Virchows Arch (1998) 433:465–470

ORIGINAL ARTICLE

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Evidence for in vivo mitosis by granule-containing mast cells from canine mastocytomas

Received: 22 April 1998 / Accepted: 14 May 1998

Abstract Mast cell accumulations are generally considered to arise almost exclusively from the recruitment of non-granulated, bone-marrow-derived, precursor cells, with the stem cell factor (SCF) reported to play a crucial role in the growth, development and maturation of granulated mast cells within specific tissue sites. In this study dog mastocytoma specimens have been examined by both immunohistochemical and ultrastructural techniques, to demonstrate that fully granulated mast cells are capable of mitotic activity. Observations showing the formation of mitotic spindles, chromosome separation and cytokinesis all support the concept that granulated mast cells are capable of proliferative activity. The ability of mature granulated mast cells to replicate provides an alternative process for local increases in mast cell numbers, at least in canine mast cell tumours. Such observations suggest the possibility that normal or neoplastic human mast cells, fully granulated, have the potential to proliferate in specific tissue sites.

Key words Canine mastocytoma · Mitosis · Mast cells

Introduction

Stem cell factor (SCF), also known as the c-kit ligand, plays a crucial part in the growth and development of normal mast cells [5, 9]. SCF is reported to be responsible for the proliferation of bone-marrow-derived mast cell precursors, their recruitment to specific tissue sites, and their subsequent development and maturation into fully granulated mast cells [5, 11, 14]. Thus, accumulations of mast cells seen in such conditions as urticaria, nasal polyps and mastocytomas may be considered to arise almost exclusively from the recruitment of non-granulated precursor cells and their subsequent development/maturation mediated by locally produced SCF, rather than as a result of mitotic activity by granulated, fully developed mast cells. Little is known about the ability of fully differentiated, granulated mast cells to undergo mitosis, especially in vivo. Indeed, it seems that the numerous tightly packed granules within such cells would be likely to physically hamper and prevent the formation of mitotic spindles, chromosome separation and cytokinesis.

The effects of SCF are mediated by c-kit, also known as the SCF receptor (SCFR) [5, 14, 15]. The expression of SCFR by mast cells in spontaneously occurring dog mastocytomas has been elegantly demonstrated by London et al. [10], but although binding of SCF by those mast cells was demonstrated it remains unclear whether fully granulated mast cells would undergo cell division, either in vivo or in vitro. Proliferation in culture is well recognised for agranular mast cell progenitors [3, 4, 7], the human mast cell leukaemia cell line (HMC-1) [1] and mouse mastocytoma cell lines, but all these cell preparations contain a paucity of mast cell granules compared with normal tissue mast cells, or indeed most canine mastocytoma cells. To examine whether the local proliferation of fully formed, granulated mast cells could also contribute to increased mast cell numbers in tissues we have examined canine mast cell tumours for mitotic activity. Mast cell tumours in dogs are not uncommon, but specimens show variable behavioural patterns with regard to growth rate, aggressiveness and metastatic potential. We have examined eight such tumours, representing the three grades of malignancy described by Patnaik et al. [12], and we present here our evidence for mitotic activity as demonstrated by granulated, fully differentiated mast cells within those lesions.

Materials and methods

Surgically excised mast cell tumours were obtained from eight dogs, previously diagnosed by needle biopsy, attending veterinary

