MAST CELL AND MYCOBACTERIUM LEPRAE IN EXPERIMENTAL LEPROSY

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ABSTRACT — The association of mast cells and lepra bacilli was studied in the foot pad skin of immunosuppressed mice inoculated with 10^5 lepra bacilli. The mice were killed at intervals varying from 2½ — 14 months postinoculation. **M. leprae** are found in large numbers in nerves, blood vessels, muscle fibres, hair follicles, sweat and sebaceous glands. Mastocytosis and extensive degranulation are seen at similar sites. Since these are the structures most affected in leprosy where the lepra bacilli and degranulating mast cells accumulate, there appears a close relationship between the two. This could be due to the composite role of mast cells in host tissue response and mastocytosis and degra-nulation may be related to this.

***Key* words: Experimental leprosy. Mycobacterium leprae.** Mast cells.

1 INTRODUCTION

Mastocytosis and degranulation of mast cells is a common feature reported in a variety of conditions like allergy, bacterial infections, neoplasms and mycobacterial diseases[1,2,5,15]. The morphology of the mast cell and its degranulation usually depend upon the type and severity of the disease. The present study furthers our observations on the structural changes in the mast cells and their close association with lepra bacilli in the foot pad of the immunosuppressed mouse inoculated with *M. leprae.*

2 MATERIAL AND METHODS

Immunosuppressed CBA mice (thymectomized and subjected to 900r total boby irradiation) of both sexes, weighing 20-25 gins, were employed throughout the experiments. They were inoculated with freshly prepared suspension of lepra bacilli (10^5) subcutaneously into right foot pad. The animals were killed at different intervals from 2½ months to 14 months. The skin biopsy was taken from the site of inoculation. The biopsies fixed in Susa solution were embedded in paraffin to cut 5-7 µ. thick sections and stained with haematoxylin and eosin, Ziehl Neelsen, Fite Faraco technique for AFB and toluidine blue by standard method.

These techniques served to confirm the diagnosis of the lesion produced in the mouse foot pad to detect the presence of bacilli and to reveal the morphology of the mast cells.

The normal mouse foot pad of same strain served as control.

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3 OBSERVATIONS

Mastocytosis was observed irrespective of the presence, absence or density of the mycobacteria. The cells presented morphological changes in shape and size, their affinity to accumulate around bacillary group, affected nerves, blood vessels, muscles and skin appendages. In granulomas, the cells appeared larger in size and the shape varied from long, thin to irregular (Fig. 1). The degranulating cells were seen in and around the nerves, muscle fibre and neurovascular bundles (Figs. 3, 4). The degree of degranulation was observed to increase with the progress of lesion. The nerves with healthy axons did not show much concentration of mast cells around them. There was an apparent increase in the density of the mast cells as compared with the control group and the cells showed massive degranulation (Fig. 2). The degranulating mast cells clustered around the thickened epineurium of the nerve bundle which was studded with bacilli (Figs. 5 and 6). The mast cells and the bacilli were seen in relation to the structures, mostly involved in lesions of leprosy. Besides the isolated mast cell granules and bacilli were also seen scattered in the dermis. Though the mast cells and the bacilli had the predilection for the same structures yet it was not a constant feature to find both of them together at the same site. Some affected nerves showed thickened epineurium and after 13 months postinoculation, the bacilli were seen in groups at this site (Fig. 6) . Mast cells were present both within and outside nerve bundles in all the foot pads, many more in number in the one which showed bacilli.

4 DISCUSSION

Lymphocytes, plasma cells, macrophages and fibroblasts have been described to play essential immunological role in leprosy infection. There is, yet, another connective tissue cell, the mast cell, which has been reported to be present in abundance in the granulomas of leprosy[16]. The mast cell has been classified into different groups on the basis of its shape, size, staining tinctorial properties and functional response to various agents. Its morphology varies from species to species and even in different tissues of same individual. The detailed morphology of mast cell was conducted in normal mouse foot pad in order to appreciate and compare the changes observed in leprosy[13]. The mast cell of the normal (control) foot pad showed round to oval shape and the cells were conventionally recognized by the presence of cytoplasmic metachromatic granules masking the nucleus. Majority of the cells were intact and showed perineural, perivascular, periglandular and perifollicular arrangement[17]. Moreover occasional cell was found elongated in shape. The normal round shape of mast cell was confirmed by culturing the mast cell which when matured had smooth surface, rounded nucleus which was masked by the cytoplasmic granules. These cells were found to be structurally and functionally same as the native cells [3,7,8,9]. The majority of the mast cells showed altered morphology in our experiments and rarely a round cell was seen. Many shapes and sizes of mast cells were observed in inoculated foot pad. It is suggested that the manifold types of mast cells when encountered in the tissue, merely represented the variant of the same cell at different stages of maturity or activity and the structural adaptations to surrounding tissues and Stimuli[18].The structural and morphological changes were also observed in mouse foot pad skin inoculated with serum obtained from lepromatous leprosy patients[14]. Thus this structural response of mast cells could be caused

