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 anaethesia (sodium nembutal, 40 mg/kg) into the submucosa of the everted pouch with 0·1 ml of a bacilli suspension containing 5×10^6 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.

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