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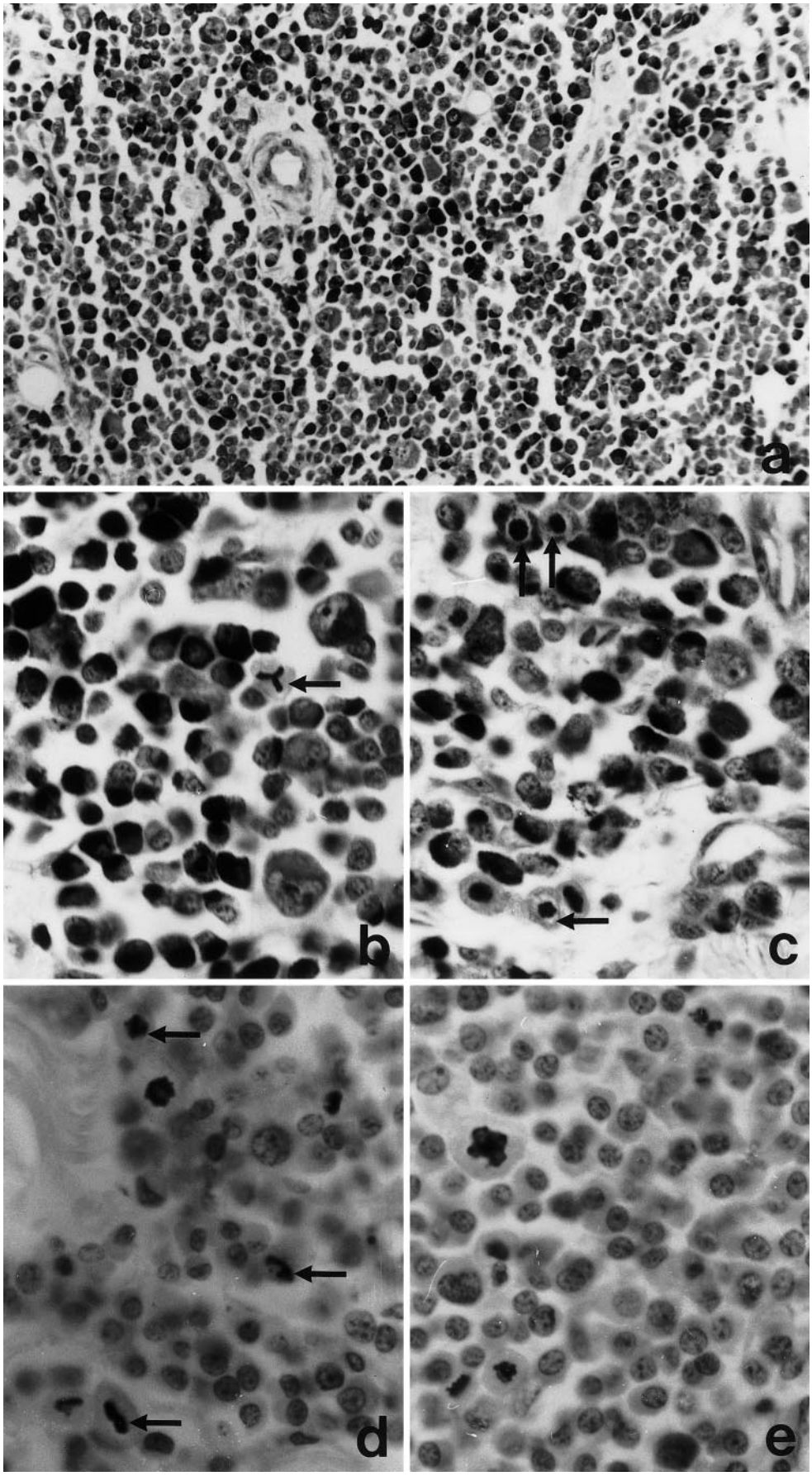

Fig. 1a–e Evidence of mitotic activity in dog mastocytomas as demonstrated by light microscopy. **a** Low-power photomicrograph of mastocytoma specimen 5, consisting of metachromatically stained mast cells. Acidified toluidine blue, ×210. **b**, **c** High-power photomicrographs from **a**, showing mitotic figures (*arrows*) in metachromatically stained mast cells. Acidified toluidine blue, ×530. **d**, **e** High-power photomicrographs of mastocytoma specimen stained for mast cell tryptase. Note that the tryptase staining intensity of cells was variable, appearing variously as **d** dark grey cytoplasm and **e** lighter grey. Mitotic figures within tryptase-positive cells are *arrowed*. Haematoxylin counterstained, ×530

practices. Specimens obtained at the time of surgical removal were placed in Dulbecco's modified Eagle's medium (DMEM), and within 1 h dissected tissue samples were fixed for light microscopy and electron microscopy.

