

Characterization of *Leishmania* sp. strains isolated from autochthonous cases of human cutaneous leishmaniasis in Santa Catarina State, southern Brazil

Edmundo C. Grisard ^{a,e,*}, Mário Steindel ^a, Jeffrey J. Shaw ^c,
Edna A.Y. Ishikawa ^f, Carlos J. Carvalho-Pinto ^a, Iriane Eger-Mangrich ^a,
Helena K. Toma ^d, José H. Lima ^a, Alvaro J. Romanha ^b, David A. Campbell ^e

^a Departamento de Microbiologia e Parasitologia, CCB, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

^b Laboratório de Parasitologia Celular e Molecular-Centro de Pesquisas René Rachou-FIOCRUZ, Belo Horizonte, MG, Brazil

^c Departamento de Parasitologia, ICB, Universidade de São Paulo, São Paulo, SP, Brazil

^d Departamento de Análises Clínicas e Toxicológicas, CCS, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

^e Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA, USA

^f Departamento de Parasitologia, Instituto Evandro Chagas-FNS, Belém, PA, Brazil

Received 30 June 1999; received in revised form 27 August 1999; accepted 9 September 1999

Abstract

Four *Leishmania* sp. samples were isolated from autochthonous human cases of American cutaneous leishmaniasis (ACL) in Santa Catarina State, southern Brazil. These strains were characterized using indirect immunofluorescence with a panel of *Leishmania*-specific monoclonal antibodies (MAbs), and by PCR amplification and hybridization assay of the mini-exon gene with group specific probes. The results obtained with the MAbs were in agreement with the genetic marker. Two isolates (MHOM/BR/89/JSC89-H1 and MHOM/BR/89/JSC89-H2) were identified as *L. (Leishmania) amazonensis* and two (MHOM/BR/96/LSC96-H3 and MHOM/BR/97/LSC97-H4) as *L. (Viannia) braziliensis*. The southernmost autochthonous cases of ACL in Brazil are due to two different *Leishmania* sp. species, confirming the spreading of ACL on the American continent. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Human cutaneous leishmaniasis; *Leishmania* sp.; Mini-exon gene; Spliced leader; Characterization; Monoclonal antibodies; PCR; Hybridization

* Corresponding author. Tel.: +55-48-331-9512; fax: +55-48-331-9258.

E-mail address: grisard@ccb.ufsc.br (E.C. Grisard)

1. Introduction

American cutaneous leishmaniasis (ACL) is a parasitic disease caused by *Leishmania* species that are widely distributed in Latin America and infect several mammalian species including man (WHO, 1990; Peraza et al., 1998).

In the Santa Catarina State, southern Brazil (Fig. 1), autochthonous ACL cases, with no mucocutaneous manifestations, were described in a 1987 outbreak that occurred in the rural areas of the Coronel Freitas and Quilombo municipalities, in the western region of the State, where two *Leishmania* sp. samples were isolated (São Thiago and Guida, 1990). Recently, two other samples were isolated from autochthonous ACL cases detected at the municipality of Chapecó and from Piçarras city in the western and northeastern regions of the state, respectively.

These patients, with ages ranging from 16 to 46 years, were inhabitants of rural areas and presented a single ulcer that cured after one single cycle of treatment with pentavalent antimonial 10 mg/Kg body weight per day (Glucantime®-Rhodia) during 20 days.

In the present work we characterize these four *Leishmania* sp. samples isolated from autochthonous ACL cases that occurred in non-endemic

areas in Santa Catarina State, southern Brazil, using different methods.

2. Materials and methods

2.1. Parasite isolation

Parasite isolation was performed through biopsy sub-inoculation in hamster footpads (*Mesocricetus auratus*). After 2–4 months skin biopsies from these animals were cultured in both liver infusion tryptose (LIT) and Schneider's insect medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). The parasite strains were coded as MHOM/BR/89/JSC89-H1, MHOM/BR/89/JSC89-H2, MHOM/BR/96/LSC96-H3 and MHOM/BR/97/LSC97-H4 and stored in liquid nitrogen. Hereafter, these strains will be designed as H-1, H-2, H-3 and H-4.

2.2. Monoclonal antibody assays

Monoclonal antibody (MAb) assays were performed as described by McMahon-Pratt et al. (1984) and Shaw et al. (1989) with 27 different anti-*Leishmania* monoclonal antibodies.



Fig. 1. Map of Brazil indicating the Santa Catarina State (shaded) and map of the Santa Catarina State showing the localities where the ACL cases were detected in southern Brazil.

2.3. Mini-exon gene assays

Using the mini-exon gene as a genetic marker in order to distinguish these isolates as previously described (Fernandes et al., 1994; Ramos et al., 1996), PCR amplification products resolved by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining were blotted onto nylon membranes. As reference strains, *L. (Leishmania) amazonensis* MHOM/BR/80/JOSEPHA and IFLA/BR/67/PH8; *L. (Viannia) braziliensis* MHOM/BR/84/LTB300 and MHOM/BR/75/M2903 and *L. chagasi* MHOM/BR/82/BA3 were used.

