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A Simplification of the Mouse Foot-pad Infection Using *Mycobacterium leprae* from Skin Scrapes

J. M. H. PEARSON*

Leprosy Research Unit, National Leprosy Control Centre, Sungei Buloh, Malaysia

In lepromatous leprosy bacilli can be obtained from skin scrapes in sufficient numbers for mouse footpad inoculation. This technique is simpler than biopsy, particularly suited to field conditions, and the method of choice in the investigation of patients suspected of developing drug resistance.

Because *Mycobacterium leprae* has not been cultured *in vitro*, bacteriological investigations on this organism have to be undertaken *in vivo* using the standardized mouse footpad technique (Shepard, 1960*b*; Rees, 1964). Although bacilli for mouse footpad inoculation have been obtained from nasal washings (Shepard, 1960*a*) or nasal secretions (Davey and Rees, 1974), the usual source of organisms has been from biopsies of skin lesions from leprosy patients. While the biopsying of skin is a relatively simple procedure it does require local anaesthesia and medical supervision. Moreover, the processing of biopsies of skin to obtain suitable suspensions of *Myco. leprae* is time-consuming, and entails the mincing and homogenizing of skin tissue and a series of centrifugations in order to separate out skin-tissue components. On the other hand, relatively small numbers of *Myco. leprae* are required for establishing the standard mouse footpad infection (an inoculum of 5.0×10^3-1.0×10^4 bacilli/footpad) and since the skin from previously untreated or actively relapsing lepromatous patients is likely to contain 10^7-10^9 bacilli/g of tissue, and routine biopsies of skin are likely to weigh between 200-400 mg, the yield of bacilli from such biopsies is far in excess of the numbers required for bacteriological investigation. Because such small numbers of *Myco. leprae* are required to establish the footpad infection compared with the high density of organisms in the skin of patients and because of the increasing use of this method in chemotherapeutic trials or for detecting the emergence of dapsone resistance, a simple alternative method of obtaining adequate numbers of bacilli was investigated.

This paper discribes a method for establishing the footpad infection in mice with *Myco. leprae* obtained from skin scrapes.

* Present address: MRC Leprosy Project, PO Box 1005, Addis Ababa, Ethiopia.
Reprint requests to J. M. H. Pearson, National Institute for Medical Research, Mill Hill, London NW7 1AA

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Materials and Methods

Myco. leprae were obtained using the standard "slit and scrape" technique which is used routinely for preparing smears for assessing qualitatively and quantitatively bacilli in the skin of patients with leprosy. The skin site to be sampled is thoroughly cleaned with spirit, which is allowed to dry, firmly pinched between the thumb and forefingers to exclude haemorrhage and then incised to the depth of about 1 mm with a scalpel without local anaesthesia. The blade is turned through 90° and the incision scraped by returning the scalpel along the line of incision. In the standard procedure the material on the scalpel blade, which in active lepromatous cases is rich in bacilli, is spread on a slide. For harvesting bacilli for footpad inoculation, the harvested material is rubbed off the scalpel blade in 2 ml of sterile saline (preferably containing 0.1% bovine albumin) in a sterile container. A rather larger amount of tissue is scraped from the skin incision for the harvesting of bacilli than is routinely used for preparing skin smears.

Samples of scraped tissues were obtained from 4 to 6 skin sites showing active lepromatous disease and each was added to the suspending fluid. The pooled crude bacillary suspension, which was slightly cloudy, was then dispersed by grinding in a glass homogenizer. The number of acid-fast bacilli in the homogenate was then assessed using the standard method of Hart and Rees (1960). By this procedure yields of 10^6-10^7 acid-fast bacilli/ml were obtained, and were adequate for mouse footpad inoculation.

Results

For the present studies bacilli obtained by this method were used to inoculate groups of mice for assessing the dapsone sensitivity of the organisms from patients who had relapsed while receiving dapsone. *Myco. leprae* harvested in this way proved to be infectious for mice and in the isolates under study, proved to be dapsone resistant (Rees, 1967).

Discussion

First and foremost the study has shown that adequate numbers of *Myco. leprae* can be obtained and prepared in a suitable form for mouse footpad inoculation by the "slit and scrape" technique from active skin lesions in patients with lepromatous leprosy. This is of more than academic interest, for under field conditions this "slit and scrape" procedure offers considerable advantages of convenience, both to patients and staff. The patients are spared the inconveniences of a biopsy—which can be considerable if they are required to travel 20 km from home to clinic to have the sutures removed. For staff, the paraphenalia of biopsies are not required; most clinics undertake "slit and scrape" procedures routinely, and both they and patients are accustomed to these tests. The only extra materials needed are a sterile container and sterile suspending fluid.

However the greatest advantage this procedure offers is in the investigation of patients suspected of developing resistance to dapsone or other drugs. In the early stages of relapse the new lesions are often very small superficial nodules, and would probably be considered unsuitable for biopsy. In such cases skin scrapes can be taken from a number of lesions, even tiny ones, and the viable bacilli will

not be diluted by large numbers from outside the active zone that are non-viable, as can happen in biopsies. Thus the likelihood of obtaining a suspension with a high proportion of viable drug resistant bacilli should be much increased; in such cases this technique is not merely more convenient, but is the method of choice, and should be employed even if biopsy facilities are available.

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References

Davey, T. F. and Rees, R. J. W. (1974). The nasal discharge in leprosy; clinical and bacteriological aspects. *Lepr. Rev.* **45**, 121.

Hart, P. D'Arcy and Rees, R. J. W. (1960). Effect of macrocyclon in acute and chronic pulmonary tuberculous infection in mice as shown by viable and total bacterial counts. *Br. J. exp. Path.* **41**, 414.

Rees, R. J. W. (1964). Limited multiplication of acid-fast bacilli in the foot-pads of mice inoculated with *Mycobacterium leprae*. *Br. J. exp. Path.* **45**, 207.

Rees, R. J. W. (1967). A preliminary review of the experimental evaluation of drugs for the treatment of leprosy. *Trans. Roy. Soc. Trop. Med. Hyg.* **61**, 581.

Shepard, C. C. (1960*a*). Acid-fast bacilli in nasal excretions in leprosy, and results of inoculation of mice. *Am. J. Hyg.* **71**, 147,

Shepard, C. C. (1960*b*). The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. *J. exp. Med.* **112**, 445.