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Phenotypic analysis of mast cell granule proteinases in normal rat bone marrow cultures

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SUMMARY

Mast cells with morphological and some biochemical properties of mucosal mast cells (MMC) proliferate and mature in rat bone marrow cultures stimulated with factors from antigen- or mitogen-activated T lymphocytes. There has been much controversy over the criteria used to distinguish the different mast cell subsets, and because histochemistry of granule glycosaminoglycans does not adequately define mast cell subsets morphologically, the proteinase phenotypes of cultured mast cells were analysed. Affinity-purified cross-absorbed monospecific $F(ab')_2$ antibodies raised against rat mast cell protease I (RMCPI) from connective tissue mast cells (CTMC) and against rat mast cell protease II (RMCPII) isolated from mucosal mast cells were used to stain granule proteinase by an immunohistochemical technique. Mast cells grown in culture from normal rat bone marrow stained exclusively with anti-RMCPII antibodies, thus providing further confirmation of their similarity to, and identity with, MMC.

Mucosal mast cells (MMC) are functionally and biochemically distinct from tissue mast cells elsewhere in the body (Enerback, 1981; Befus *et al.*, 1982; Jarrett & Haig, 1984). Cytochemical distinction between connective tissue mast cells (CTMC) found in skin, muscle and peritoneal cavity, and MMC that predominate in the mucosa of the gastrointestinal tract, is based on the absence of granule heparin in MMC, and on the failure to detect MMC glycosaminoglycans in formalin-fixed tissues (Enerback, 1981; Newlands, Huntley & Miller, 1984). Neither of these techniques adequately defines mast cell heterogeneity. An alternative approach to identifying mast cell subsets is to determine the proteinase phenotype of mast cell granules. Biochemical studies have shown that rat mast cell protease I (RMCPI) predominates in CTMC and RMCPII in MMC (Woodbury, Gruzenski & Lagunoff, 1978) and, more recently, immunohistochemical analyses have confirmed that RMCPII is located exclusively in cells of the MMC phenotype and that RMCPI is present in CTMC (Gibson & Miller, 1986).

Mast cells with the morphological and biochemical properties of MMC appear and proliferate to form the predominant cell type in rat bone marrow cultures stimulated with factors from antigen- or mitogen-activated lymphocytes (Haig *et al.*, 1982, 1983). Although these cultured mast cells have been shown, biochemically, to contain and secrete RMCPII (Haig *et al.*, 1982, 1983), they have not been characterized by immunohistochemical techniques. The phenotype of these cells was, therefore, further characterized using monospecific $F(ab')_2$ antibodies, which were employed to demonstrate immunohistochemically the presence of RMCPI and RMCPII. Rat peritoneal exudate cells were used as a source of CTMC for

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Table 1. Comparison of mast cell subsets stained with toluidine blue/Leishman anti-RMCPI and anti-RMCPII in peritoneal exudate cells (PEC) and cultured normal bone marrow (NBM)

Day of culture	Cell type	% cells stained Tol. blue/Leishman	% cells stained anti-RMCPI	% cells stained anti-RMCPII
NA*	PEC†	11·6	10·8	0
0	NBM	1·8	1·6	0
NA*	PEC†	9·6	7·7	0
7	NBM	48·7	1·6	44·4
NA*	PEC†	13·9	15·6	0
30	NBM	94·1	0	93

The procedures for immunohistochemical detection of proteinases and toluidine blue/Leishman staining of mast cells were as described in the text.

* NA, not applicable.

† A pool of peritoneal exudate cells was prepared from three animals. Bulk cultures of normal bone marrow were prepared as described in the text. Cell counts were performed on cytospin preparations with five fields of view being counted/slide and 100 cells counted/field at × 40. Results are expressed as a percentage of total cells counted/slide.

comparison. This work was done to define further the mast cell subset(s) present in the normal bone marrow cultures.