Hybridizations were individually performed with oligonucleotides S-1593 (5'-A(A/G)(C/T)GGCACCCCTCACA(G/A)CGACCTGGGCA-3') specific for the *Viannia* subgenus group species, S-1595 (5'-GGGCG(C/A)CGGC(A/G)-GCCGTGAC(A/G)CGTGG(C/T)CCGG-3') specific for the New World dermatropic group species and S-1698 (5'-CGGCCATGGTGGTGAC(G/A)CGCGGGCCCGTGC-3') specific for the viscerotropic group species, labelled by [γ - 32 P]ATP (Ramos et al., 1996).

3. Results

MAB assays revealed that strains H-1 and H-2 were only recognized by the M2 and M3 MABs that are specific for *L. (Leishmania) amazonensis* and WA2 that recognizes both *L. (Leishmania) amazonensis* and *L. (Leishmania) mexicana*.

Strains H-3 and H-4 were recognized by the B12 MAB whose epitope is found in other species such as *L. (Viannia) naiffi*, *L. (Viannia) peruviana* and *L. (Viannia) guyanensis*. The expression of this epitope in the former two species and *L. (Viannia) braziliensis* is the same but different from *L. (Viannia) guyanensis*. Both strains were also positive with B16 MAB which has been found to be specific for all Brazilian isolates of *L. (Viannia) braziliensis*. No reaction was observed with the other MABs tested.

The mini-exon PCR products for samples H-1

and H-2 showed a similar migration pattern of *L. (Leishmania) amazonensis* reference DNA (*La*) (Fig. 2(A)) and only hybridized with the S-1595 probe (Fig. 2(B)). We discarded the possibility that H-1 and H-2 bands can be due to *L. (Leishmania) mexicana* since none of the specific MABs for this species gave positive reaction. Products obtained from samples H-3 and H-4 clearly showed a similar migration pattern as observed for the *L. (Viannia) braziliensis* reference DNA (*Lb*) (Fig. 2(A)) and only hybridized with the probe S-1593 (Fig. 2(C)). None of the strains revealed the same migration pattern of the *L. chagasi* control DNA (*Lc*) and showed no hybridization with the S-1698 specific probe (Fig. 2(D)). All PCR products were recognized by the *Leishmania*-specific intron probe LtMEIn (5'-GTTCCGGAAGTTTCGCATAC-3') (data not shown).

4. Discussion

Traditionally, Santa Catarina State is considered a non-endemic area for human leishmaniasis (Grimaldi Jr. et al., 1987). The use of monoclonal antibodies, PCR amplification and hybridization of the mini-exon gene with group-specific probes allowed us to identify *L. (Leishmania) amazonensis* and *L. (Viannia) braziliensis* as the two different etiological agents causing the southernmost cases of ACL in Brazil. No other confirmed report of autochthonous human infection due to these parasites in these areas has been reported so far.

Additionally, preliminary studies on the phlebotomine sandfly fauna in both peridomiciliar and sylvatic environments revealed the presence of *Lutzomyia neivai* in Piçarras city, and *L. neivai*, *L. fischeri* and *L. migonei* in the western region of the state.

This study shows that the mini-exon gene PCR is a useful tool to detect and characterize DNA of both *Leishmania* subgenera, as also recently reported by Harris et al. (1998) and Aviles et al. (1999) targeting kDNA minicircles.

