INOCULATION OF THE MYCOBACTERIUM LEPRAE INTO THE HAMSTER CHEEK POUCH

Sir,

The lack of in vitro techniques for the cultivation of Mycobacterium leprae and the fact that M. leprae multiply and produce disease only in a limited number of species represents an important barrier to progress in leprosy research. The inoculation of mycobacteria into the footpads of immunologically intact mice remain the basic tool for assessing the activity of drugs against the bacilli. Unfortunately, this animal model has limitations because of the long duration of the experiments due to the very slow rate of growth of M. leprae. Immuno-deficient animals are little used in experimental leprosy due to the high cost of the animals and difficulties of their maintenance; furthermore, mortality is high before dissemination of the disease.1

In view of these data, we decided to study the behaviour of viable M. leprae inoculated into the cheek pouch of hamsters. This structure is an invagination of oral mucosa, where the lack of lymphatic drainage cuts the afferent arm of immune response.2 In addition, we compared the histological aspects of lesions induced by viable M. leprae inoculated into the pouch and into the footpad, an area rich in lymphatics.

Suspensions of viable M. leprae were prepared from lepromatous nodules, as described by Shepard.3 The mycobacterial identification was done through bacteria inoculation in a culture medium (Loewenstein–Jensen) and into the footpads of balb/c mice.3

Two-month-old male hamsters (Mesocricetus auratus) were divided into 2 groups. Group 1 (34 animals) were inoculated, under anaesthesia (sodium nembutal, 40 mg/kg) into the submucosa of the everted pouch with 0·1 ml of a bacilli suspension containing \(5 \times 10^6\) viable bacilli/ml. Group 2 (18 animals) were inoculated into the footpad with the same dose of bacilli. A minimum of 3 hamsters were killed by ethyl ether inhalation 30, 60, 120 and 150 days post-inoculation (pi). After death, samples from the pouch and inoculated footpads were collected, formol fixed, embedded in paraffin, cut and stained by hematoxin–eosin and Fite–Faraco.

No gross alterations were observed in the footpad of group 2 animals. Histologically, in 5 out of 8 hamsters studied 30 days pi, the mycobacteria evoked focal epithelioid granulomas, with giant cells, lymphocytes and very few, or no, bacilli. No macroscopic or histological alterations were observed in the footpad of animals killed after 30 days.

In 7 out of 34 hamsters inoculated into the pouch there was nodular infiltration 3–5 mm in diameter that were removed for histological study. From animals which did not present gross alterations, 3 random fragments were collected. Histological alterations were observed in 16 out of 34 of the pouch-inoculated hamsters; it is possible that the absence of lesions in the remaining animals was related to the lack of gross alterations and that the fragments submitted to histology did not represent the inoculation site. In order to confirm this possibility, further experiments are being done, i.e. tattooing with Indian ink 1 cm above and 1 cm below the site of inoculation.

In the pouches that showed lesions, the reactions were represented by accumulations of large grossly vacuolated macrophages containing numerous bacilli, without any epithelioid transformation. This pattern persisted up to 150 days pi and were similar to that observed in anergic forms of human disease.

The ability of M. leprae to evoke epithelioid cell granulomas in the footpad, but not in the cheek pouch, an immunoprivileged site, confirms that, in leprosy, the epithelioid granulomas are directly related to the development of immune response to M. leprae.1

Moreover, since M. leprae grows easily and rapidly (about 30 days) in the pouch,
this model may represent a good alternative for the study of new antileprosy drugs and drug resistance.

Faculdade de Ciencias, UNESP  
M. S. P. DE ARRUDA, R. N. FLEURY &  
Av. Edmundo Coube, S/N  
M. E. S. NOGUEIRA  
CEP 17033-360 Bauru, SP  
Brasil

References


PROTECTIVE FOOTWEAR FOR LEPROSY PATIENTS WITH SOLE SENSORY LOSS OR ULCERATION OF THE FOOT

Sir,

For many years it has been accepted that the management of patients with sole sensory loss and/or ulceration of the foot must include the wearing of suitable protective footwear, usually on a lifetime basis. This advice appears in publications from the World Health Organisation\(^1,2\) and is included in recent guidelines from the International Federation of Anti-Leprosy Association (ILEP): Prevention of Disability. Guidelines for Leprosy Control Programmes.\(^3\) At the recent 14th International Leprosy Congress in Orlando, Florida\(^4\) a number of papers supported this view and many different types of protective footwear were on display.

In this Institute, the need for protective footwear has long been recognized and our staff includes a full-time shoemaker with appropriate tools and equipment. During the past 10 years, we have attempted to provide a pair of shoes made from microcellular rubber, tyre soles and soft leather straps, for all patients with significant sole sensory loss or ulceration of the foot. They have been instructed in the proper care and use of the shoes and on the need to report back when repair is needed, as also on the self-care of their feet, essentially as described in the above ILEP document.

We have recently reviewed our results with regard to footwear, with particular attention to the provision of 158 pairs of shoes during the past 4 years, including the necessary repair services. The results have been far from satisfactory. Enquiries amongst our health staff and social workers have revealed that many patients do not wear the shoes once they leave hospital, whilst others wear them for a short time and then discard them, or fail to report back when repair is obviously needed. Our re-admission rate for foot ulceration is high, doubtless related to deficiencies in self-care and the proper use of shoes. Interestingly enough, however, there are a number of patients who, from their own account and from the observations of field workers, have used the shoes as directed, thus suggesting that footwear does not give protection under all circumstances.

We have discussed the possible reasons for these disappointing results with staff members and come to the conclusion that there are, at least in this part of India, a number of factors which seriously undermine the potential effectiveness of the advised strategy. These include:

Design. The ‘MCR design’ has been shown to be technically satisfactory in many parts of India and elsewhere, but the use of such shoes in a village is unusual in that they do not resemble