Mast cells in leprosy skin lesions

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Abstract
The variability of mast cell density within and between leprosy skin lesions was examined as a basis for future studies, and whether the number of mast cells in the lesion was determined by local or systemic factors was evaluated. The mast cell density in the granuloma, skin appendages, and intervening dermis was assessed by counting mast cells in glycol methacrylate sections stained with Giemsa stain and relating these counts to area measurements obtained by planimetry. In biopsy specimens taken from the edge of established lesions, the density of mast cells within the granulomata was considerably higher than that in the intervening dermis and was comparable with that found in the appendages. No major differences in mast cell density were found between unaffected skin and the centre or edge of individual lesions. Mast cell densities in biopsy specimens from the edge of different lesions on the same patient were also similar, suggesting that the mast cell density within the granulomata is independent of the site of the lesion and is determined systemically.

The skin lesions of leprosy contain many if not all of the cell types which occur in normal skin, in addition to those which migrate into the lesions from peripheral blood as part of the granulomatous process. The contribution of cells normally present in the dermis to granuloma formation and progression is uncertain. Mast cells have received little attention in leprosy recently, but evidence linking them with the development of delayed hypersensitivity reactions raises the possibility that they might be of some importance in leprosy lesions. Mast cells are heterogeneous with respect to their morphology, biochemistry, and function. The differences between them appear to depend on the tissue and species from which they are derived, so that is is not possible to apply the results of studies of mast cell distribution or function in one site or animal to another with any certainty.

Type 1 reversal reactions are thought to reflect increased delayed hypersensitivity to Mycobacterium leprae and account for much of the nerve damage which occurs in borderline leprosy patients. Clinically there is inflammation of both skin lesions and affected nerves, with pain, swelling, and tenderness on palpation. Histologically, intercellular oedema is prominent, there is an influx of lymphocytes, fibroblast proliferation is evident, and giant cell formation occurs. Patients of the same classification on the Ridley-Jopling scale vary in their susceptibility to reversal reactions.

It has long been known that mast cells are intimately concerned with the production of oedema, one of the features of reversal reaction. Their possible role in the pathogenesis of such reactions was investigated by Chowdhury and Ghosh, who found a lower density of mast cells in tuberculoid lesions in reaction compared with non-reactive tuberculoid lesions. There is also some evidence of mast cell degranulation in the cutaneous form of leprosy, for example, those due to Mycobacterium leprae.

The aim of this investigation was to examine the variability of mast cell density within and between leprosy skin lesions as a basis for future studies, and to ascertain whether the number of mast cells in the lesion is determined by local or systemic factors.

Three methods of staining mast cells are currently in use: (a) metachromatic staining of their granules; (b) histochimical demonstration of enzymes; and (c) immunoperoxidase staining using monoclonal antibodies. As methods (b) and (c) both require frozen sections metachromatic staining was the only option available for this study. The method developed by Vogt et al. using glycol methacrylate sections stained with Giemsa stain was chosen for this study because it combined the excellent morphological detail of plastic embedding with a reliable method of showing the presence of metachromatic granules.

Methods
Two groups of 4 mm skin punch biopsy specimens were obtained under local anaesthesia with lignocaine from patients with leprosy in India and Bangladesh. All of the patients were untreated or had received treatment (WHO multiple drug therapy regimen) for less than one month. Multiple biopsy specimens were taken from individual patients; for the examination of intralesional variation in mast cell numbers, specimens were taken from the same lesion, the edge of the lesion, and from the apparently unaffected skin outside the lesion at a point 2 cm from the nearest identifiable margin of the lesion in 22 patients (biopsy series 1); for the study of interlesional variation specimens were taken from the edge of two different lesions on the same patient in 35 cases (biopsy series 2). An effort was made to choose lesions of disparate size and appearance
where possible to determine the maximum variation which might occur. The number, age, and sex distribution, and Ridley-Jopling classification of the patients in each series is shown in table 1.

After being transported to Dundee in 4% buffered formaldehyde each biopsy specimen was bisected: one half was embedded in paraffin wax, the other in glycol methacrylate.11 Paraffin wax sections (5 μm) were stained with haematoxylin and eosin and by the Wade-Fite method for diagnostic purposes, while 2 μm sections were cut from the glycol-methacrylate embedded half of each biopsy specimen and stained for mast cell quantitation using the Giemsa method developed by Vogt et al.11

The dermal area and the areas of the section occupied by granuloma and by skin appendages (sebaceous glands, sweat glands, and hair follicles) were measured by planimetry.10 Mast cell counts were performed on non-overlapping fields at ×400 magnification by scanning the whole section using a 25 square eyepiece grid. To avoid overestimation of mast cell density cells were counted only if part of the nucleus could be seen, and anucleate fragments were ignored.

Mast cell counts were expressed for each component (dermis, granuloma, and appendage) as cells per mm². The results for paucibacillary and multibacillary cases were compared using the Mann-Whitney U test for non-parametric data. As lesion selection may have occurred when taking biopsy specimens from different lesions (biopsy series 2) the results in this group were randomised before the relation between mast cell densities in individual patients was assessed by linear regression analysis.

