Chymase expressing bone marrow mast cells in mastocytosis and myelodysplastic syndromes: an immunohistochemical and morphometric study


Background: Two cell specific neutral proteases, tryptase and chymase, are produced by human mast cells (MC). Tryptase is constitutively expressed by all MC, whereas chymase is found only in an MC subset. Very little is known about chymase expression in MC proliferative disorders (mastocytosis).

Aims and Methods: Routinely processed, formalin fixed, and paraffin wax embedded bone marrow trephine biopsy specimens obtained from patients with various subtypes of mastocytosis (n = 47) and myelodysplastic syndromes (MDS; n = 28) were immunostained with antibodies against chymase and tryptase. Normal/reactive bone marrow specimens with intact haemopoiesis (n = 31) served as controls. The numbers of chymase expressing (C+) and of tryptase expressing (T+) MC were assessed morphometrically using a computer assisted video camera system.

Results: In normal/reactive bone marrow, the numbers of C+ MC (median, 8/mm²; maximum, 159/mm²) were in the same range as those of T+ MC (median, 4/mm²; maximum, 167/mm²). Because normal MC express both chymase and tryptase, these findings indicate that the common phenotype of bone marrow MC in normal/reactive states is MCC, (MC expressing both tryptase and chymase). In contrast, in MDS and mastocytosis, the bone marrow exhibited far more T+ MC than C+ MC in almost all cases.

Conclusions: According to these findings, the predominant MC type in the bone marrow in neoplastic states such as MDS and mastocytosis is MC, (MC expressing only tryptase). Although the pathophysiological basis of this apparent lack of chymase expression in most neoplastic MC in mastocytosis and MC involved in MDS remains unknown, this study has produced further evidence of the superior value of antitryptase antibodies in the diagnosis of mastocytosis.

MATERIAL AND METHODS

Our study was performed on bone marrow trephine biopsies taken from the iliac crest. There were 47 patients with various subtypes of mastocytosis, 28 patients with myelodysplastic syndromes (MDS), and 31 patients with normal/reactive bone marrow (controls), most of whom had undergone bone marrow biopsy during staging of a lymphoma of high grade malignancy. A diagnosis of mastocytosis was established only when at least one dense MC infiltrate comprising a minimum of 15 cells, irrespective of their shape, was detected. The diagnoses were based on current systems of classification of haematological malignancies, especially the French–American–British criteria. The biopsies were routinely processed, fixed in 5% buffered formalin, subject to mild decalcification overnight in acetic acid/EDTA, and embedded in paraffin wax. Serial sections were cut and immunostained by the avidin–biotin–peroxidase complex method of Hsu et al., using antibodies against tryptase (AA1; DakoDiagnostic, Hamburg, Germany) and chymase (B7; Chemicon, Temecula, California, USA). A detailed description of the technical procedures has already been published. The numbers of tryptase positive (T+) and C+ cells were assessed with a computer assisted video camera system. The two tailed Fisher’s exact test, the Kruskal-Wallis test, and the Wilcoxon rank sum test were used for statistical analysis.

Abbreviations: C+, chymase expressing; MC, mast cells; MCC, mast cells expressing only chymase; MC+, mast cells expressing only tryptase; MCC+, mast cells expressing both tryptase and chymase; MDS, myelodysplastic syndromes; T+, tryptase expressing
RESULTS

General aspects

MC almost always exhibited intense staining of the intracytoplasmic granules for tryptase and/or chymase and could therefore be easily identified (fig 1). In a considerable proportion of the cases of mastocytosis some of the cytomorphologically atypical, often non-metachromatic MC exhibited weaker immunoreactivity because of their reduced granule content. However, all the immunoreactive cells could be easily identified, irrespective of the intensity of staining. In reactive non-mastocytotic bone marrow and in many cases of MDS, MC were loosely distributed throughout the marrow and exhibited a round to oval shape, a low nuclear–cytoplasmic ratio, and inconspicuous nucleoli. In cases of mastocytosis, the MC shape showed greater variability: spindle shaped cells were frequent and were the predominant cell type in most cases. Except in a few cases of MDS, in which a minority of blast cells were labelled by antitryptase, no haemopoietic cells other than MC expressed appreciable amounts of chymase or tryptase.

Morphometric and statistical analysis

Table 1 summarises the morphometric findings concerning the numbers of C+ and T+ cells in mastocytosis, normal/reactive bone marrow, and MDS. The main finding was a highly significant difference (p < 0.001) between the very high numbers of T+ cells in mastocytosis (median, 446/mm²) and the relatively low numbers in normal/reactive marrow (median, 4/mm²) and MDS (median, 32/mm²). However, the difference in numbers of T+ cells between MDS and normal bone marrow was not significant. In contrast, the numbers of C+ MC in MDS (maximum, 46/mm²; median, 0/mm²) and mastocytosis (maximum, 993/mm²; median, 0/mm²) were lower in most cases, and only rarely exceeded 30/mm². Altogether, the number of T+ cells far exceeded that of C+ cells in most cases of mastocytosis and MDS. No significant differences in the numbers of C+ MC could be found among the diagnostic groups (median for mastocytosis, 0/mm²; median for non-mastocytotic states, 4/mm²). In normal/reactive bone marrow the number of C+ MC (median, 8/mm²) was similar to, or at least in the same range as that of T+ cells.

