - | *L(L) mexicana* |
| MPER/MX/94/PO11 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MPER/MX/94/P8 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/93/Or14b | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/93/OrK6 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/93/Or12a | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/93/OrN1 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/94/OrF9 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/94/OrC8 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/94/OrD9 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MORY/MX/OrC9 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MOTO/MX/93/OtK5 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MOTO/MX/94/Ot6a | + | + | - | - | - | - | - | *L(L) mexicana* |
| MOTO/MX/94/OtB4 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MOTO/MX/94/OtC4' | + | + | - | - | - | - | - | *L(L) mexicana* |
| MOTO/MX/94/OtS9 | + | + | - | - | - | - | - | *L(L) mexicana* |
| MOTO/MX/94/OtQ9 | + | + | - | - | - | - | - | *L(L) mexicana* |

in the same focus. Because of its potential high virulence, the transmission cycle of *L.* (*V.*) *braziliensis*, its response to therapy, and the prognosis in the focus should be studied.

The present study identified *L.* (*L.*) *mexicana* in four species of wild rodents: the black-eared rice-rat, *O. melanotis*; the hispid cotton-rat, *S. hispidus*; the big-eared climbing rat, *O. phyllotis;* and the Yucatán deer-mouse, *P. yucatanicus*. In Belize, eight *O. phyllotis* and one *S. hispidus* have been found infected (Lainson & Strangways-Dixon 1964, Disney 1968). The species of the parasite was never characterized but according to its behaviour, was thought to be *L.* (*L.*) *mexicana* (Lainson 1987). In Campeche, *L.* (*L.*) *mexicana* had only been identified in two samples from wild rodents: one *S. hispidus* and one *O. melanotis* (Chablé-Santos et al. 1995). Thus, the present study confirmed the presence of *L.* (*L.*) *mexicana* in *S. hispidus* and *O. melanotis*, and identified the same parasite in two new species of rodents *O. phyllotis* and *P. yucatanicus* which is an endemic species of the peninsula of Yucatán.

However, the direct incrimination of a mammal as a primary reservoir host requires that it is demonstrated that the parasite population needs this particular mammal for its maintenance in that specific focus. This demands extensive ecological studies and direct incrimination is generally not possible. For this reason WHO (1990) has estab-

lished some criteria to define a primary reservoir host. One of them and probably the most important, is to demonstrate that the strain of parasite in a given focus is of the same biotype in both the mammal reservoir and human. In the present study, *L.* (*L.*) *mexicana* was characterized, by IFA using Mabs, in both 96% (N=25) of the human cases and 100% (N=21) of the rodent samples which demonstrated that the strains were from the same biotype and that this specific LCL is a wild zoonosis in the State of Campeche. Thus, one or more species of wild rodents might act as a reservoir host for *L.* (*L.*) *mexicana.* The four other criteria to define a primary reservoir are under study.

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