Preliminary Communications

Human Leprosy in Normal Mice

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Summary: It has now been shown that normal mice can be used as models for studying the early stages in the development of leprosy. Inoculation into the foot pads of mice of as few as 10^4 leprosy bacilli leads to infections which spread to distant sites via the blood stream and after two or more years give rise to granulomata and neural damage at the sites of inoculation. Where the tissue response had fully developed it reproduced exactly the histological features of human leprosy in the borderline range.

Introduction

Experimental research in human leprosy has been severely handicapped until recently because the causative organism, *Mycobacterium leprae*, could not be grown either in vitro or in animals. Shepard (1960a, 1960b), since confirmed by others (see Rees, 1964), was the first to achieve success in growing the organism in animals, using mouse foot pads. But growth was severely restricted and localized, and the lesions did not resemble histologically any of the disease forms seen in man. Since the bacillary growth reached a peak after 8 to 10 months and then apparently died out, it was assumed that the infection was self-limiting.

In leprosy, however, time is the paramount factor which cannot be ignored, for it is unique among infectious diseases in that it develops over a period of years rather than days or weeks. Here we present evidence that in normal mice the infection described by Shepard is not, in fact, self-limiting, but that bacillary multiplication continues in the normal mouse for two or more years. Towards the end of this time not only has the infection spread to distant sites, which are the same as those commonly involved in man, but, in addition, the tissue response in the foot pad gives a histological picture identical with that of human leprosy in the borderline range.

Material and Methods

In an extensive series of experiments normal female CBA mice aged 2 months were infected with *Myco. leprae* by injection of 10^4 bacilli into the hypodermis of both hind foot pads or both foot pads and ears. Bacilli were obtained either by homogenizing fresh biopsy specimens from patients with untreated lepromatous leprosy or as passage strains from mice. Progress of the infection was observed over a period of up to 27 months, which is about the normal life span of these mice.

To assess the rate of multiplication of organisms bacterial counts were made from one foot pad and/or ear of each animal; other tissues were sometimes counted. Histological examination of the other foot pad and/or ear was carried out routinely by light, fluorescence, and electron microscopy. Other tissues examined included the sciatic nerves, nose, trunk muscles, spleen, liver, lung, trunk skin, and lymph nodes. This material was compared with biopsy specimens obtained from patients in the Leprosy Research Unit, Sungei Buloh Leprosarium, Malaysia.

Observations

Bacteriological.—One foot pad of each mouse was homogenized at intervals and the number of bacilli were counted (Rees, 1964). The maximum yield (mean $2{\cdot}5 \times 10^6$) was obtained six to nine months after inoculation. Thereafter the number gradually fell and by two years was below the limit of detectability ($<10^5$).

Histological.—At two years, despite the very low bacterial counts, there were pronounced histological changes, showing that the infection had not died out. Small numbers of bacilli were to be found chiefly in dermal neurovascular bundles, lying in epineurial histiocytes, and sometimes in perineurial and Schwann cells. There were also macrophages containing bacilli infiltrating the dermis. But the most striking feature of the infection was the presence in the hypodermis and among muscle fibres of an epithelioid-cell granuloma containing very few bacilli and surrounded in places by loosely packed lymphocytes (Fig. 1A). Clusters of epithelioid cells were present in every mouse, but they were sometimes too small to be classified as granulomata. Foci of lymphocytes, often surrounding bacilli, were always present. Where the tissue response had fully developed it exactly reproduced the histological features of human leprosy in the borderline range, including neural damage characteristic of leprosy (Fig. 2A).