For light microscopy, tissue samples were placed in Carnoy's fixative for 2–4 h, depending on size, and were routinely processed to paraffin blocks. Sections were cut at 3–4 µm, placed on glass slides coated with poly-L-lysine (Sigma), dewaxed in Histoclear, rehydrated in a graded series of ethanol, and stained with haematoxylin and eosin for histological assessment. All specimens were confirmed as mast cell tumours by staining with acidified toluidine blue, and were graded according to criteria described by Patnaik et al. [12].

Monoclonal antibodies to MC tryptase (Biogenesis, Poole, UK) effectively stained both canine and human mast cells and were also used for the identification of MCs. Tissue sections were incubated with 10% (v/v) nonimmune rabbit serum for 30 min and then treated with primary antibody to MC-tryptase for 2 h at room temperature, followed by overnight incubation at 4° C. After washing in Tris-buffered saline (TBS, 3×10 min) the tissue sections were incubated with secondary biotinylated rabbit anti-mouse antibody for 45 min. After further washing in TBS, StreptABComplex alkaline phosphatase (AP) conjugate (Dako) was applied for 45 min, the sections were washed again in TBS, and alkaline phosphatase was developed using New Fuchsin [13]. Tissue sections were counterstained with Mayer's haematoxylin and mounted in Histomount (Mensura Technology, Wigan, UK). All histologically stained tissue sections were examined with a Zeiss photomicroscope III and photographed using TMAX 100 pro film (Kodak).

For ultrastructural studies mast cell tumour tissues were fixed in 5% (v/v) cacodylate-buffered glutaraldehyde, post-fixed in 1% (w/v) osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in a graded series of ethanol and embedded in Agar 100 resin (Agar Scientifice). Semi-thin (1 µm sections were cut from the resultant polymerised blocks and stained with toluidine blue to select areas for ultrathin sectioning. Ultrathin sections were cut on a Reichert OMU4 Ultracut ultramicrotome, mounted on copper grids and stained with uranyl acetate and lead citrate. Grids were examined in an AEI EM801 electron microscope.

Results

All eight specimens showed a preponderance of mast cells as judged by metachromatic staining with acidified toluidine blue on light microscopy. However, the intensity of metachromatic staining for individual cells was variable, both in different tumours and within each mastocytoma. Whereas all specimens had a major proportion of the cells intensely stained with acidified toluidine blue, some cells showed weaker metachromasia; observations suggestive of a variable granule or heparin content (Fig. 1 a).

Examination of the specimens at high magnification showed clearly discernible mitotic figures in four of the eight specimens. Indeed, even within a single microscopic field (×16 objective) it was possible to observe several cells in the process of cell division. The demonstration that such mitotic figures were present within metachromatically stained cells gave the first indications that granule-containing mast cells were undergoing mitotic activity (Fig. 1b, c). Confirmation of mast cell proliferation was subsequently demonstrated by showing that the mitotic cells were also positively stained for the mast-cell-specific enzyme, tryptase (Fig. 1d, e).

Table 1 provides a summary of the histopathological features of each specimen and their grades as defined by Patnaik [12], and shows that most cells in each specimen were tryptase-positive, and that mitotic figures observed within four of the specimens were usually associated with cells showing metachromatic and/or tryptase-positive features. Interestingly, evidence for mast cell mitotis was observed in all three grades of canine mastocytoma, and this was subsequently confirmed by electron microscopy.