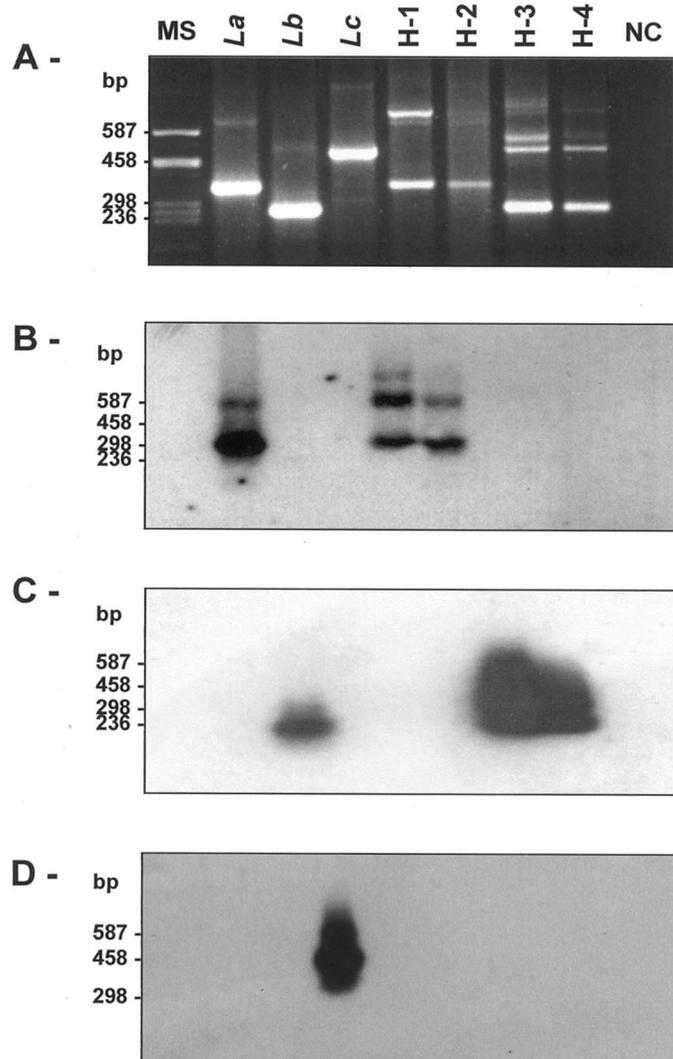


Fig. 2. (A) Amplification products of *Leishmania* sp. isolates obtained with the oligonucleotides ME-L/ME-R in 1.5% agarose gel electrophoresis stained with ethidium bromide. La = *L. (Leishmania) amazonensis*; Lb = *L. (Viannia) braziliensis*; Lc = *L. chagasi*, and H1-4 *Leishmania* sp. isolates (MHOM/BR/89/JSC89-H1, MHOM/BR/89/JSC89-H2, MHOM/BR/96/LSC96-H3 and MHOM/BR/97/LSC97-H4) obtained from patients of Santa Catarina State, Brazil. MS = molecular size marker (pUC 18-*Hae*III digested); NC = negative control (no DNA added). Hybridization of PCR-amplified mini-exon repeats of *Leishmania* sp. isolates with oligonucleotides S-1595 (B), S-1593 (C) and S-1698 (D).

This report characterizes *Leishmania* sp. parasites isolated from human cases in an area where the disease has previously not been described, improving the knowledge of ACL spreading pattern, and reinforcing the needs of surveillance, control and prevention of new foci.

Acknowledgements

To Nancy R. Sturm for her helpful suggestions on the experiments and for critical reading this manuscript. To Dr D. McMahon-Pratt, Dr D. Snary and Dr C.A. Hanham who kindly donated

the monoclonal antibodies used in this study, and to Fundação Nacional de Saúde — FNS (Brazilian Ministry of Health) for their valuable help in field works. E.C. Grisard is a grantee of Capes — Brazilian Government Agency.

References

- Aviles, H., Belli, A., Armijos, R., Monroy, F.P., Harris, E., 1999. PCR detection and identification of *Leishmania* parasites in clinical specimens in Ecuador: a comparison with classical diagnostic methods. *J. Parasitol.* 85, 181–187.
- Fernandes, O., Murthy, V.K., Kurath, U., Degraive, W.M., Campbell, D.A., 1994. Mini-exon gene variation in human pathogenic *Leishmania* species. *Mol. Biochem. Parasitol.* 66, 261–271.
- Grimaldi, G. Jr., David, J.R., McMahon-Pratt, D., 1987. Identification and distribution of new world *Leishmania* species characterized by serodeme analysis using monoclonal antibodies. *Am. J. Trop. Med. Hyg.* 36, 270–287.
- Harris, E., Kropp, G., Belli, A., Rodriguez, B., Agabian, N., 1998. Single-step multiplex PCR assay for characterization of New World *Leishmania* complexes. *J. Clin. Microbiol.* 36, 1989–1995.
- McMahon-Pratt, D., Jaffe, C.L., Grimaldi, G. Jr., 1984. Application of monoclonal antibodies to the classification of *Leishmania* species. In: *Genes and Antigens of Parasites*. Fundação Oswaldo Cruz, Rio de Janeiro, pp. 121–135.
- Peraza, J., Urbina, A., Zeledón, R., 1998. Zymodeme and serodeme characterization of *Leishmania* isolates obtained from Costa Rican patients. *Mem. Inst. Oswaldo Cruz* 93, 283–287.
- Ramos, A., Maslov, D.A., Fernandes, O., Campbell, D.A., Simpson, L., 1996. Detection and identification of human pathogenic *Leishmania* and *Trypanosoma* species by hybridization of PCR-amplified mini-exon repeats. *Exp. Parasitol.* 82, 242–250.
- São Thiago, P.T., Guida, U., 1990. Leishmaniose tegumentar no oeste do Estado de Santa Catarina, Brasil. *Rev. Soc. Bras. Med. Trop.* 23, 201–203.
- Shaw, J.J., Ishikawa, E.A.Y., Lainson, R., 1989. A rapid and sensitive method for the identification of *Leishmania* with monoclonal antibodies using fluorescein-labelled avidin. *Trans. R. Soc. Trop. Med. Hyg.* 83, 783–784.
- WHO, 1990. Control of the Leishmaniasis. Report of a WHO Expert Committee. World Health Organization, Geneva, Switzerland. Technical Report Series no. 793.