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 in a limited number of species represents an important barrier to progress in leprosy research. Mycobacterial inoculation into the footpad of immunologically intact mice remains the basic tool for assessing the effectiveness or otherwise 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*. Immunodeficient animals are less often used in experimental leprosy due to the high cost of the animals and difficulties in their maintenance; furthermore, mortality is high before dissemination of the disease.[1]

We therefore decided to study the behaviour of viable *M. leprae* inoculated into hamster's

cheek pouches. 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] Their mycobacterial identification was done by inoculation of the bacteria into culture medium (Loewenstein–Jensen) and into the footpads of balb/c mice.[3]

Male hamsters (*Mesocricetus auratus*), 2 months old, were divided into 2 groups: in group 1, 34 animals were inoculated, under anaesthesia (sodium nenbutal, 40 mg/kg) into the submucosa of the everted pouch with 0·1 ml bacilliary suspension containing 5×10^8 viable bacilli/ml. In 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 postinoculation (pi). Postmortem samples of inoculated pouch tissue and inoculated footpads were taken, formol fixed, embedded in paraffin, cut and stained using hematoxin & eosin and Fite-Faraco.

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

In 34 hamsters inoculated into the cheek pouch, 7 showed nodules 3–5 mm in diameter, and these were removed for histological study. From animals which did not present gross alterations, 3 randomly selected fragments of cheek pouch tissue were taken.

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 the fragments submitted to histology did not represent the inoculation site. In order to check this possibility, further experiments are being done by tattooing with Indian ink 1 cm above and 1 cm below the inoculation site.

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

The ability of *M. leprae* to evoke epithelioid 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 readily in the pouch, this model may represent a better alternative for the study of new antileprosy drugs and drug resistance.

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