Table 1 Histological assessment of dog mastocytoma specimens: mast cell staining properties and presence of mitotic figures

Case no.	Histopathology	Histological grade[a]	Staining for tryptase	Mitotic figures[b]
1	Well-differentiated monomorphic tumour cells with ample distinct cytoplasm, arranged in rows and small groups in hyalinised stroma and along skin adnexa	1	+	
2	Moderately pleomorphic tumour cells arranged in groups and sheets within fibrovascular stroma. Focal oedema present	2	+	+
3	Tumour cells as in specimen 1, arranged in small groups and in rows	1	+	
4	As 3	1	+	
5	Mostly solid tumour with scanty fibrovascular stroma. Tumour cells show marked cellular pleomorphism with numerous epithelioid cells, some with bizzare nuclei. Numerous mitotic cells. Some perivascular lymphocytic infiltrates	3	+	+
6	Medium and small tumour foci and some dispersed infiltrates at the edge of the tumour consisting of monomorphic, well-differentiated cells with distinct cytoplasm. Stroma partially hyalinised	1	+	+
7	Tumour cells as in specimen 1. Focal marked oedema. Moderate inflammatory infiltrate	1	+	
8	Solid tumour with moderate to high cellular pleomorphism. Moderate amount of delicate fibrovascular stroma. Multifocal oedema, haemorrhage and necrosis	3	+	+

[a] Grades 1–3 represent histological prognostic features as defined elsewhere [12]
[b] All demonstrated in either metachromatically stained and/or tryptase positive mastocytoma cells

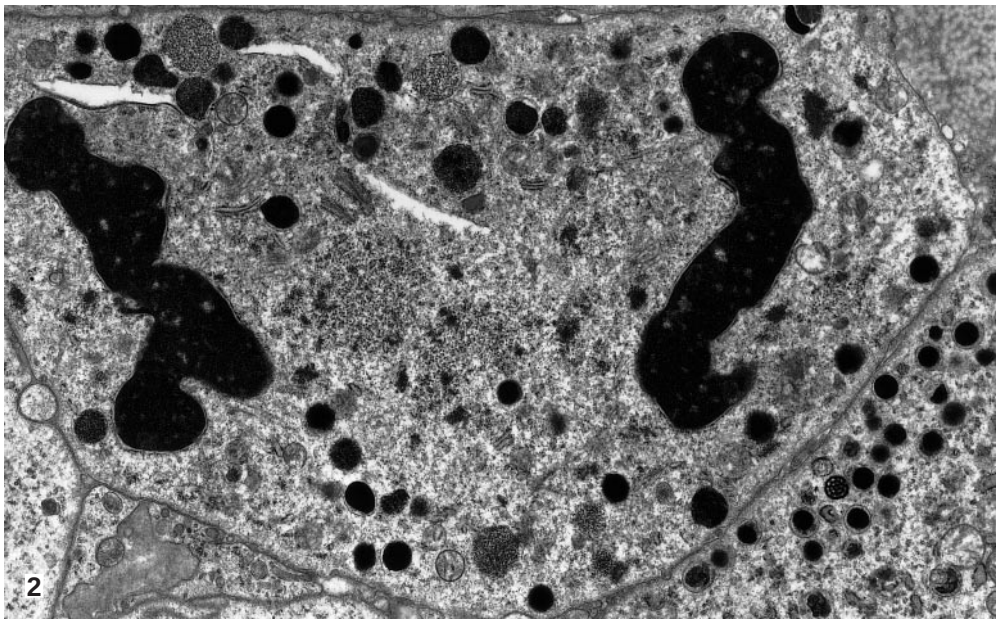

Fig. 2 Electron micrograph of a mitotic cell in mastocytoma specimen 5. The cell shows condensed chromatin on opposite sides and therefore represents a late anaphase or early telophase stage of mitosis. Note mast cell granules around the periphery of the cell and the nuclear envelope around each piece of condensed chromatin. ×8300