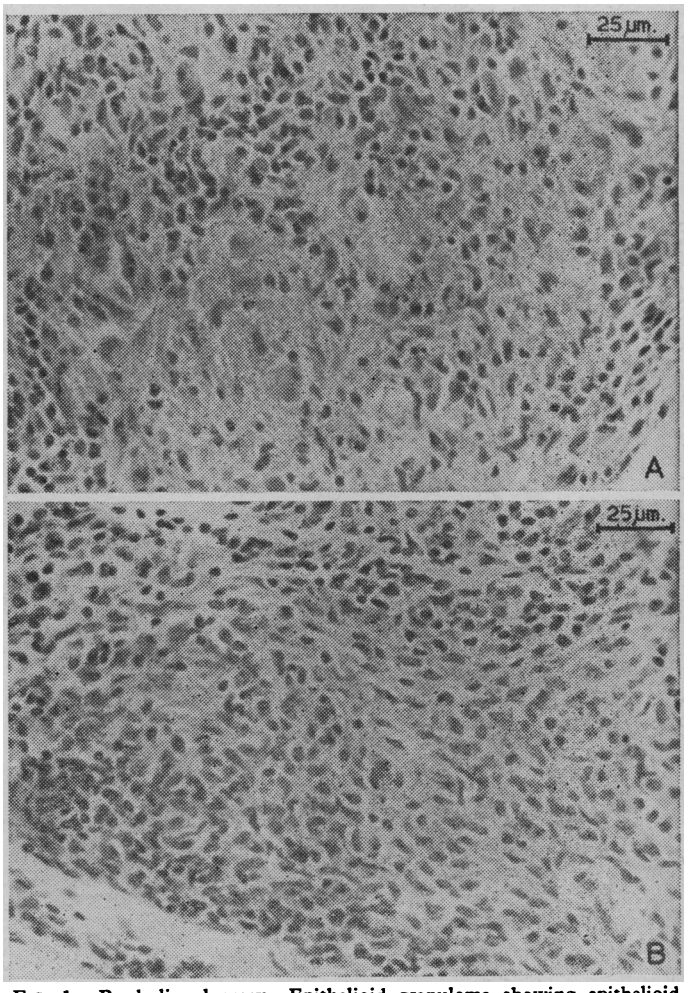

FIG. 1.—Borderline leprosy. Epithelioid granuloma showing epithelioid cells in deep dermis surrounded by lymphocytes. (A) Mouse: foot pad skin. (B) Man: upper arm skin.

Another feature of the late infection was dissemination. There was good evidence from the distribution of the bacilli in the foot pad that they had entered blood vessels at a very early stage of the infection. Moreover, at two years and thereafter counts were not infrequently positive in the nose or in un-

inoculated foot pads, though at these distant sites the histological features of human borderline leprosy had not fully developed.