Bulk cultures of F344 rat bone marrow (rats obtained from an inbred colony at the University of Glasgow Veterinary School) were prepared in Iscove's medium (Gibco, Paisley, Renfrewshire) + 20% horse serum (Flow Laboratories, Irvine, Ayrshire) at a cell concentration of 5×10^5 cells/ml in 80 cm^2 tissue culture flasks. The cells were stimulated with conditioned medium prepared from immune mesenteric lymph nodes taken on Day 14 after infection with *Nippostrongylus brasiliensis* and stimulated *in vitro* with 2 μg/ml of concanavalin A, and the conditioned medium was harvested after 48 hr of culture. The cultured bone marrow cells were harvested on Day 7 and Day 30 after plating. Total cell counts were performed and cytospin preparations were made (Cytospin 2, Shandon Southern Ltd, Runcorn, Cheshire). The slides were then fixed in methanol and stored at $-20°$. Peritoneal exudate cells were obtained by peritoneal lavage of F344 rats (a pool of three animals was used per experiment). A total cell count was performed and cytospin preparations were made and fixed as for the cultured cells.

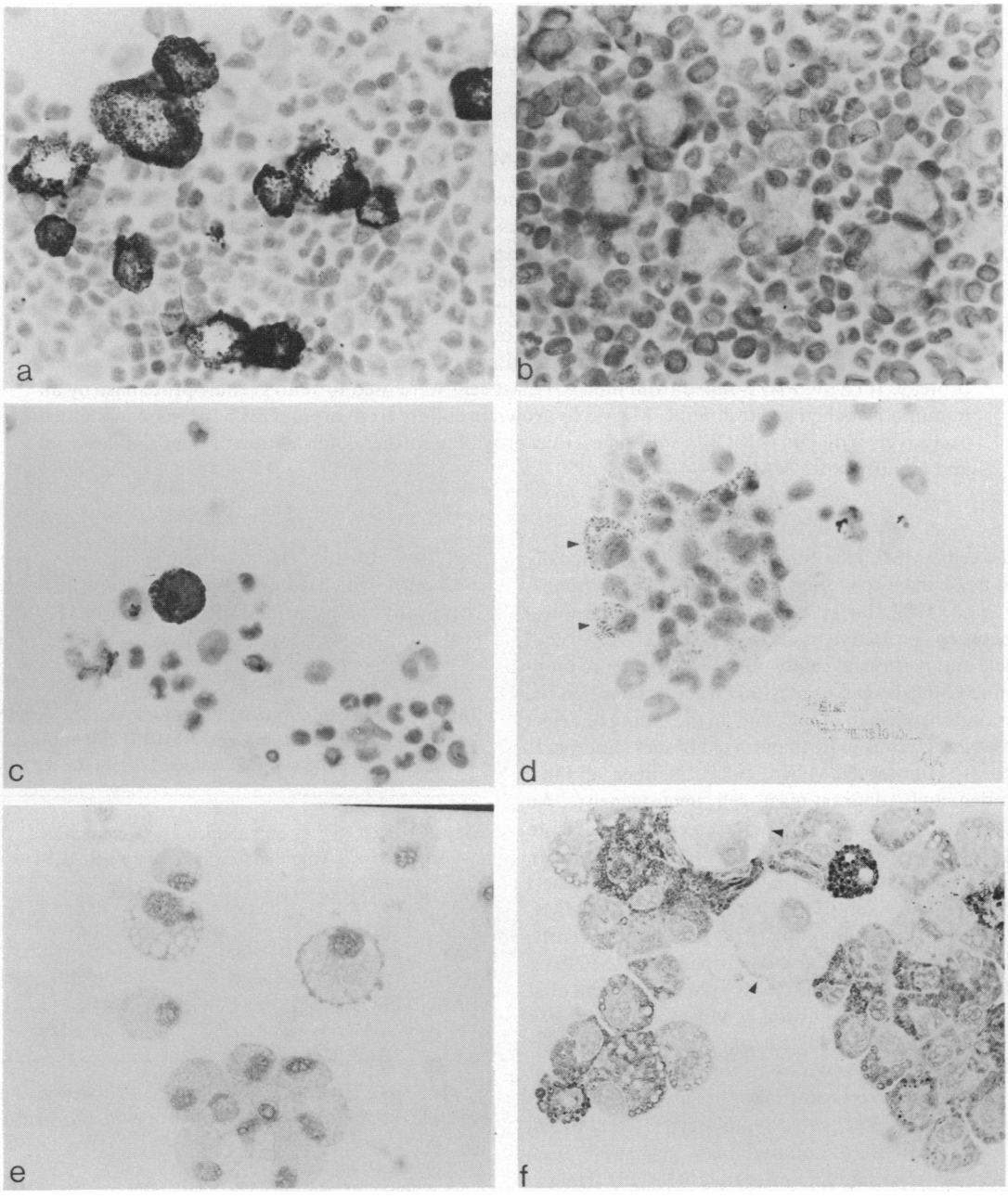

Figure 1. Detection of mast cells in rat peritoneal washings and rat normal bone marrow cultures with monospecific rabbit $F(ab')_2$ anti-RMCPI or II. Peritoneal exudate cells stained with (a) anti-RMCPI (× 430), and (b) anti-RMCPII (× 430). Normal bone marrow on Day 7 of culture stained with (c) anti-RMCPI (× 430), and (d) anti-RMCPII (× 430) positive cells are arrowed. Normal bone marrow on Day 30 of culture stained with (e) anti-RMCPI (× 430), and (f) anti-RMCPII (× 430). Note that in (f) all cells are stained, but to varying degrees, except those arrowed.

RMCPI and RMCPII were demonstrated in the cell preparations using affinity-purified rabbit $F(ab')_2$ anti-RMCPI and anti-RMCPII that had been rendered monospecific for each enzyme by affinity chromatography and cross-absorption (Gibson & Miller, 1986). Development was accomplished using sheep Fab anti-rabbit $F(ab')_2$-peroxidase conjugate and 3-amino-9-ethylcarbazole as substrate (Newlands *et al.*, 1984). Toluidine blue (pH 0·5) and Leishman staining were also performed on the cytospin preparations.

The numbers of mast cells stained with toluidine blue and Leishman were counted. Comparable slides were then assessed for numbers of mast cells stained with anti-RMCPI and anti-RMCPII after the immunoperoxidase reaction. Five fields of view were counted per slide, with at least 100 cells counted per field. The results are expressed as a percentage of total cells counted per slide.

