

Short reports

Mastocytosis: reactive or neoplastic?

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Abstract

Mast cells are now known to derive from CD34⁺ haemopoietic stem cells in the bone marrow. However, it has not yet been established whether the various types of mastocytosis, which involve tumour-like proliferation of mast cells, are true neoplastic disorders or reactive/hyperplastic conditions. In this study, tissue specimens (five bone marrow, two spleen, one skin) from female patients with histologically confirmed mastocytosis were investigated with a recently developed polymerase chain reaction assay for the determination of clonality of female cells using the human androgen receptor gene (HUMARA). Mast cells purified to near homogeneity from hysterectomy specimens served as a control. The findings in bone marrow and skin either were not reproducible, or indicated polyclonality. However, both spleen specimens exhibited monoclonality. In addition, DNA analysis by flow cytometry was performed and revealed a diploid chromosome content with proliferation indices of under 8% in all the specimens. This is the first molecular biological study to indicate that mastocytosis is indeed neoplastic in nature.

(*J Clin Pathol* 1997;50:525-527)

Keywords: HUMARA; mastocytosis; clonality; polymerase chain reaction

Mast cells originate from haemopoietic tissue in the bone marrow, and have now been shown to be derived specifically from CD34⁺ progenitor cells.¹ However, it has not been established whether the various types of mastocytosis are true neoplastic disorders or reactive/hyperplastic conditions. This study was undertaken to investigate this problem in tissue specimens from patients with histologically confirmed mastocytosis, using a recently developed assay for the analysis of clonality at the human androgen receptor (HUMARA) locus.^{2,3}

Methods and results

The HUMARA assay was performed on formalin fixed, paraffin wax embedded specimens of bone marrow (iliac crest biopsy speci-

Table 1 Diagnoses and results of the HUMARA assay in the tissue investigated

Case	Diagnosis	Tissue	Monoclonality
1	Malignant mastocytosis	Spleen	+
2	Malignant mastocytosis	Spleen	+
3	Malignant mastocytosis	Bone marrow	
4	Malignant mastocytosis	Bone marrow	
5	Systemic mastocytosis	Bone marrow	
6	Systemic mastocytosis	Bone marrow	
7	Systemic mastocytosis	Bone marrow	
8	Urticaria pigmentosa	Skin	

mens, n = 5), skin (n = 1), and spleen (n = 2) from patients with mastocytosis (table 1). Tissue sections of 4 µm thickness were cut from these specimens and stained with an antibody against tryptase (AA1; Dako, Hamburg, Germany) to confirm mast cell infiltration (fig 1A). Ideally, simultaneous analysis of non-neoplastic cells of the same type, from the same individual, should be performed with the HUMARA assay. However, this is not possible in mastocytosis for several reasons: infiltration in mastocytosis is diffuse, not focal, so non-involved tissue from the same organ cannot be obtained; generally, the entire mast cell population in patients with mastocytosis is considered lesional, so that it is not possible to obtain "normal" mast cells from the same individual for comparison. Even if this were not the case, normal mast cells are not present in any organ in sufficient numbers to be investigated in small biopsy specimens. Therefore, in this study native mast cells from hysterectomy specimens obtained from other patients were used as control cells. These cells were isolated to a purity of more than 95% with a magnetic cell sorter⁴ using magnetic microbeads coated with an antibody against CD117 (c-kit). In addition, normal spleen (n = 2) from other female patients was used as control tissue.

Sections of 8 µm thickness were cut from each specimen, dewaxed, and incubated with proteinase K. DNA was extracted with phenol:chloroform:isoamyl alcohol and alcohol precipitation, and tested for structural integrity and amplifiability with primers for the β globin gene. The HUMARA assay was then performed.³ A third of the extracted DNA from each specimen was digested with the restriction

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Accepted for publication 3 February 1997

