Dermal Mast Cells in Mastocytosis: Fixation, Distribution and Quantitation

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The mast cell distribution and number were studied in skin biopsies of 18 mastocytosis patients and 10 controls. The biopsies were stained for mast cells with toluidine blue at pH 0.5. The number in the upper dermis of lesional abdominal skin was at least twice as high as that of normal adjacent skin. Fixation in iso-osmotic 0.6% formaldehyde and 0.5% acetic acid, revealed more mast cells than conventional 4% formaldehyde fixation. Staining for 5 days, when compared to the normal for 30 min, increased the number of demonstrable mast cells just as did the change in fixation. Conventional formaldehyde fixation thus partially blocks the dye-binding of cutaneous mast cells, about 20% of the cells escaping detection. The degree of aldehyde blocking was similar in lesional and normal skin. A more pronounced blocking of dye-binding has been demonstrated previously in gut mucosal mast cells. Whether the blocking of dye-binding is an expression of heterogeneity in dermal mast cells remains to be determined. (Received April 4, 1985.)

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Mastocytosis is a dithe disease is localized to the skin, it is called urticaria pigmentosa (UP). In UP, the mast cell increase in the skin occurs mainly in the subpapillary layers of the dermis (1). The mast cells are sometimes found in tumour-like aggregates, but are most often more evenly spread and sometimes even relatively sparse. In the absence of other histological changes and if the clinical picture is ambiguous, the diagnosis of mastocytosis may be difficult in these cases. In order to assess the mast cell number in an objective way, it is essential to know the distribution and number of mast cells in normal and diseased skin, and the optimal techniques for their preservation and demonstration in the tissue.

In normal dermis the mast cells are mainly found in the vicinity of capillaries. Since most capillaries are localized to the subpapillary region and around the cutaneous appendages, mast cells are observed in great number in these areas (2, 3). The mast cell density in normal skin is greatest immediately below the dermo-epidermal junction, gradually falling to a minimum density in the deeper layers of the dermis (4). Consequently, when mast cells are counted in skin biopsies, the depth of the biopsy must be taken into consideration, as more superficial biopsies will tend to show more mast cells per unit tissue area than deep ones.

The demonstration of mast cells in tissue sections may be effected by the histotechnical procedure, especially the tissue fixation (5). Aldehyde based fixatives are commonly used in routine histopathology and are also usually satisfactory for the demonstration of mast cells in human tissues. However, aldehydes may interfere with the dye-binding of the granular glycosaminoglycans by their fixation of structurally associated protein (6). This blocking effect of aldehyde may be overcome by very long staining times (6), or by the use of special fixatives such as an iso-osmotic low concentration mixture of 0.6% formaldehyde and 0.5% acetic acid (IFAA) (7). The aldehyde blocking of dye-binding, however, may be used as one means of detecting heterogeneities in the mast cell system which are now becoming increasingly evident (8). Thus, mucosal mast cells, which appear to

constitute a distinct mast cell phenotype (9), are especially sensitive to this blocking of dye-binding, both in the rat (6) and in man (10, 11).

This paper reports the distribution of mast cells in mastocytosis lesions and control skin, and their reaction to aldehyde fixation. Furthermore, the paper examines the practical conditions for their quantitative assessment in the tissue.

MATERIAL AND METHODS

Patients

Eighteen patients with the clinical diagnosis of UP were studied, 9 women and 9 men. Six of these patients had systemic mastocytosis, verified by mast cell increase in bone marrow biopsy and increased urinary excretion of tele-methylimidazoleacetic acid (MeImAA), which is the main histamine metabolite (12). Only one of the patients with systemic mastocytosis had symptoms which could be related to systemic involvement of the disease, these being in the form of diarrhea and malabsorption. The ages ranged from 19 to 69 years with a mean age of 41 years. Two to 4 biopsies from lesional and uninvolved skin were taken from each patient for fixation in IFAA or 4 % neutral formaldehyde (see below).

For controls, 4 skin biopsies were obtained from each of 10 patients undergoing a hernia operation. These patients were not known to have any other diseases nor were they taking any drugs such as cytostatics, corticosteroids or antihistamines. Their mean age was 52 years with range from 17 to 72 years.