**Table 1  Age and sex distribution of patients and Ridley-Jopling classification**

<table>
<thead>
<tr>
<th>Age (Mean (SD))</th>
<th>Sex ratio (M:F)</th>
<th>Ridley-Jopling classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Idt  TT  BT  BB  BL  LL  Total</td>
</tr>
<tr>
<td>Series 1 36 (13-0) 16:6</td>
<td>1 0 14 0 3 4 22</td>
<td></td>
</tr>
<tr>
<td>Series 2 38 (15-1) 28:7</td>
<td>0 1 19 2 1 12 35</td>
<td></td>
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</table>

Idt = indeterminate; TT = tuberculoid; BT = borderline-tuberculoid; BB = borderline; BL = borderline-lepromatous; LL = lepromatous.

**Results**

The appearance of the mast cells in leprosy lesions is very variable. Several morphologically distinct variants were present: “active mast cells” with large ovoid nuclei, prominent nucleoli, and extensive cytoplasm with many granules; “fusiform mast cells” with elongated dark-staining fusiform nuclei and little cytoplasm; and “resting mast cells” with small dark nuclei, scanty cytoplasm, and few granules. These cell types were most commonly found in inflammatory foci (fig 1), applied to sebaceous or sweat glands (fig 2), and in the unaffected dermis (fig 2), respectively.

There were too many cells of intermediate morphology, however, to allow these types of mast cell to be differentiated and counted separately on morphological grounds alone.

**BIOSPY SERIES 1**

The average mast cell density in biopsy specimens from unaffected skin was 17.6 per mm². Specimens taken from the centre of the lesion had an average of 18.7 mast cells per mm², while at the edge of the lesion the average mast cell density was 19.5 per mm². There was no significant difference between the densities in either of these sites, suggesting that the total number of mast cells in the skin does not change greatly within the lesion and is similar to that in normal skin. When the component mast cell densities from different sites in the same lesion (granuloma, appendages, and intervening dermis) were compared in each biopsy specimen there was no discernible pattern in either paucibacillary or multibacillary patients.
BIOPSY SERIES 2
The overall mast cell densities in biopsy specimens from the edge of different lesions on the same patient showed some similarity \( (r = 0.559, \text{slope} = 0.534) \) (fig 3a), despite the differences in mast cell density known to occur between different anatomical locations in normal human skin. \(^{11}\) When the component mast cell densities were compared between different lesions, the strongest correlation was found within the granulomata \( (r = 0.854, \text{slope} = 0.827) \) (fig 3b), and there was little correlation in the mast cell densities from the appendages \( (r = 0.107, \text{slope} = 0.007) \) or intervening dermis \( (r = 0.481, \text{slope} = 0.354) \).

To examine possible relations between mast cell density and Ridley-Jopling classification, the results from edge biopsy specimens from different lesions (biopsy series 2) were summed and considered with the results from those from the edge of the lesion in biopsy series 1 (table 2). In those from the edge of the lesion the density of mast cells was greatest in the granulomata (average density = 99.7 per \( \text{mm}^2 \)), but large numbers were also found around skin appendages (average density = 72.8 per \( \text{mm}^2 \)). In the intervening dermal connective tissue mast cells were uncommon (average density = 9.6 per \( \text{mm}^2 \)) and were usually of “resting” morphology. There was no significant difference between the overall mast cell densities in biopsy specimens from the edge of paucibacillary or multibacillary patients (table 2). The granuloma mast cell density (fig 4) is reduced in multibacillary patients compared with paucibacillary patients (Mann-Whitney \( U \)-test, \( p < 0.04 \)), as is the mast cell density in the intervening dermis compared with the granuloma mast cell density (Mann-Whitney \( U \)-test, \( p < 0.02 \)). The mast cell densities within the appendages showed no appreciable difference between paucibacillary and multibacillary patients. To investigate the possibility that mast cells might be moving into the granuloma from either the appendages or the intervening dermis, the relation between component mast cell densities was examined, but there was no inverse correlation between the granuloma, appendage, and intervening dermal mast cell densities in individual patients.

Discussion
The methacrylate sections stained with Giemsa \(^{1} \) used in this study provided excellent histological detail. All cells containing metachromatic granules could be counted easily, even if the cells were partially degranulated and only a few granules were present. Although Vogt et al state that human basophils can be detected using this method, their differentia-

<table>
<thead>
<tr>
<th>Grade</th>
<th>n</th>
<th>Granuloma</th>
<th>Appendage</th>
<th>Dermis</th>
<th>Total skin</th>
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<tr>
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<td>114.7</td>
<td>77.3</td>
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<tr>
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<td>79.6</td>
<td>65.6</td>
<td>8.5</td>
<td>17.2</td>
</tr>
<tr>
<td>MBL</td>
<td>57</td>
<td>99.7</td>
<td>72.8</td>
<td>9.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>79.0</td>
<td>70.8</td>
<td>5.5</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Table 2 Average mast cell densities (per \( \text{mm}^2 \)) in skin and component parts of specimens taken from edge of leprosy lesions.