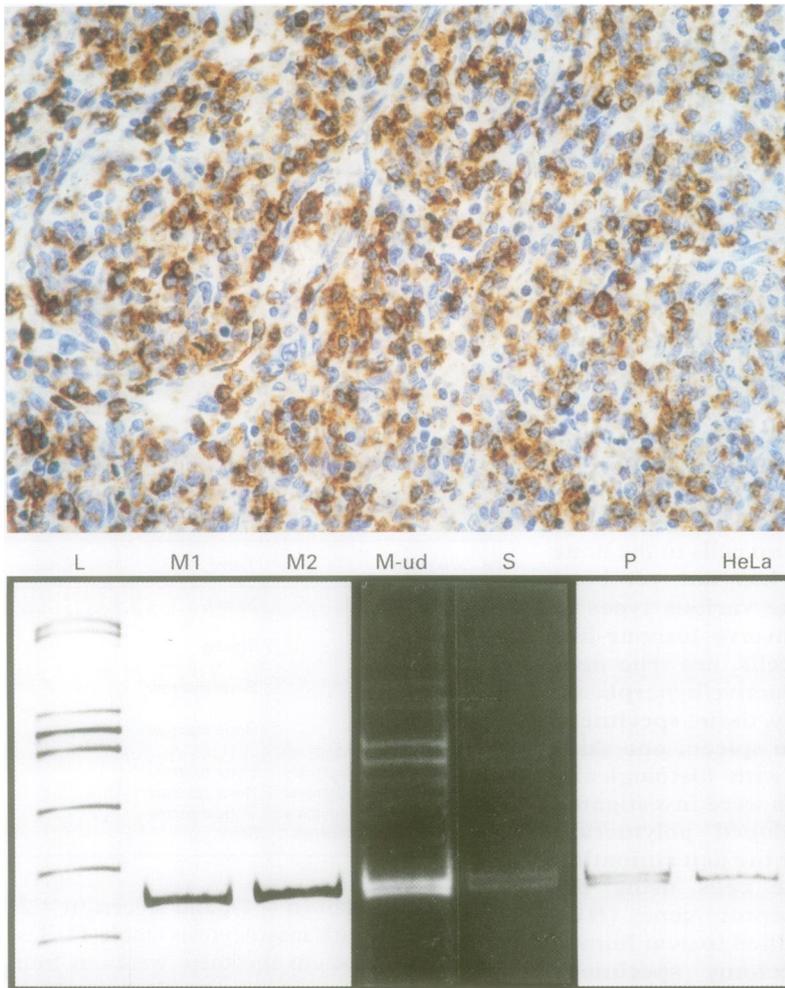


Figure 1 The spleen in malignant mastocytosis. (Top) There is diffuse infiltration of the red pulp by mast cells, which are stained by an antibody directed against mast cell specific tryptase. (Bottom) Gel electrophoresis of the amplification products of the HUMARA PCR assay in two cases of malignant mastocytosis (M) without an associated myelodysplastic/myeloproliferative syndrome reveals monoclonality. Two bands are seen when undigested DNA is used (M-ud). Normal splenic tissue (S) and control mast cells (P) exhibit polyclonality with two equally strong bands. HeLa, positive control; L, length marker.

enzyme *HpaII*, a third was digested with *HhaI*, and a third was left undigested. The three fractions thus obtained formed the template DNA for the polymerase chain reaction (PCR) with two primer pairs that bind to the first exon of the HUMARA gene. The specificity of the amplification products was tested by direct sequencing. Suspensions of cell nuclei for DNA analysis were prepared from all the spleen samples and the native mast cells, and their DNA content investigated by flow cytometry.⁵

Amplifiable DNA was obtained from all the specimens of bone marrow, spleen, and skin. DNA from the bone marrow and skin of the mastocytosis cases exhibited either polyclonality or no reproducible bands for the HUMARA locus. However, in the spleen specimens involved in mastocytosis, the DNA preparations that had been digested with *HpaII* exhibited over-representation of one of the inactive X-chromosome HUMARA genes with monoclonal bands (fig 1B). Heterozygosity was demonstrated in the form of two bands in the undigested DNA preparations from both these

specimens. The control specimens (spleen and native uterine mast cells) exhibited polyclonality.

Flow cytometry revealed a diploid nuclear DNA content and a proliferation index of less than 8% in both the normal mast cells and those obtained from the splenic tissue from mastocytosis cases.

