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# Granule proteinases define mast cell heterogeneity in the serosa and the gastrointestinal mucosa of the mouse

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## SUMMARY

In order to define further mast cell heterogeneity in the mouse, affinity-purified antibodies against a 28,000 MW serine proteinase from mouse intestinal mast cells (IMCP) and against rat mast cell proteinase I (RMCPI) were used to characterize mast cell cytoplasmic granules immunohistochemically. On Western blot, anti-IMCP cross-reacted with RMCPI and with a 25,000 MW antigen from isolated mouse serosal mast cells (SMC). Anti-RMCPI did not react with IMCP, although it identified the same 25,000 MW antigen from SMC. Isolated SMC (85–90% pure) lacked the 28,000 MW IMCP on Western blot, even though, immunohistochemically, the cells were stained with both anti-RMCPI and anti-IMCP. Anti-IMCP stained the granules of more than 85% of all mast cells detected with toluidine blue in the tongue or gastrointestinal mucosa. The specificity of anti-RMCPI which, in the rat, detects very few mucosal mast cells was almost identical to that of anti-IMCP for murine tongue and gastric and large intestinal mucosae, but a significant proportion of cells in distal jejunal, ileal and caecal mucosae were not stained with this antibody. The immunohistochemistry of the large numbers of mast cells recruited to jejunum following infection 10 days previously with 300 *Trichinella spiralis* muscle larvae was similar to that of uninfected control mice. The results show that considerable mast cell heterogeneity exists within the gastrointestinal mucosa of the mouse and indicate that there are both similarities and differences between mouse and rat in the distribution of mast cells and of their granule proteinases.

## INTRODUCTION

Mast cell heterogeneity (reviewed by Lee *et al.*, 1985) is significant not only because each mast cell subset may be functionally distinct (Befus *et al.*, 1982) and, therefore, responsive to different stimuli or drugs, but also because there is increasing evidence that one mast cell phenotype can switch to another under the influence of local environmental factors (Davidson *et al.*, 1983; Kitamura *et al.*, 1986; Levi-Schaffer *et al.*, 1987).

Mast cell heterogeneity in the mouse is currently defined by histochemical analysis of the staining and fixation properties of granule glycosaminoglycans (GAGS) (Crowle & Phillips, 1983; Kitamura *et al.*, 1986) and by biochemical characterization and comparison of granule proteoglycans in serosal mast cells (SMC) and in bone marrow-derived mast cells (BMMC) grown in culture (Sredni *et al.*, 1983; Levi-Schaffer *et al.*, 1987). Since BMMC contain predominantly chondroitin sulphate E, and SMC heparin, the former are thought to be the equivalent of the 'mucosal mast cell' phenotype in the rat (Sredni *et al.*, 1983; Kitamura *et al.*, 1987) and SMC equivalent to rat connective tissue mast cells (CTMC) (Kitamura *et al.*, 1987).

Although the histochemical and fixation properties of mast cell GAGs in rat intestine are uniform (Enerback, 1981), immunocytochemistry of the mast cell granule proteinases RMCPI and II demonstrates that there is heterogeneity even within such an apparently uniform cell population (Gibson *et al.*, 1987). Proteinase heterogeneity has been identified also in human intestinal mucosa (Irani *et al.*, 1986), even though GAG histochemistry again suggests a relatively uniform cell population (Enerback, 1987).

The influence of local tissue environment on mast cell differentiation has been studied intensively in the mouse, but the emphasis has been on the nature of the granule GAGs in skin and gastric mucosa and muscularis (Kitamura *et al.*, 1987). The purpose of the present study is, therefore, to compare the distribution of granule proteinases in mast cells in the serosa with those in the gastrointestinal mucosa of normal and parasitized mice.

## MATERIALS AND METHODS

### *Animals*

Random bred 8–10-week-old Swiss White mice were raised at Moredun Research Institute, and inbred (6–8-week-old) NIH

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mice were purchased from OLAC (Bicester, Oxon). Mice were fed and watered *ad libitum* and NIH mice were maintained in positive-pressure isolators. Antisera were raised in New Zealand White Rabbits and in Wistar rats, both from the Moredun Research Institute.

*Parasite infection*

NIH mice were infected orally with 300 *Trichinella spiralis* muscle larvae and the latter were recovered by pepsin digestion from NIH mice infected several months previously (Alizadeh & Wakelin, 1982). Mice killed on Day 10 of infection were bled out and jejunum was taken for immunohistology.