Figure 3 Correlations (with regression lines shown) between mast cell densities in biopsy specimens from the edge of two separate lesions on the same patient. (biopsy series 2).
(a) Total skin mast cell densities \( (r = 0.559, p < 0.001) \)
(b) Granuloma mast cell densities \( (r = 0.854, p < 0.001) \).

Figure 4 Comparison of granuloma mast cell densities for edge biopsy specimens from paucibacillary (PBL) and multibacillary (MBL) patients, showing pronounced variation in granuloma density between patients. The granuloma mast cell is significantly lower in multibacillary patients \( (p < 0.04) \).
Mast cells in leprosy skin lesions

From mast cells in human tissues is problematical and relies on their polymorphonuclear morphology, which is not easily appreciated, even in thin sections. Unequivocal basophils were not seen in the sections examined during this study and all cells containing metachromatic granules were included in the counts.

The role of mast cells in delayed hypersensitivity reactions has attracted considerable interest in recent years from those studying mast cell function in experimental animals. Interleukin 3 (IL-3) production by T lymphocyte is implicated in mast cell precursor maturation and that T lymphocytes may promote mast cell division and that other mediators cause mast cell degranulation which is not IgE dependent.

In leprosy mast cells were first investigated by Sen Gupta and Ghosh, who published their observation that mast cell granules could be stained by acid fast methods such as the Fite-Faraco method. Using toluidine blue stained paraffin wax sections, Chowdhury and Ghosh showed that the density of mast cells within tuberculoid lesions was lower if the lesions were in reaction. They noted that these observations mirrored the histamine content of non-reactional lesions and those in reaction. Mast cells which have partially degranulated, however, possess few remaining granules and may be missed in paraffin wax sections stained with toluidine blue. Their results probably indicate widespread degranulation of mast cells in reactions. The presence of oedema may also be important as this would increase dermal volume and result in an apparent decrease in mast cell density. A similar increase in dermal volume in multibacillary patients is likely to be the cause of the lower granuloma mast cell density in these patients compared with paucibacillary patients.

Mast cells are a heterogeneous population of cells. In different tissues they exhibit very different biochemistry and show functional differences. In the biopsy specimens examined here they exhibited a wide range of morphological types which seemed to be common in different sites. Although total mast cell degranulation occurs in vitro, mast cell activation in tissues is thought to provoke partial degranulation and continuous production of various inflammatory mediators, including prostaglandins and histamine. Their functional activity in the lesion may well be more important than their overall number, and further studies of mast cell morphology in leprosy lesions using both light and electron microscopy would be worthwhile.

The quantitative data show that in biopsy specimens from the edge and centre of leprosy lesions and outside the lesion, the total number of mast cells present does not change significantly. Although it was not possible to compare the results of this study with those from normal subjects, the mast cell density in clinically unaffected skin was found to be 17-6 per mm², a figure comparable with the mast cell density found in normal skin by other workers, if allowances were made for methodological differences. The average number of mast cells at the centre and the edge of the lesion is apparently slightly smaller than it is outside the lesion, although this may reflect the loss of skin appendages at the centre of tuberculoid and borderline lesions. The degree of migration of mast cells into leprosy granuloma, either from the surrounding dermis or as precursor cells from the blood, may therefore be small in relation to the overall numbers present in the skin. Comparison of the component densities at different sites within the lesions showed no rise or fall in mast cell density within the granuloma, appendages, or intervening dermis. This finding contrasts with both granuloma fraction and apoptotic density, both of which are highest at the edge of the lesion.

Biopsy specimens from the edge of different lesions in the same patient showed considerable variation in overall mast cell density, in keeping with previous studies of the distribution of human mast cells in skin. The correlation in mast cell densities within the granuloma from different lesions, however, is unexpectedly strong (r = 0.854), with a slope close to that which would be produced by complete agreement. This suggests that the number of mast cells within leprosy granuloma is independent of the site of the lesion, and that the mast cell density within granuloma is mainly determined by the systemic response to infection by M lepra and not by local factors.

The density of mast cells within the granuloma is considerably higher than that in the intervening dermis and is comparable with that found in the appendages. Several of the cases studied show much higher mast cell densities within their granuloma than average (fig 4). In the absence of sequential studies of biopsy histology during treatment and reactions in leprosy the clinical importance of these differences is not clear, but the fact that such differences exist is of interest, and the results of the seminal study by Chowdhury and Ghosh suggest that differences in mast cell density might be related to a tendency to develop reactions. The hypothesis that those patients with higher granuloma mast cell densities might be susceptible to more damaging reversal reactions due to the release of mediators from the mast cells seems attractive and is worthy of further study.

We are most grateful to LEIPRA for funding the collection of the biopsy specimens. We thank Dr J Mehta, C A Gardiner, C Fisher, and R Cochrane and R Hart for their assistance. We also thank Mr R Fawkes, Mrs S Gibbs, and Mr S MacPherson for preparation of the figures.

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