Discussion

This study was undertaken to investigate the hypothesis that mastocytosis exhibits a monoclonal growth pattern. This was performed with an assay for non-random X chromosome inactivation at the first exon of the HUMARA gene.^{2,3} The PCR technique used involves analysis of naturally occurring allelic polymorphism. This polymorphism is reflected in the presence or absence of cleavage sites for a particular restriction endonuclease (restriction fragment length polymorphism; RFLP). The loss of genetic material can be identified by reduced intensity of the PCR products of one of the two corresponding alleles in the tumour DNA. The HUMARA assay for clonality uses PCR to determine the relative proportions of maternal and paternal inactive X chromosomes. Therefore, this method can be used to identify monoclonal cell proliferation in females. Each member of a monoclonal cell population in a female contains the same (non-random) copy of the inactivated X chromosome, unlike those of the surrounding polyclonal tissues, which possess, on average, equal (random) numbers of paternal and maternal inactive X chromosomes. The first exon of the HUMARA locus contains two cleavage sites each for *HpaII* and *HhaI* upstream from polymorphic trinucleotide repeats. Methylation of these cleavage sites correlates with X chromosome inactivation.^{2,3} Primers are prepared that flank both the methylation sensitive cleavage sites and the trinucleotide repeats. Digestion with methylation sensitive restriction enzymes followed by PCR of the polymorphic region produces a single band, in the case of monoclonality, and two bands, in the case of polyclonality. The HUMARA assay has proved to be a sensitive method for determination of the clonality of various tumours and tumour precursor cells.^{3,6,7} Other authors have also shown that this method can be used on formalin fixed, paraffin embedded material because fixation with formalin does not interfere with DNA methylation.⁸ The great advantages of analysis of clonality at the HUMARA locus lie in its high degree of allelic variability (informativeness > 90%) both in the form of a variable number of trinucleotide repeats, and the proximity of the differentially methylated restriction sites to these repeats. These are necessary criteria for the analysis of clonality.⁶

The HUMARA assay revealed monoclonality in both spleen samples from cases of malignant mastocytosis without an associated myeloproliferative/myelodysplastic syndrome. Monoclonality could not be demonstrated in any of the other mastocytosis specimens or

controls. To the best of our knowledge, this is the first investigation in which monoclonality in mastocytosis has been demonstrated directly by a molecular biological technique. Our findings are consistent with those of two recently published studies, in which point mutations of the proto-oncogene *c-kit* were found in peripheral blood cells⁹ and mast cells of both skin and spleen¹⁰ in mastocytosis. Additional DNA analysis by flow cytometry revealed no quantitative DNA abnormality severe enough to be detectable. The proliferation index of less than 8% indicates that malignant mastocytosis is a relatively slow growing tumour, compatible with the finding that, in this disease, the extent of mast cell infiltration often remains stable in serial biopsies.

No definite conclusions can be drawn about the biological nature of the mastocytoses from this study because of the small number of cases included and the fact that it may vary among the various different subtypes of mastocytosis. Further molecular biological investigations on defined subtypes are therefore planned.

The authors thank Dako Diagnostica GmbH, Hamburg, Germany, for contribution towards the cost of reproduction of the colour artwork.

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Small intestine in lymphocytic and collagenous colitis: mucosal morphology, permeability, and secretory immunity to gliadin

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Abstract

There is a recognised association between the "microscopic" forms of colitis and coeliac disease. There are a variety of subtle small intestinal changes in patients with "latent" gluten sensitivity, namely high intraepithelial lymphocyte (IEL) counts, abnormal mucosal permeability, and high levels of secretory IgA and IgM antibody to gliadin. These changes have hitherto not been investigated in microscopic colitis. Nine patients (four collagenous, five lymphocytic colitis) with normal villous architecture were studied. Small intestinal biopsies were obtained by Crosby capsule; small intestinal fluid was aspirated via the capsule. IEL counts were expressed per 100 epithelial cells, and intestinal IgA and IgM antigliadin antibody levels were measured by ELISA. Small intestinal permeability was measured by the lactulose:mannitol differential sugar permeability test. IEL counts were

normal in all cases, median 17, range 7-30. Intestinal antigliadin antibodies were measured in six cases and were significantly elevated in two patients (both IgA and IgM). Intestinal permeability was measured in eight cases and was abnormal in two and borderline in one. These abnormalities did not overlap: four of nine patients had evidence of abnormal small intestinal function. Subclinical small intestinal disease is common in the two main forms of microscopic colitis. (*J Clin Pathol* 1997;50:527-529)

Keywords: lymphocytic and collagenous colitis; coeliac disease; intraepithelial lymphocyte counts; IgA; IgM

The increasing use of colonoscopy has allowed the identification of a group of patients with chronic watery diarrhoea who have minimal macroscopic changes but microscopic evidence of mucosal inflammation. Read *et al*¹ coined the term "microscopic colitis" to describe this

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Accepted for publication 25 February 1997