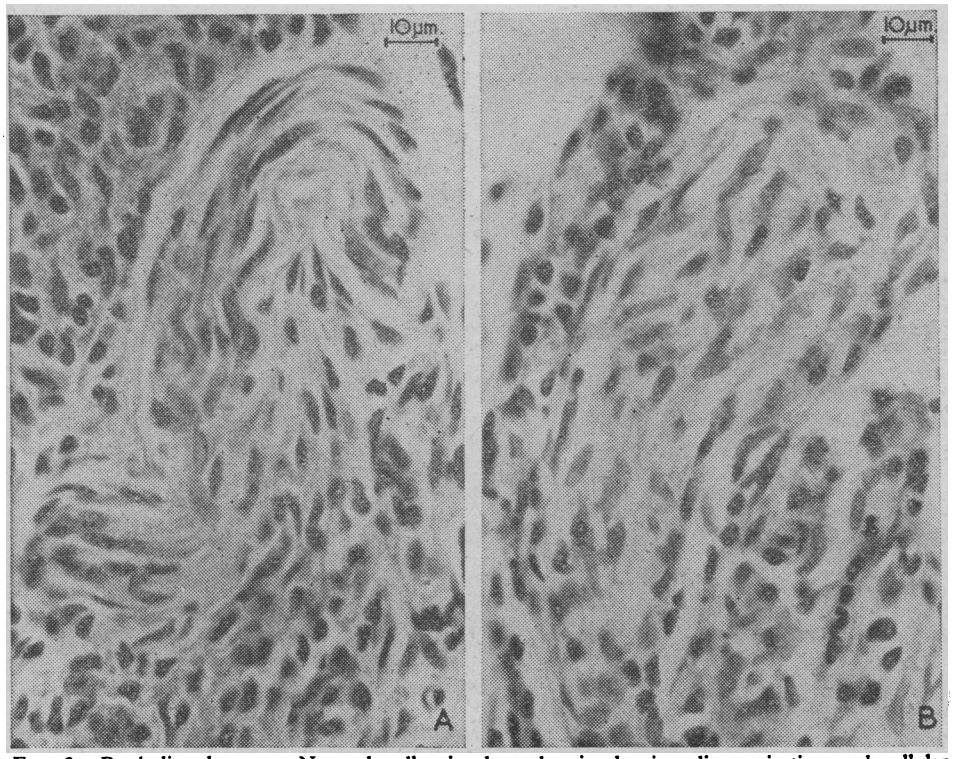

FIG. 2.—Borderline leprosy. Nerve bundles in deep dermis showing disorganization and cellular infiltration. (A) Mouse: foot pad skin. (B) Man: upper arm skin.

DISCUSSION

To appreciate the significance of these findings it might be helpful to give a brief outline of what is known of leprosy in man. Human leprosy presents a wide range of clinical and histological appearances, depending on the immunological status and capacity of the patient. The extremes of the spectrum are tuberculoid (high resistant) and lepromatous (low resistant) leprosy, but between these polar forms there is a range of intermediate types which are called "borderline" or "dimorphous" leprosy. They are characterized clinically by the presence of multiple annular lesions and plaques, usually anaesthetic, and histologically there is an epithelioid granuloma with rather scanty and loosely arranged lymphocytes, occasional bacilli, and always neural involvement. Borderline leprosy tends to be an unstable and transient form of the disease, often deteriorating towards lepromatous leprosy if untreated and tending to revert to the tuberculoid end of the spectrum during successful treatment.

Little is known of the early stages in the development of leprosy. Even the route by which the bacilli enter the body is not known, and in such a slowly developing disease the difficulty of dating the initial infection is very great. Nevertheless, it is usually believed that the minimal incubation period is of the order of two years, which is in reasonable agreement with the estimated multiplication time for *Myco. leprae* of about 12 days (Shepard and McRae, 1965). It follows, therefore, that even the earliest lesions seen in man are the result of infections which are likely to have been present for many months or even years. Hence an infection in a 2- to 3-year-old mouse at the end of its life span would correspond to a very early one in man.

In normal mice the progress of the infection is consistent with the pattern of development of human leprosy so far as this is known. In the first months after inoculation of leprosy bacilli there was a period of multiplication during which there was no specific host response. It was not until two or more years after inoculation that the mice developed granulomata resembling those in human leprosy in the borderline range. These lesions were found only at inoculation sites, but not every lesion seen in the foot pad was a fully mature granuloma. Nevertheless, Figs. 1 and 2 show how exactly the mouse infection reproduces the human disease.

From these observations it is clear that the normal mouse provides an accurate model for studying the early stages in the pathogenesis of leprosy. In particular there is a long incubation period, corresponding to estimates of that in man, before a lesion develops which corresponds to human leprosy. In addition, granulomata and nerve destruction develop locally and in many cases resemble those of human leprosy in the borderline range. Even in animals in which the local histopathological picture of borderline leprosy has not fully matured nerve fibres are involved and the lesion tends towards the borderline rather than the lepromatous type. Furthermore, there is an early spread of the infection via the blood stream to distant sites, particularly those most often involved in man. The histopathology of these sites, though not fully developed, tends towards that of borderline leprosy.

We have already shown (Rees and Weddell, 1968) that when normal mice are injected intravenously an infection develops which is in the lepromatous range, though falling short of a fully developed granuloma. We have also reproduced lepromatous leprosy, though only in mice subjected to thymectomy and whole-body irradiation (900 r) (Rees, 1966; Rees *et al.*, 1967). The fact that human borderline leprosy can be produced in normal mice greatly increases the significance of the findings in immunologically handicapped animals. Although tuberculoid leprosy has not yet been reproduced, it is now certain that the mouse can be used as a model for most of the range of human leprosy infections.

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