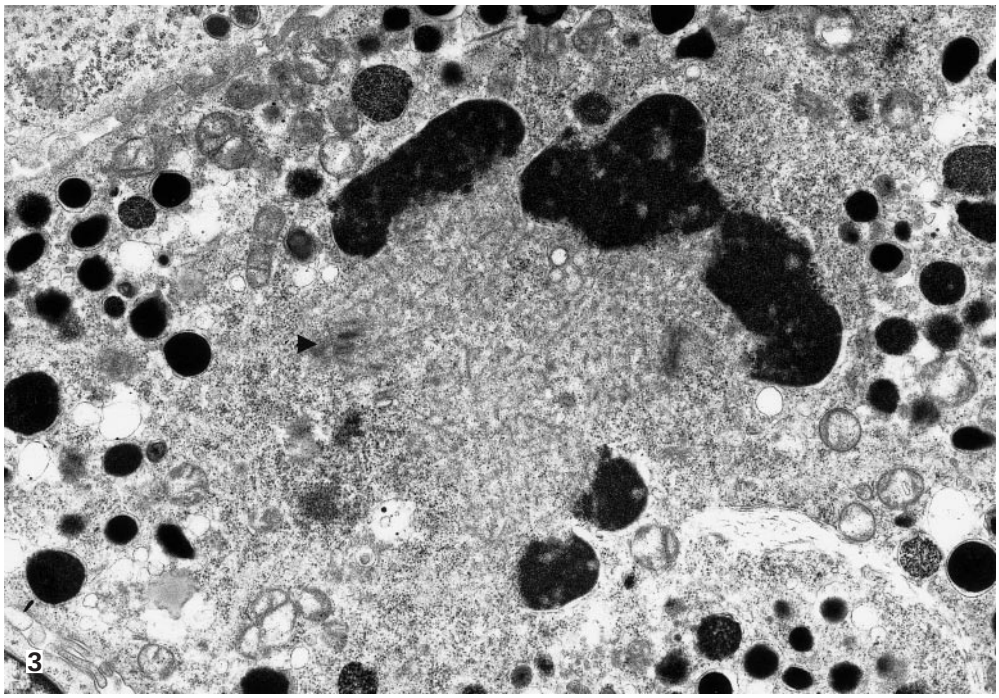

Fig. 3 Low-power electron micrograph showing a mitotic mast cell with condensed chromatin and spindle microtubules radiating from a spindle pole (*arrowhead*). Note abundant mast cell granules in the peripheral cytoplasm. ×11000

Ultrastructural analysis of the mast cell tumour specimens confirmed mast cell mitosis in those specimens identified by light microscopy studies. Abundant mitoses were observed within specimen 5, in which mast cells containing characteristic granules were seen at various stages of the mitotic cycle (Figs. 2–5). Metaphase and anaphase stages were the most easily recognised. In those mitotic stages the nuclear envelope was fragmented, profiles of the condensed chromosomes were apparent, and the microtubular elements of the mitotic spindle were easily recognised (Figs. 2–4). At the telophase stage a double membrane was observed around individual condensed chromosomes; presumably this would form the nuclear envelope of the daughter cells.

Some mast cells were observed undergoing cytokinesis. Figure 4 shows the final stages of cell division, where the plasma membrane has formed a deep cleavage furrow perpendicular to the spindle axis and between the two sets of daughter chromosomes. The narrow bridge of cytoplasm left between the cleavage membrane shows the remains of spindle microtubules. Figures 2–5 provide evidence that mature mast cells with a significant content of fully differentiated granules have the ability to undergo mitosis, observations suggesting that such granules are not detrimental to the mitotic process.