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<th>T+</th>
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<tr>
<td></td>
<td>Max</td>
<td>75th</td>
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<tr>
<td>Mastocytosis (n=47)</td>
<td>5343</td>
<td>1113</td>
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<td>MDS (n=28)</td>
<td>493</td>
<td>53</td>
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<tr>
<td>Normal BM (n=31)</td>
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25th and 75th refer to the 25th and 75th centiles, respectively.
reaching a maximum of 159/mm². In mastocytosis, the number of T+ cells always exceeded that of C+ cells. About 30% of the mastocytosis cases exhibited more than 1000 T cells/mm², with a maximum of 5343/mm².

Table 2 summarises the numbers of C+ and T+ bone marrow MC in the main subtypes of mastocytosis—namely, indolent and aggressive systemic mastocytosis—compared with normal/reactive bone marrow and MDS. No significant difference was detected between the indolent and aggressive subtypes of mastocytosis and non-mastocytotic conditions. The numbers of T+ MC also did not differ significantly between the indolent (median, 427/mm²; maximum, 5343/mm²) and aggressive (median, 808/mm²; and maximum, 2835/mm²) subtypes of mastocytosis, although the median was considerably higher in aggressive mastocytosis.

DISCUSSION

Our study focuses on the immunohistochemical identification and morphometric enumeration of C+ bone marrow MC in reactive and neoplastic states, with special reference to MC proliferative disorders/mastocytosis and MDS. MDS exhibits increased MC numbers in most cases, but it is not known whether these MC are reactive, thus representing a hyperplastic state, or belong to the malignant cell clone. In each of our cases, the numbers of C+ MC were compared with those of T+ cells in serial sections.

The main findings were the following:

1. When the numbers of C+ MC in mastocytosis, irrespective of the subtype, were compared with those seen in the non-mastocytotic states (MDS and normal/reactive bone marrow) no significant differences could be found, with the median value even being lower in mastocytosis (0/mm²) than in non-mastocytotic states (4/mm²). The numbers of C+ MC were in the same range as those of T+ MC in most (67%) normal/reactive bone marrow specimens.

2. However, the number of T+ cells proved to be significantly higher (p < 0.001) in mastocytosis (median, 532/mm²) than in MDS (median, 32/mm²) and normal/reactive bone marrow (median, 4/mm²).

From these findings it can be concluded that the principal phenotype of neoplastic bone marrow MC in most cases of mastocytosis, especially the aggressive variant, is that of an MC. However, the major phenotype of MC in normal/reactive states and in many cases of indolent systemic mastocytosis is that of an MCc. These findings also confirm the superior value of antitryptase antibodies for the recognition of atypical, especially non-metachromatic MC, and thus for the diagnosis of the aggressive and leukemic subtypes of mastocytosis.

Only a few quantitative immunohistochemical analyses of the tissue distribution of T+ and C+ MC have been published. Kankkunen and colleagues reported a preponderance of T+ MC over C+ MC in malignant breast tumours, particularly at the invasion front. In contrast, benign breast lesions contained almost equal numbers of C+ and T+ MC. Jeziorska and colleagues found a predominance of MCc in atherosclerotic lesions in human carotid arteries at all stages of development. Jarvikallio and colleagues investigated T+ and C+ MC in non-lesional and lesional skin of patients with atopic dermatitis and nummular eczema. They found a concentration of MC in the upper dermis, with a predominance of MCc.

Our study clearly shows that the main phenotype of the neoplastic bone marrow MC in mastocytosis is that of a mast cell that only expresses chymase (MCc). This is consistent with earlier findings concerning the high diagnostic value of antitryptase antibodies in mastocytosis.

Take home messages

- The main phenotype of the neoplastic bone marrow mast cell in mastocytosis is that of a mast cell that only expresses chymase.
- This is consistent with earlier findings concerning the high diagnostic value of antitryptase antibodies in mastocytosis.
- Mast cells that express only chymase may be present in normal or reactive bone marrow.

On the basis of our morphometric findings of a slight predominance of C+ MC over T+ MC in a considerable number of cases with normal/reactive bone marrow, it could be speculated that, apart from the well recognized phenotypes of MCc and MCw, MC that express only chymase (MCc) may also exist. Because the numbers of C+ MC never exceeded those of T+ MC in mastocytosis, this “C only” phenotype appears to be of minor importance in MC neoplasms involving the bone marrow. The existence of an “MCc” is still a matter of debate.

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Table 2 Numbers of tryptase expressing (T+) and chymase expressing (C+) bone marrow (BM) mast cells (MC)/mm² in the two main subtypes of mastocytosis and non-mastocytotic states (myelodysplasia/MDS and normal/reactive BM)

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25th and 75th refer to the 25th and 75th centile, respectively. ASM, aggressive systemic mastocytosis; ISM, indolent systemic mastocytosis.
REFERENCES


4. Li L, Krillis SA. Conditioned media obtained from a human mastocytosis cell strain induce mast cells expressing chymase but not tryptase from human progenitors. Int Arch Allergy Immunol 1997;113:289–90.