Skin biopsy procedure, fixation and staining

Skin biopsy specimens were taken with a disposable punch biopsy instrument for biopsies 3 mm in diameter. For local anesthesia, 1 % xylocaine without adrenaline was injected around the area to be biopsied. Skin biopsies were taken from lesions and normal skin in the subcostal abdominal region and from sites less than 1 cm apart in each patient. In control patients biopsies were taken from the lwoer abdominal region.

Biopsies were either fixed in 4% formaldehyde for 1–2 days, and/or in IFAA for 24 hours. All biopsies were embedded in paraffin. Sections approximately 4 µm in thickness were cut as near the middle of the biopsy as possible and perpendicular to the skin surface. The sections were stained with 0.5% toluidine blue at pH 0.5 for 30 min. Formaldehyde fixed sections, cut as close as possible to the other section, were also stained with toluidine blue but for 5 days.

Mast cell counts

The mast cell counts were carried out with a microscope equipped with a ×40 objective lens and a ×125 ocular, the ocular having an eye-piece graticule in order to insure that overlap and double counting did not occur. All counts were done by the same person. The whole section was counted, but records were also made of the number of mast cells in the upper 0.4 mm layer of the biopsy including the epidermis. All mast cell profiles were counted, regardless of whether the nucleus could be identified or not, but the counting of small cell fragments was avoided. Three sections from each biopsy were counted and each section was counted twice. The mean of the two counts was used in all calculations. The methodological error calculated from the coefficient of variation of duplicate determinations was 6%.

The area of the sections was obtained by drawing the outlines of the biopsy on a paper by utilizing a camera lucida microscope in ×250 magnification. The outline of the upper 0.4 mm layer of the epidermis and dermis was also drawn on the paper. The total and upper 0.4 mm areas were then calculated with a HJouston Instrument Hipad Digitizer attached to a computer, programmed for area calculation. The coefficient of variation from duplicate area readings was 0.4%. The mast cell density was expressed as the number of cells per mm^2 of the whole section or of the upper 0.4 mm area.

The apparent mast cell density may be effected by volume chganges during fixation and staining. However, no differences were found between the mean section area of biopsies fixed in IFAA and formaldehyde, or between formaldehyde fixed sections stained for 30 min or for 5 days.

Aldehyde blocking of dye-binding

The degree of blocking was assessed by determining the fraction of cells counted in sections stained for 5 days (not for 30 min) in formaldehyde fixed tissue, or as the fraction of cells counted in sections of IFAA fixed tissue but not in formaldehyde fixed tissue (staining time 30 min).
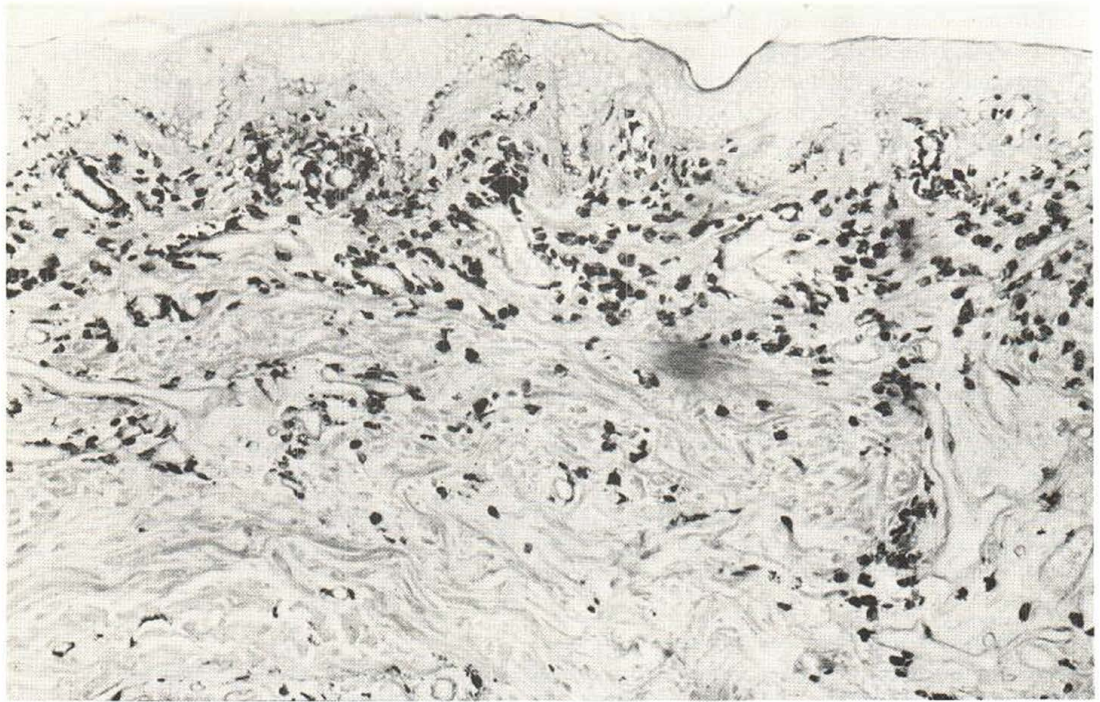
Fig. 1. Mast cell infiltration in the upper dermis of mastocytosis lesions. fixed in 4% formaldehyde and stained with 0.5% toluidine blue for 5 days. ×125.