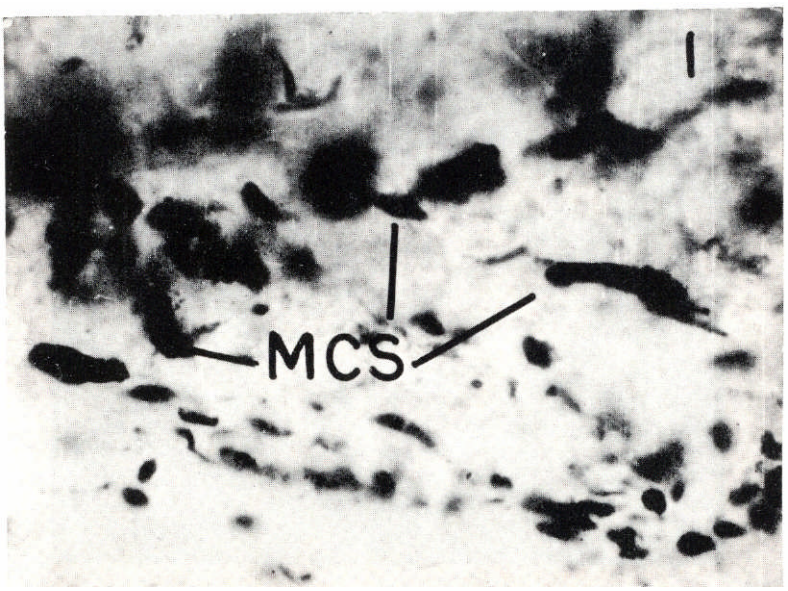

FIGURE 1 - Photomicrograph of the skin of the mouse foot pad (immunosuppressed and inoculated with lepra bacilli) — The different shape and size of mast cells. Stain toluidine blue x 400. *

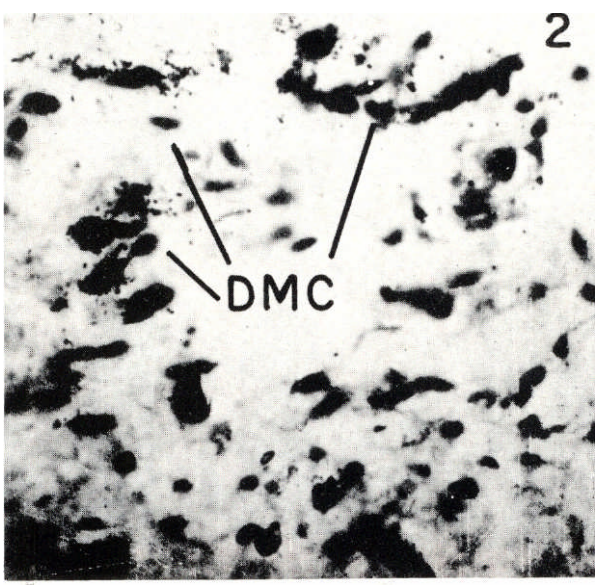

FIGURE 2 — Photomicrograph of the skin of the mouse foot pad (immunosuppressed and inoculated with lepra bacilli) — Degranulating mast cells. Stain toluidine blue x 400.

(*) Mast cells (MCS).Degranulating mast cells (DMC).

