Mem. Inst. Oswaldo Cruz, Rio de Janeiro,
Vol. 82(4): 579, out./dez. 1987

RESEARCH NOTE

THE RELEVANCE OF CHARACTERIZING *LEISHMANIA* FROM CUTANEOUS LESIONS. A SIMPLE APPROACH FOR ISOLATION

ALDINA BARRAL*, ROQUE P. ALMEIDA*, AMELIA R. DE JESUS*, EDUARDO MEDEIROS NETO**, ILMA ANDRADE SANTOS* & WARREN JOHNSON JR.***

*Universidade Federal da Bahia – Hospital Prof. Edgard Santos, Rua João das Botas s/n, 40140 Savador, BA, Brasil **Faculdade de Ciências da Saúde, Universidade de Brasília ***Cornell University Medical College, New York, USA

Isolation and correct identification of leishmanial parasites are very important for the diagnosis of leishmaniasis. Recent reports have described the isolation of parasite species not suspected as the agent causing certain clinical pictures (Barral et al., 1986, *Am. J. Trop. Med. Hyg., 35* :732-734; Bellazong et al., 1985, *Ann. Parasitol., 60* :1-3; Schnur et al., 1985; *Trans. R. Soc. Trop. Med. Hyg., 79* :134-135). Such isolation relies on direct *in vitro* culturing of biopsy material or on injecting this material into hamsters (Cuba Cuba et al., 1980, *Bol. Of. Sanit. Panam., 89* :195-206; Grimaldi et al., 1984, *Trans. R. Soc. Trop. Med. Hyg., 78* :560; Marsden et al., 1975, *Rev. Soc. Bras. Med. Trop., 9* :309-326; Mayrink et al., 1979, *Ann. Trop. Med. Parasitol., 73* :123-127). Contamination of cultures directly seeded with biopsy material is an important limitation of the techniques and maintenance of hamsters or mice is not feasible for most clinical personnel in the endemic areas.

We report a simple method for obtaining material which proved successful in isolating parasites in the majority of cases investigated without contamination. Patients received 2% xylocaine, infiltrated in the area of the lesion as a local anesthetic. Half a milliliter of sterile saline was injected directly into the lesions with a 21 G needle, using a 10ml syringe. The needle was removed with gentle rotatory movements while aspirating the blood-stained saline. The material was then placed in NNN agar slants of the following formulation (Bacto agar 3.5g; sodium chloride 1.5g; Tryptose peptone 1.25g; distilled water 225 ml, plus 25% defibrinated rabbit blood) overlaid with 1 ml of modified LIT medium (sodium chloride 4.0g; 10X concentrated RPMI 1640 liquid culture medium and 10X concentrated medium 199, 10ml of each; penicillin 100 IU/ml; streptomycin 100 μg/ml and distilled water to 1,000 ml). Cultures were kept at 25°C and examined then twice weekly. Negative cultures for 4 weeks after seeding, were transferred to new NNN slants and observed for a period of two months. In some patients a biopsy of the lesion border was also taken and the material was macerated and cultured in the same medium.

Cultures from aspirated material were performed in 54 patients with leishmaniasis and were positive in 33 of them (61.1%). Positivity was much higher in the group with cutaneous lesions (CL; 25 positives out of 33 patients or 75.7%) than in patients with mucocutaneous lesions (MCL; 8 positives out of 18 cases or 44%). Isolates were characterized by a panel of monoclonal antibodies (courtesy of Dr. G. Grimaldi, FIOCRUZ, Rio de Janeiro) being 13 of the *L. mexicana* complex (11 from CL cases and 2 from MCL patients); 20 of the *L. braziliensis* complex (14 CL, 6 MCL). In 21 cases it was not possible to isolate parasites and the diagnosis of leishmaniasis was made by Montenegro reaction and/or immunofluorescence assay to *Leishmania* antigen (17 cases) or by histopathological examination (4 cases).

In 24 patients a comparison between aspirated and macerated lesion material was made. In these cases aspirated material gave 15 positive cultures (62%) without bacterial or fungal contamination. Cultures from macerated material were positive on 12 occasions (50%), but 3 exhibited bacterial contamination reducing the percentage of useful cultures to 37.5% (9/24 cases).

The high positivity and low contamination rate obtained with the technique described here should proven to be an important instrument in isolating *Leishmania* stocks from cutaneous lesions.

Acknowledgements: We thank Dr. Phillip Marsden for critical comments.

This work received support from the Brazilian National Research Council (CNPq) and from US PHS Service Grant AI-16282.

Received July 10, 1987.
Accepted September 17, 1987.