Statistical analysis

Comparisons of the mast cell density form different fixations and stainings and between involved and uninvolved skin, were carried out using the Student's t-test. A paired t-test was used when applicable for the comparison, otherwise the t-test for two means was used. A two-tailed test was used in all cases. *p-Values* of less than 0.05 were considered significant.

RESULTS

Toluidine blue stain at pH 0.5 resulted in distinct staining of mast cell granules, while the nucleus and the surrounding structures were virtually unstained, greatly facilitating the identification and counting of the mast cells. When stained for 30 min, the granules stained metachromatic violet, while staining for 5 days made the granules appear dark blue. The mast cell granules were easily distinguished from melanophage granules (Fig. 1).

The mast cells were found at all levels of the dermis but were most numerous in the papillary and subpapillary layers. No mast cells were found in the epidermis. The mast cells were usually evenly distributed in the upper dermis but, in some cases, they were found to lie in dense aggregates. The nucleus was usually spindle-shaped or oval but, in the mast cell aggregates, a cuboidal nuclear appearance was sometimes observed, and the cells seemed somewhat smaller.

The results from the mast cell counts are shown in Figs. 2–4. The mean mast cell density in the involved skin of the mastocytosis patients was up to 7 times higher (IFAA fixation upper dermis) than in the control patients. Furthermore, the mean mast cell density in involved skin of these patients was 4 to 6 timnes higher than in adjacent uninvolved skin.
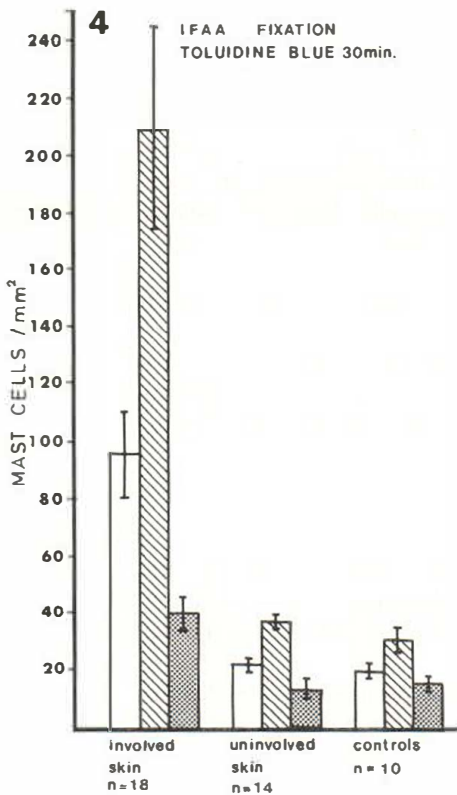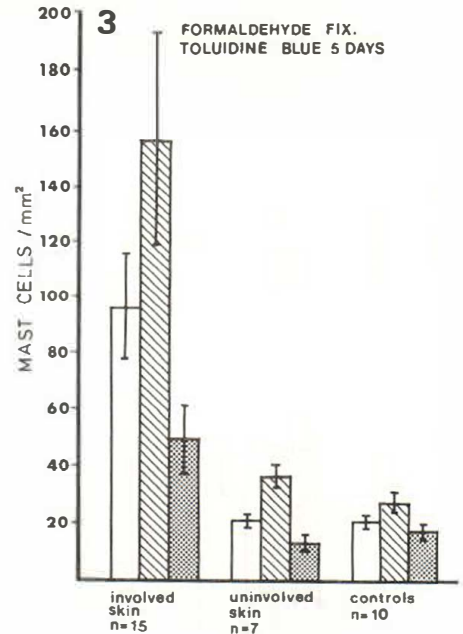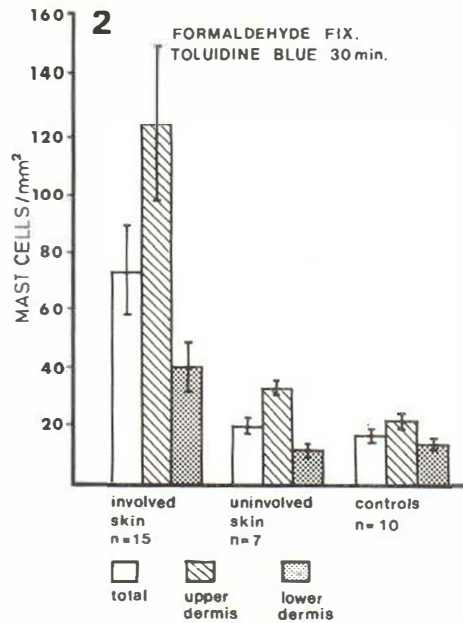
Fig. 2. Mast cell density in the involved and uninvolved dremis of mastocytosis patients and controls. Biopsies were fixed in 4 % formaldehyde and stained with toluidine blue for 30 min. Mast cells were counted per mm² whole section area (total) and per mm² of a 0.4 mm thick superficial region (upper dermis).