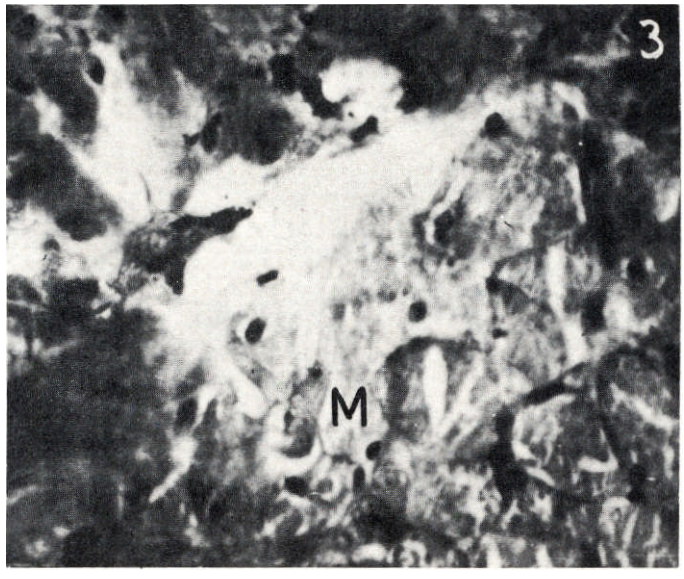

FIGURE 3 - Photomicrograph of the skin of the mouse foot pad (immunosuppressed and inoculated with lepra bacilli) — Degranulating mast cells are seen in relation to muscle fibre. Stain toluidine blue x 400. *

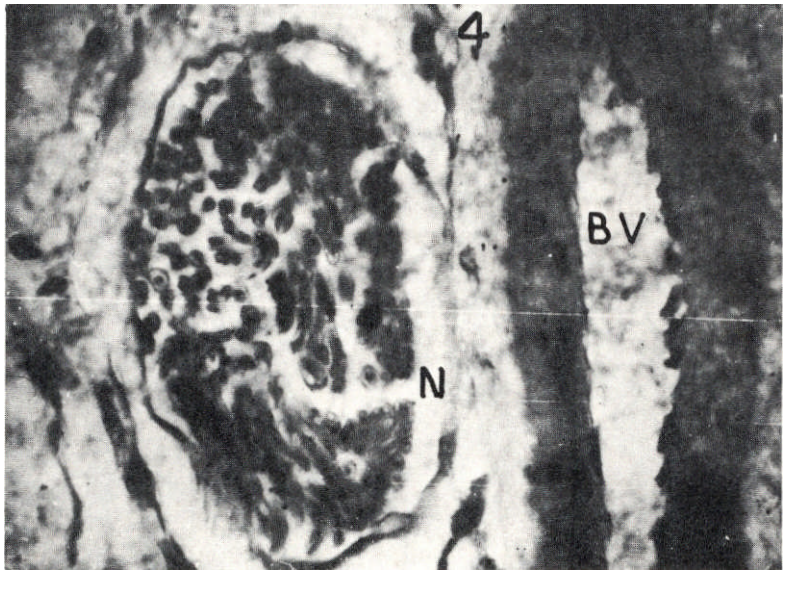

FIGURE 4 - Photomicrograph of the skin of the mouse foot pad (immunosuppressed and inoculated with lepra bacilli) — Degranulating mast cells are seen in relation to neurovascular bundle. Stain toluidine blue x 400. *

(*) Muscle fibre (M). Nerve bundle (N). Blood vessel (BV).

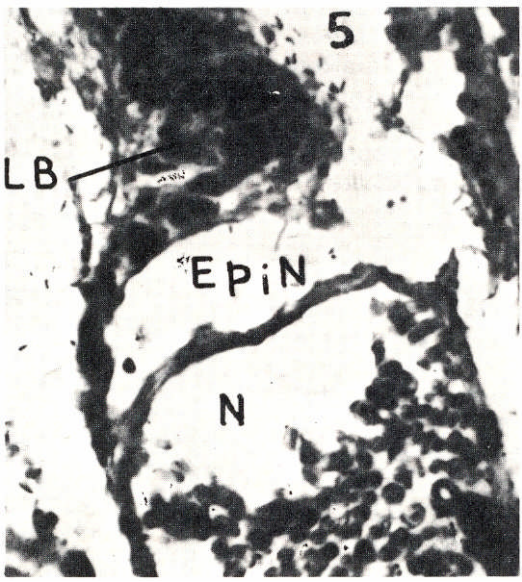

FIGURE 5 — Photomicrograph of the skin of the mouse foot pad (immunosuppressed and inoculated with lepra bacilli) — Thickened epineurium studded with lepra bacilli. Stain Fite Faraco x 400.

