Increased Numbers of Mast Cells in Pemphigus Vulgaris Skin Lesions

A Histochemical Study

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We have used a histochemical technique to study mast cells (MC) in skin biopsies of 8 patients suffering from pemphigus vulgaris (PV) and from 4 control volunteers. The MC were stained for 30 min with 0.5% toluidine blue, pH 0.5, counted and then restained for 5 days under the same conditions. This staining method allows the identification of two groups of MC, one that stains promptly (30 min) and one that stains after longer incubation times (5 days). After 30 min of staining, a slight increase was found in the number of MC in PV sections, in comparison with normal controls. However, when the 30 min stained sections were reincubated under the same conditions for 5 days, a significant increase in the number of MC in PV was found in comparison with 5-day-stained normal skin sections ($p < 0.005$) and in comparison with 30-min-stained PV sections ($p < 0.005$). The MC were distributed throughout the dermis and were concentrated in the upper dermis near hair follicles and vessels. The possible importance of the increased numbers of MC in PV is discussed.

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In early lesions of pemphigus vulgaris (PV), little inflammatory reaction accompanies the acantholytic process and few eosinophils are seen within the bulla or in the dermis. In older lesions, the number of inflammatory cells, including eosinophils and plasma cells, may be considerable (1). Although it seems that the basis of the blistering is an autoimmune process, it is clear that inflammatory processes are also involved in the pathogenesis of PV. Mast cells (MC) are the key cells involved in allergic reactions but also play a pivotal role in inflammation (2).

The present study was carried out to evaluate, using an histochemical method, the extent of mast cell involvement in PV, since activated MC have been reported to be an important component in recruiting the eosinophils and possibly other inflammatory cells in bullous pemphigoid, another blistering autoimmune skin disease (3).

MATERIALS AND METHODS

Patients

Eight male patients with the clinical and histological diagnosis of PV were studied. Their ages ranged from 20 to 70. As controls, skin biopsies were obtained from 4 individuals with no overt disease who were to undergo plastic surgery. The biopsy sites were similar in the PV and normal subjects.

Skin biopsy procedure, fixation and staining

Skin biopsy specimens were taken with a disposable 3-mm biopsy punch. For local anesthesia, 1% lidocaine without adrenalin was injected. Pemphigus vulgaris biopsies were taken from typical skin lesions.

Biopsies were fixed in 4% formaldehyde for 1–2 days and embedded in paraffin wax. Sections of 5–6 μm were cut. The sections were stained, after deparaffinization, with 0.5% toluidine blue (no. T–3260, Sigma, St. Louis, Mo., USA) pH 0.5, according to the procedure described by Wingren & Enerbäck (4) with slight modifications as follows. The sections were deparaffinized and stained for 30 min and preserved with Entellan (Merck, Darmstadt, Germany). After MC counts, the preparations were immersed in xylene and the coverslips removed. The slides were subsequently washed in decreasing concentrations of ethanol to distilled water, and stained for an additional 5 days with the same solution of toluidine blue. This technique allowed direct comparison between MC counts at 30 min and at 5 days on the same specimen.

Mast cell counts

The MC counts were carried out with a microscope equipped with a x40 objective lens and a x10 ocular. All the counts were performed twice by two different observers. The mean of the two counts was used in all calculations; the two results differed by less than 5%. All MC profiles were counted in the entire section. In case of doubt, a single MC was examined at x1000 by oil immersion.

Statistical analysis

Comparisons of the MC counts after different staining times and between pemphigus lesions and normal skin specimens were carried out using Student's *t*-test. *p*-values of less than 0.01 ($p < 0.01$) were considered significant.

RESULTS

Staining of the sections with toluidine blue (pH 0.5) for 30 min resulted in a distinct violet metachromatic staining of the MC cell granules against a virtually unstained background. Incubation of the 30-min-stained sections for an aditional 5 days in toluidine blue resulted in the orthochromatic staining of the MC present in the skin specimens.