Fig. 3. Mast cell density in the involved and uninvolved dermis of mastocytosis patients and controls expressed as in Fig. 2. Biopsies were fixed in 4% formaldehyde and stained with toluidine blue for 5 days.

Fig. 4. Mast cell density in the involved and uninvolved dermis of mastocytosis patients and controls expressed as in Fig. 2. Biopsies were fixed in IFAA and stained with toluidine blue for 30 min.
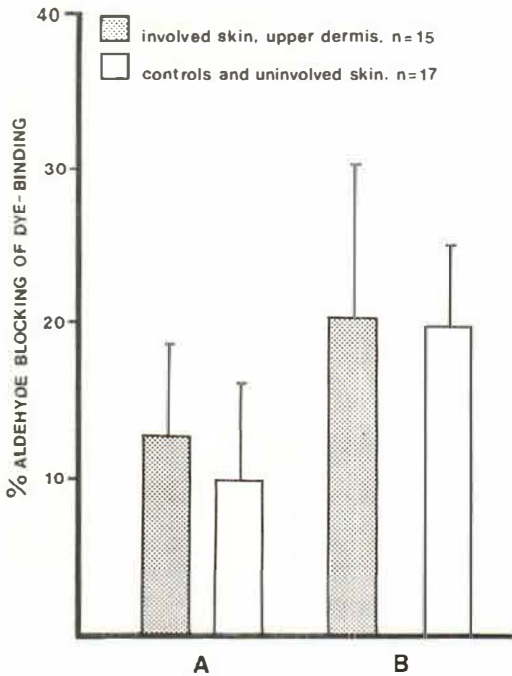
Fig. 5. The degree of dye-blocking in mastocytosis patiens and controls calculated as the percentage of mast cells not stained when biopsies were fixed with formaldehyde and stained with toluidine blue for 30 min as compared to staining for 5 days (A), or to fixation in IFAA (toluidine blue 30 min) (B).

In paired observations in individual patients, the mast cell increase between involved and uninvolved skin ranged from 2–17 times.

The mast cell numbers counted were effected by the mode of fixation and staining time. In general, more mast cells were detected with IFAA fixation than after fixation in formaldehyde and stained for 30 min. This IFAA/formaldehyde difference was most marked in the upper 0.4 mm layer of the dermis where about 40% more mast cells were detected ($p<0.01$). Staining of formaldehyde fixed biopsies with toluidine blue for 5 days rather than for 30 min also resulted in a greater number of mast cells. Again, the staining time difference was most marked in the upper dermis of mastocytosis lesions, where approximately 20% more mast cells were detected ($p<0.05$).