*Mast cell proteinases*

Mouse intestinal mast cell proteinase (IMCP) was recovered from intestines of *T. spiralis*-infected mice using a slight modification of the original technique (Newlands *et al.*, 1987). Briefly, intestines were homogenized at 4° in 20 mM Tris-HCl (pH 7·5) and, after centrifugation (15,000 *g* for 1 hr), the supernatant was applied to CM fractogel (Merck, Darmstadt, FGR) equilibrated with the same buffer. Bound enzyme was eluted with a linear 0–0·5 M NaCl gradient. Samples containing activity against CBZ-tyrosine substrate were pooled and desalted over a Sephadex G25 column (40 × 2 cm) equilibrated with 20 mM phosphate buffer (pH 6·0). Active fractions were again pooled and applied to a Pharmacia FPLC Mono S cation exchange column equilibrated with 20 mM phosphate buffer (pH 6·0). IMCP was recovered as a complex of three peaks after elution with a linear gradient of 0·075–0·150 M NaCl in 20 mM phosphate buffer, pH 6·0. An enzymatically active fraction from the middle of the third and largest peak was diluted 10-fold with 20 mM phosphate buffer, pH 6·0, and reapplied to the Mono S cation exchanger. This resolved as a single peak after elution with a 0·075–0·150 M NaCl linear gradient. Two milligrams of IMCP were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Milton-Keynes, Bucks) according to the manufacturer's instructions.

The recovery and purification of RMCPI from rat peritoneal mast cells was as described by Gibson & Miller (1986).

*Specific antibodies against IMCP and RMCPI*

Antibodies raised in rats and rabbits against IMCP (Newlands *et al.*, 1987) were isolated by affinity chromatography on IMCP–Sepharose 4B and, together with the original antisera, were repeatedly cross-absorbed on RMCPI–Sepharose 4B (Gibson & Miller, 1986). Rabbit anti-RMCPI, purified by affinity chromatography on RMCPI–Sepharose 4B, as well as the original whole serum, were repeatedly cross-absorbed against both RMCPII– (Gibson & Miller, 1986) and IMCP–Sepharose 4B. All antibodies were concentrated to 100–300 μg/ml, aliquoted, and stored at −70°. Antisera were stored at −20°.

*SDS–PAGE*

Discontinuous SDS–PAGE of enzyme preparations on 12% acrylamide slab gels was as described previously (Gibson & Miller, 1986; Newlands *et al.*, 1987).

*Nitrocellulose blotting*

Enzyme preparations judged to be pure by SDS–PAGE, or pellets of purified SMC (see below) extracted directly into reducing SDS–PAGE sample buffer, were electroblotted on nitrocellulose membranes (Newlands *et al.*, 1987). The nitrocellulose was then washed 6 × 2 min in wash buffer and cross-absorbed rat or rabbit anti-IMCP, or anti-RMCPI antisera (250 μl in 10 ml wash buffer containing 0·5% Tween 20) (T. W. Tan and A. Taylor, personal communication), were applied for 1 hr. After a further 6 × 2 min washes the blots were incubated with sheep $F(ab)_2$ anti-rabbit Fab-peroxidase (Gibson & Miller, 1986) or sheep IgG anti-rat IgG-peroxidase (10 μg/ml) (Sera Lab, Crawley, Sussex) in wash buffer containing 0·5% Tween 20 for 1 hr. After further washings peroxidase activity was revealed with diaminobenzidine and $H_2O_2$ (Graham & Karnovsky, 1966).

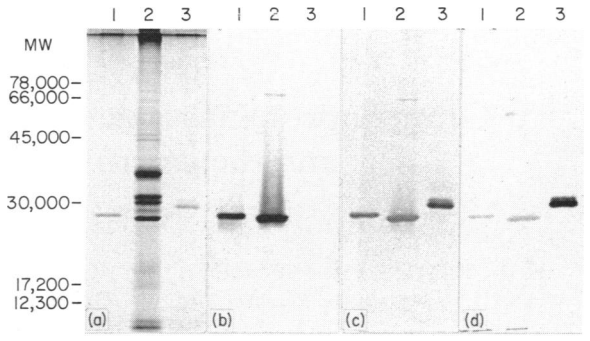


**Figure 1.** One-dimensional electrophoresis and Western blot of mast cell proteinases: (a) Silver-stained SDS–PAGE showing (lane 1) purified RMCPI (250 ng protein), (lane 2) extract from $5 \times 10^4$ (85–90% pure) peritoneal mast cells and (lane 3) 140 ng of purified IMCP. (b–d) Western blot in which the sample electrophoresed in (a) have been transferred to nitrocellulose and incubated with cross-absorbed antisera: (b) rabbit anti-RMCPI, (c) rabbit anti-IMCP, (d) rat anti-IMCP. Blots were developed with appropriate immunoperoxidase conjugates and stained with diaminobenzidine.