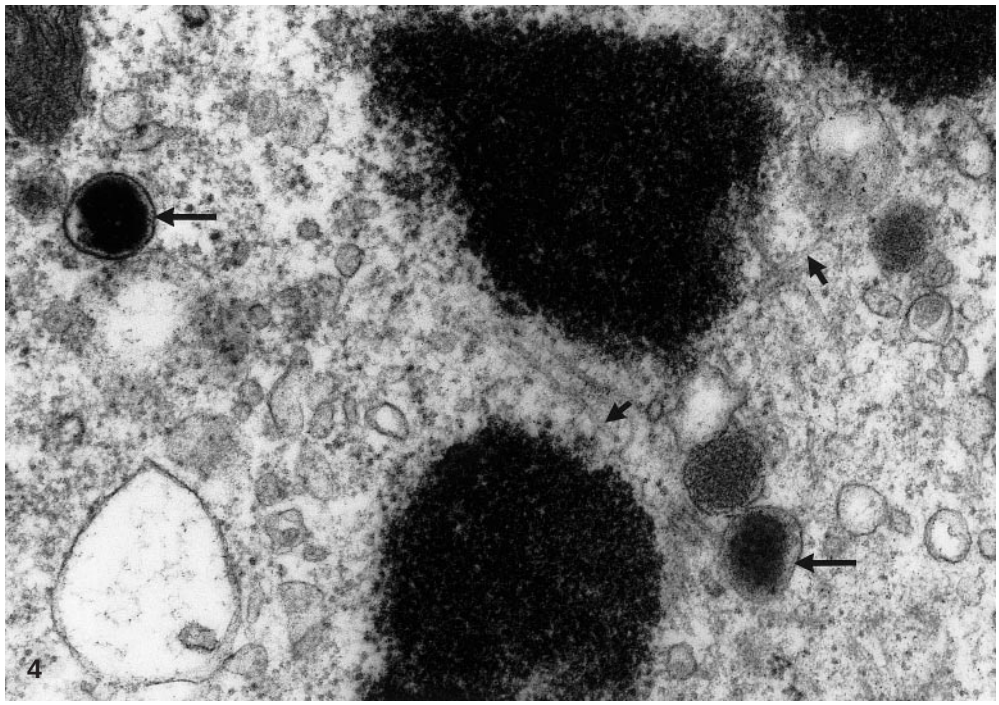

Fig. 4 High-magnification electron micrograph showing condensed chromatin and spindle microtubules (*arrowheads*). Note presence of mast cell granules (*arrows*). ×49250