The mast cell density in uninvolved skin from mastocytosis patients was slightly higher than in the controls, however, this difference was not statistically significant.

After having demonstrated the adverse effect of normal aldehyde fixatioon on the mast cells, we assessed the degree of aldehyde blocking of dye-binding as indicated in Material and Methods. On the average, 13–27% of the mast cells could not be detected after normal aldehyde fixation and toluidine blue staining (Fig. 5).

DISCUSSION

The hallmark of the mast cell is the metachromasia of its granules. This is best shown with thiazine dyes, such as toluidine blue. At low pH, the mast cell granules are the only cell elements stained and the background appears virtually unstained, greatly facilitating the identification and counting of the mast cells.

Even if the conventional 30 min toluidine blue staining makes mast cells easily identifiable, our results show that the longer staining times, in the order of 5 days, reveal more mast cells. The same results were obtained when IFAA fixation was used instead of aldehyde fixation.

The calculation of the degree of aldehyde blocking, indicates that approximately 20% of the dermal mast cells cannot be demonstrated after conventional aldehyde fixation and staining. No significant difference was found between lesional and normal skin in the afflicted and the control patients. Aldehyde blocking of dye-binding is one of the characteristics observed in the preparation of mucosal mast cells in rats and mice (7, 9). In addition, it has been shown that aldehydes similarly block the dye-binding of mucosal mast cells in the human gut. As a result in human intestinal mucosa, 70–80% of the mast cells cannot be visualized after normal aldehyde fixation (10, 11). Evidently cutaneous mast cells do not show such a high degree of blocking of dye-binding and, most importantly, the mast cells of the UP lesions do not differ in this respect from normal mast cells. Whether this partial blocking of dye-binding is an expression of heterogeneity among the dermal mast cells remains to be clarified.

In normal skin, mast cells are mainly distributed in the papillary and subpapillary layer of the dermis (4). In UP, the mast cells are increased throughout the whole dermis, the proportionally greatest increase being in the upper dermis (Figs. 1–3). It is therefore important to take the depth of the biopsy into account when the mast cell number is assessed. We chose to draw a line parallel to the surface of the biopsy at 0.4 mm depth, as this was found to be convenient for microscopical purposes, the diameter of the field of vision being 0.4 mm in the lenses used.

Most earlier studies on the distribution of skin mast cells have shown that the density varies considerably with localization on the body (13, 14, 15) and, furthermore, that the density decreases with age (15). It is also well known that many factors affect the cell counts in solid tissue (15, 16), such as the thickness of the sections, the fixation procedures and the staining methods. Because of these variabgles it is often difficult to compare the results of mast cell counts reported in different studies. Therefore, it is unpractical to try to establish reference values for normal mast cell densities. It is appropriate, however, to standardize the localization of the biopsy site in order to gain experience regarding the normal variations of mast cell numbers in that particular area. According to our experience, most UP patients have lesions in the upper abdominal region which, therefore, may be used as a standardized biopsy site. In uncertain cases, mast cell numbers in lesional biopsies can be compared to those of uninvolved skin close to the lesions. In our unselected material of UP, the mast cell density of abdominal skin lesions was at least twice as high as that of adjacent unaffected skin.

There was a slight increase in mast cell density in the uninvolved skin of the UP patients compared to the skin of the controls. Although this is in accordance with previous findings (17), the difference in the present material was not statistically significant. Moreover, this finding is somewhat supported by the histamine content of uninvolved skin in mastocytosis which has also been reported to be elevated compared to controls (12).

Mast cell increases in skin have been described in a number of skin diseases, such as pretibial myxoedema, Paget's disease of the nipple, atopic dermatitis, mycosis fungoides, neurofibromatosis, lichen simplex chronicus, lichen planus and psoriasis (1, 14, 18, 19). However, such increases do not appear to be of the same order of magnitude as that seen in mastocytosis where, in addition, the infiltrate consists almost exclusively of mast cells. Furthermore, these diseases can usually be distinguished from UP clinically.

ACKNOWLEDGEMENTS

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