*Purification of mouse serosal mast cells (SMC)*

Peritoneal cells were recovered by lavage from 20 adult Swiss White mice and were purified to 80–90% SMC by density-gradient centrifugation over metrizamide, as described by Hamaguchi *et al.* (1987). Cells were then washed and resuspended in Hanks' solution/0·1% (w/v) gelatin (HG).

*Immunohistochemistry*

Although several fixation methods were tested, including 4% paraformaldehyde (6 hr) (Newlands, Huntley & Miller, 1984) and iso-isotonic formalin acetic acid (24 hr) (Enerback, 1966), Carnoys fluid (24 hr) (Enerback, 1966) proved to be the best fixative for detection of both mast cell proteoglycans and granule proteinases in tissues. Fixed samples were placed in 70% alcohol for 24 hr before dehydration and embedding in paraffin wax (Alizadeh & Wakelin, 1982). Sections (5 μm) were cut, dewaxed in xylene and rehydrated. Cytocentrifuge preparations of SMC ($5 \times 10^5$/ml HG) were prepared and fixed for 30 min in 4% paraformaldehyde at 45°. They were post-fixed for 30 min in 70% alcohol before rehydration.

Endogenous peroxidase activity in both sections and smears was inhibited with periodic acid/sodium borohydride (Heyderman & Neville, 1977) and all slides were treated with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min before overnight incubation with affinity-purified rabbit anti-IMCP or anti-RMCPI or with rat anti-IMCP antibodies

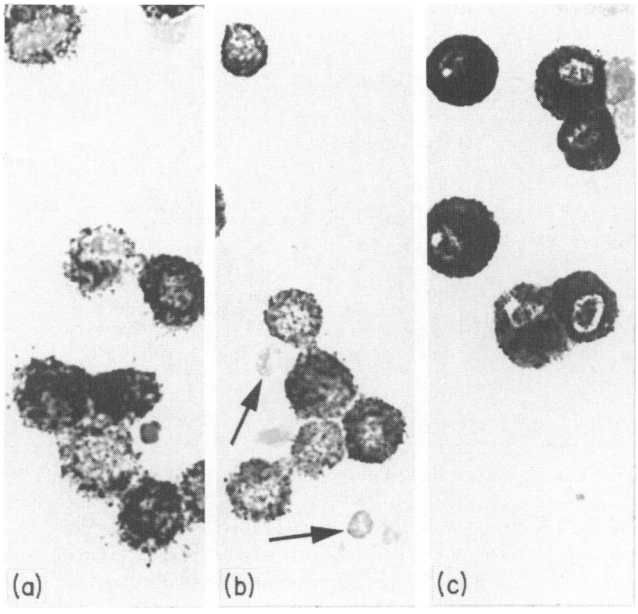


**Figure 2.** Photomicrographs of serosal mast cells (SMC) isolated from the peritoneal cavity and purified to 85–90% homogeneity over a metrizamide gradient. Immunoperoxidase staining of SMC with rabbit antibodies against (a) RMCPI and (b) IMCP. Note the distinct granule-associated peroxidase reaction and the absence of specific staining of contaminating peritoneal cells (arrows). The cytocentrifuge preparations were counterstained with haematoxylin. The mast cell morphology of the isolated Leishman-stained cells is shown in (c) ( × 160).