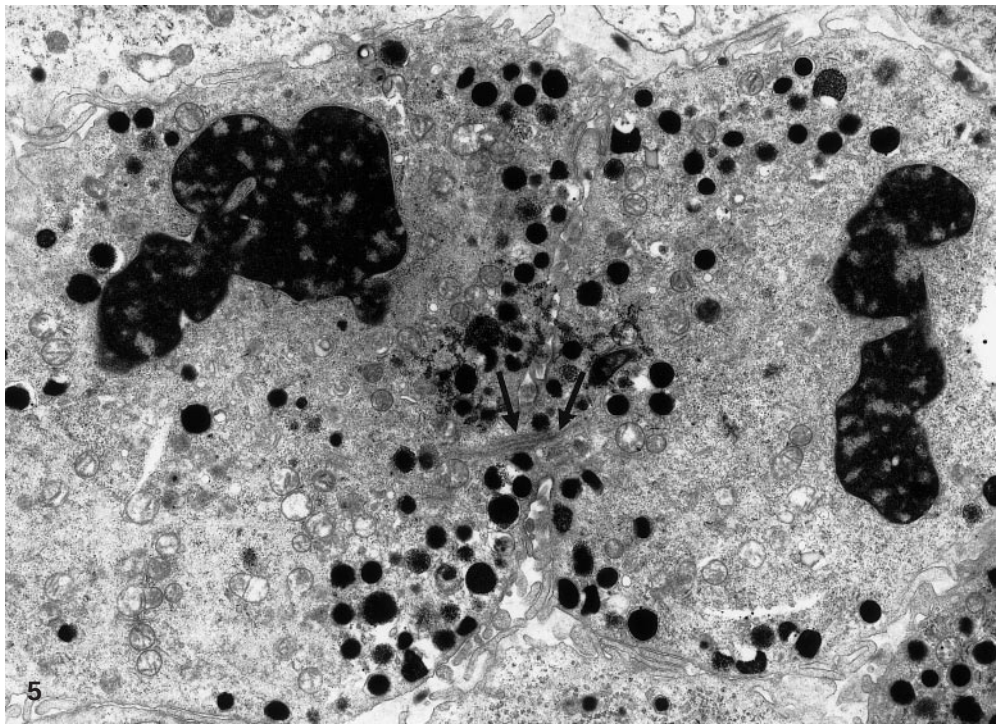

Fig. 5 Electron micrograph of a mast cell undergoing cytokinesis. Note the remains of spindle microtubules (*arrows*) in the bridge of cytoplasm between the two daughter cells and abundant mast cell granules. ×7000

Discussion

In vivo, mast cell progenitors derived from the haematopoietic system are recruited to tissue sites, where they differentiate and mature under the influence of the microenvironment into phenotypically and functionally mature mast cells characterised by numerous intracellular granules containing potent mediators [2]. The nature of the microenvironmental factors that stimulate mast cell recruitment and maturation is an active area of research, with interleukin-3 (IL-3), c-kit ligand (SCF), laminin and transforming growth factor-β_1 (TGF-β) reported as important mast cell chemoattractants [5, 6, 9]. The local maturation of mast cell precursors within tissue sites, recognised by the synthesis of numerous intracellular granules, has usually provided the explanation of how histologically recognised mast cell accumulations develop in various pathological tissues. Little consideration appears to have been given to the possibility that mature, granulated mast cells could undergo mitosis and thereby con-

tribute to local mast cell proliferation. The observations reported here demonstrate that the process of mitotic activity by granulated mast cells is possible, indicating that a relatively dense intracellular distribution of fully formed granules does not necessarily interfere with the formation and function of the mitotic spindle, chromosome separation or cytokinesis.

We accept that these observations are derived from mast cells in canine mastocytomas, cells that may be considered transformed and show aberrant behaviour. Nevertheless, the high frequency of mitotic figures observed in these granulated mast cells indicates that their ability to replicate provides an alternative process for local increases in mast cell numbers, at least in canine mast cell tumours. These observations are based on histological analyses of relatively small portions of each tumour specimen, probably no more than 10% of the whole in any one case, and certainly less than 1% of any specimen for electron microscopy. Thus the frequency of mast cell mitosis throughout any one specimen remains uncertain, and it is possible that a more complete analysis of those specimens rated as negative in Table 1 might reveal other locations containing mast cell mitotic activity by granulated mast cells. At present we do not know what the mitogenic factors in such locations might be, but previous studies have emphasised the importance of SCF [3, 5], and dog mastocytoma cells are known to express c-kit (SCFR).

A recent immunohistochemical study used the monoclonal antibody PCNA/PC10 in conjunction with α-chloroacetate esterase, tryptase or Giemsa staining to examine mast cell proliferation in a variety of human mast cell pathologies [8]. Evident mast cell mitosis was reported as approximately 1% in urticaria pigmentosa, mastocytoma and systemic mastocytosis, with up to 4.0% in malignant mastocytosis [8]. However, Horny et al. [8] noted that PC10-positive mast cells, although strongly positive for the cytoplasmic chloroacetate esterase reaction, contained only sparse metachromatic granules in Giemsa stain. We believe that the present study, supported by in vivo ultrastructural evidence, shows that fully granulated mast cells are potentially capable of proliferative activity. Whether this is mimicked to some extent in the mast cell accumulations observed in the conditions of urticaria or nasal polyps is unknown, but is currently being investigated.

Acknowledgements We thank Mrs. P. Rowland for excellent technical assistance; and veterinary surgeons D. Simpson and B. Hargreves, Withington, Manchester, for the supply of tissue specimens.

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