The number of MC per field in the PV sections after 30 min staining was 5.1 ± 0.8 vis-à-vis 3.6 ± 0.4 in normal skin sections ($p < 0.05$). When the staining was continued for 5 days, the number of MC detected in PV was significantly much greater than that in normal skin, viz. 13.7 ± 2.0 vs. 5.6 ± 0.5, $p < 0.005$. The numbers of MC stained in the PV at 30 min and 5 days were compared, showing the latter count to be significantly greater than that at 30 min ($p < 0.005$). In normal skin sections, the number of MC per field after 5 days' staining increased from 3.6 ± 0.4 to 5.6 ± 0.5 ($p < 0.01$).

When we compared the number of MC that were stained after 5 days, following subtraction of the number of cells stained after 30 min, we found 8.6 ± 1.3 MC per field in PV as compared with 2.0 ± 0.45 in normal skin ($p < 0.0005$).

We studied the shape and distribution of MC after 30 min and 5 days of staining, in PV and in normal skin. In both the PV and normal skin specimens, regardless of the staining time, the distribution of the stained MC was similar and consistent in all the sections studied. The MC were found at all levels of the dermis but were most numerous in the papillary dermis, especially around hair follicles and blood vessels. The shapes of the MC were variable, some round and some elongated. Also, their state of granulation was heterogeneous. Some MC, especially in the upper dermis, appeared partly degranulated. Granules were occasionally detected in the epidermal basal layer. In contrast, only very few MC were found degranulated in the control group.

DISCUSSION

Two subclasses of MC have been described in the rat and probably exist in the human, the 'connective tissue' type and the 'mucosal' type (5). In the rat, Enerbäck was the first to characterize 'mucosal' and 'connective tissue' type mast cells by their differing morphological and histochemical appearances. He found that, following fixation in 4%-neutral buffered formalin, rat mucosal MC (unlike connective tissue MC) stain poorly with toluidine blue and must be left in this stain for at least 5 days in order to be visualized (6).

More recently, Olfasson et al. reported the presence of 5-day-stained MC and 30-min-stained MC in normal human skin and in skin of patients with mastocytosis (7). Heterogeneity of the human skin MC as detected by their degree of blocking of dye-binding by formaldehyde and by the critical electrolyte concentration (CEC) of dye binding (both properties depending on the type of proteoglycan contained in the MC) (8,9) as well as by staining with alcian blue-safranin (10) and by the presence of tryptase and a chemotryptic proteinase (11) has also been recently shown.

The presence of numerous MC in the oral as well as in the skin lesions of patients with PV has been reported by Green et al. (12). However, the subtype of these MC has not been examined. In our study, the presence of MC in PV skin lesions has been studied qualitatively as well as quantitatively. The MC have been demonstrated in tissue sections by staining their granular proteoglycan with 0.5% acidic toluidine blue for 30 min and for 5 days, as described by Wingren & Enerbäck (4). We found that PV lesions contain increased numbers of MC compared with normal skin and most of them stained only after 5 days, therefore displaying at least one of the properties of the human mucosal MC, i.e. a high degree of blocking of dye-binding by formaldehyde (8,9). In a comparative study of two groups of patients suffering from psoriasis and bullous pemphigoid, we found a similar increase in the 5-day-stained MC population (unpublished data). To define the phenotype of these 5-day-stained MC as 'mucosal', studies intended to determine the CEC, the type of proteoglycan and of proteinase they contain should be undertaken. In PV lesions, MC were distributed throughout the dermis, most often near hair follicles and blood vessels and many of them showed signs of degranulation. No MC were found in the epidermis as was previously described (12).

The role of the significantly greater number of MC in PV skin lesions is not yet clear. MC granular

mediators include, among others, the eosinophil chemotactic factor of anaphylaxis (13) which in PV may be responsible for the recruitment of the large numbers of eosinophils (1), as in bullous pemphigoid (3).

MC might play a role in the PV acantholysis, whose mechanisms are not completely defined as yet. It has been suggested that the IgG fraction of PV patients' blood may be responsible for the acantholysis (14). Farb et al. (15) demonstrated that the acantholytic-inducing effect of the pemphigus antibodies could be inhibited with soybean trypsin inhibitor and suggested that these antibodies, when combined with an antigen in epidermal surfaces, induce the release of some esterases which can cause acantholysis. MC are known to be a very rich source of serine esterases, accounting for up to 50% of their total protein (13). Whether in PV the MC are the source of such esterases remains to be established.

Further work is necessary to elucidate the role of the increased numbers of MC in the pathogenesis and/or in the inflammatory changes of PV.

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