On Day 0, when the bone marrow was plated, cells containing RMCPII were not present, and those few mast cells (1·8% of total) that stained with toluidine blue had the histological appearance of CTMC and contained RMCPI exclusively (Table 1). By Day 7, cells containing RMCPII made up almost half of the total culture, with a small percentage of anti-RMCPI stained cells still remaining (Table 1, Fig. 1). The latter were residual CTMC present in the normal bone marrow but had virtually disappeared within 7–10 days in culture. By Day 30 the cells containing RMCPII made up more than 90% of the total culture and no RMCPI-positive staining cells remained.

The connective tissue type of mast cell in peritoneal exudate cell preparations stained with anti-RMCPI, and this was used as the control (Fig. 1). The CTMC present in the Day 7 culture were the typical large cells densely packed with uniform granules by comparison to the granules of the RMCPII containing cells at both Day 7 and Day 30 of culture. The granules of RMCPII-positive cells were sparser and varied in size (Fig. 1).

Using previously available criteria to distinguish MMC from CTMC (Enerback, 1981; Jarrett & Haig, 1984), it appeared that the mast cells growing in culture were of the mucosal type. However, none of these criteria alone was sufficient to identify the cells as MMC with any degree of certainty. The present approach, analysing proteinase phenotypes (Gibson & Miller, 1986), however, has provided an alternative method of distinguishing the mast cell subsets. Thus, RMCPI was present only in cells that by previous histochemical criteria had been defined as CTMC, i.e. the peritoneal exudate cells and the mast cells present in normal bone marrow before culture. The cells that grew and differentiated in the normal bone marrow cultures were exclusively stained by anti-RMCPII. Thus, from these results it can be stated that the mast cells grown in culture from normal rat bone marrow stimulated by factors present in conditioned medium made from mesenteric lymph node cells from *Nippostrongylus brasiliensis* infected rats are *in vitro* analogues of the cells identified *in vivo* by comparable technique (Gibson & Miller, 1986) as mucosal mast cells.

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