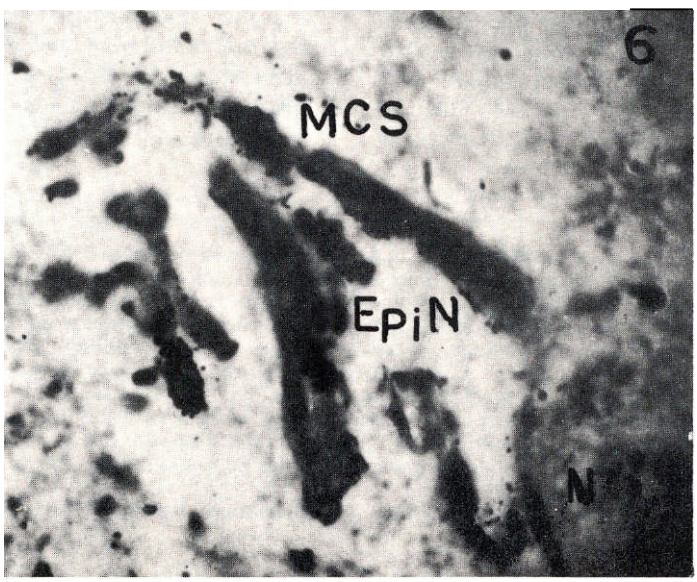

FIGURE 6 — Photomicrograph of the skin of the mouse foot pad (immunosuppressed and inoculated with lepra bacilli) — Degranulating mast cells around the lepra bacilli in thickened epineurium. Stain toluidine blue x 400. *

(*) Nerve bundle (N). Lepra bacilli (LB). Epincurium (EPiN). Mast cells (MCS).

by either the infection or by the bacilli directly. The morphological changes, mastocytosis and massive degranulation was the response observed in all the experimental groups which appeared to be an integral part of the physiopathology of this chronic infective process of leprosy. This response was observed irrespective of the presence or absence of the bacilli.

Abundance of eosinophils and mast cells have been reported in the granuloma of tuberculoid leprosy[16]. Mastocytosis has also been reported in murine and human leprosy [6,10,11,19]. Increased density and extensive degranulation was a constant feature in our experiments where the cells were mainly seen degranulating around affected nerves, muscle fibres and blood vessels. Lepra bacilli have been found in Schwann cells either as single or in clusters[4,12,14]. They have the tendency to settle in the nerves more than other structures which was clearly seen in our experiments. The isolated bacilli have also been seen inside the muscle fibre and in relation to blood vessels. Similar distribution of the degranulating mast cells was also observed in our study. This indicates the close association of lepra bacilli with the mast cells which either accumulated around the bacilli or were lying in relation to the structures severely affected in leprosy i.e. nerves, blood vessels, muscles, hair follicles and glandular elements[4].

It is certain that mast cells do not contribute to the formation of the granuloma but appear to be definitely one of the activated connective tissue cells in them around the affected nerves, muscles and blood vessels. It is indeed difficult to decide whether the observed mast cell changes are the cause or the result of the morbid lesion in leprosy. The structural changes, mastocytosis and excessive degranulation of mast cells in the mouse foot pad of the mouse in leprosy can be explained on the basis of defence mechanism against the invading mycobacterium Their presence around the group of *Mycobacterium leprae, is* to be considered, if they are advantageous to the host or to the organism and this needs further investigations.

RESUMO — A associação de mastócitos e bacilos de Hansen foi estudada na pele do coxim plantar de camundongos timectomizados e submetidos a 900r de radiação total inoculados com 10^5 **M. leprae.** Os camundongos foram mortos em intervalos que variaram de 2 e meio a 14 meses após a inoculação. Bacilos foram encontrados em grande quantidade nos nervos, vasos sangüíneos, fibras musculares, folículos pilosos, glândulas sudoríparas e sebáceas. Mastocitose e granulação extensa foram observadas nesses sítios. Já que estas estruturas são as mais afetadas na hanseníase, nas quais os bacilos e os mastócitos granulosos se acumulam, parece haver uma estreita correlação entre os dois. Isto pode ser devido ao desempenho múltiplo dos mastócitos na resposta dos tecidos hospedeiros; a mastocitose e a granulação podem estar relacionadas com esses fatos. **Tradução do Editor.**

Palavras chave: Hanseníase experimental. **Mycobacterium leprae.** Mastócitos.

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