**Table 1.** Distribution of mast cells in the proximal and distal jejunum of normal and *T. spiralis*-infected NIH mice

| | | Cells/villus–crypt unit ± SE | | |
|---|---|---|---|---|
| | | IMCP | RMCPI | Tol. blue |
| Normal | | | | |
| Proximal | Intra-epithelial | 6·0 ± 1·3 | 1·4 ± 0·4 | 4·4 ± 0·2 |
| | Lamina propria | 0·5 ± 0·2 | 0·7 ± 0·2 | 0·7 ± 0·1 |
| Distal | Intra-epithelial | 2·7 ± 0·4 | 0·4 ± 0·3 | 2·9 ± 0·4 |
| | Lamina propria | 0·2 ± 0·1 | 0·1 ± 0·1 | 0·5 ± 0·2 |
| Infected | | | | |
| Proximal | Intra-epithelial | 45·0 ± 8·8 | 9·5 ± 7·6 | 42·8 ± 8·1 |
| | Lamina propria | 2·3 ± 0·4 | 4·7 ± 1·1 | 6·3 ± 1·1 |
| Distal | Intra-epithelial | 27·9 ± 2·0 | 0·3 ± 0·3 | 24·0 ± 2·2 |
| | Lamina propria | 0·6 ± 0·2 | 0·4 ± 0·4 | 2·6 ± 0·2 |

Adjacent 5 μm serial section were stained with rabbit anti-IMCP, with anti-RMCPI followed by peroxidase conjugate, or with toluidine blue. A minimum of 20 villus–crypt units, from identical regions of the jejunum in each of the three sections, were counted and the numbers recorded per villus–crypt unit. Mast cells located within epithelium were counted separately from those in lamina propria. There were five animals in each group.

(20 μg/ml) in 5% BSA/PBS. Slides were rinsed 3 × 10 min in 5% BSA/PBS and incubated with sheep $F(ab)_2$ anti-rabbit Fab-peroxidase conjugate (Gibson & Miller, 1986) or with sheep IgG anti-rat IgG-peroxidase conjugate (Sera Lab.) for 1·5–2 hr. After rinsing 3 × 10 min in 5% BSA/PBS, peroxidase activity was developed with diaminobenzidine and $H_2O_2$ (Graham & Karnovsky, 1966).

Slides were lightly counterstained with haematoxylin. For control purposes, initial overnight incubation of slides was with normal rabbit IgG (20 μg/ml in 5% BSA/PBS), purified on protein A, or with normal rat serum diluted 1/200–1/1000 with 5% BSA/PBS. Subsequent treatment of slides, and incubation with appropriate peroxidase conjugates, was as described above.

For paired immunofluorescence, dewaxed and rehydrated sections, treated for 30 min in 10% BSA/PBS, were incubated for 2–4 hr with rabbit anti-RMCPI (20 μg/ml) in BSA/PBS. After 3 × 10 min washes in PBS, sections were incubated for 1 hr with sheep IgG anti-rabbit IgG–rhodamine (TRITC) conjugate (Sera Lab.) diluted 1:50 in 10% BSA/PBS. After further 3 × 10 min washes, sections were incubated overnight with rat anti-IMCP (20 μg/ml) in 10% BSA/PBS and were washed 3 × 10 min before incubation for 1 hr with sheep IgG anti-rat IgG fluorescein (FITC) conjugate diluted 1:50 in 10% BSA/PBS. Normal rabbit IgG (20 μg/ml) and normal rat serum diluted 1/200–1/1000 with 10% BSA/PBS were substituted for specific antibodies for control purposes. After final washing, sections mounted in citifluor AF1 mountant (Citifluor Ltd, London) were viewed under a Leitz Orthplan microscope (Leitz, FRG) sequentially excited with blue and green incident light.

*Comparative histochemistry*

Serial sections from Carnoy-fixed tissues were used alternately for immunohistochemical detection of proteinases and for detection of mast cells by staining with toluidine blue. Granule GAGs were identified after staining sections overnight with 0·5% toluidine blue, pH 0·5 (Wingren & Enerback, 1983). Cytocentrifuge preparations of SMC were air-dried and stained with Leishman's stain or, following paraformaldehyde fixation, were subjected to immunoperoxidase labelling.

*Tissue distribution of mast cells containing different proteinases*

The numbers of mast cells in normal and parasitized jejunal mucosa of NIH mice were counted per villus/crypt unit as described previously (Miller & Jarrett, 1971; Gibson *et al.*, 1987); jejunal mast cells were identified with toluidine blue, immunoperoxidase staining, or immunofluorescence. Tongue, gastric mucosa, small and large intestinal mucosae and rectal mucosa from outbred Swiss White mice were also examined. The total numbers of mast cells stained with anti-IMCP, anti-RMCPI, or toluidine blue were recorded for each of the adjacent sections. This allowed, by simple regression analysis, a direct comparison of the mast cell populations in adjacent sections (Gibson *et al.*, 1987).

## RESULTS

### Antibody specificities by Western blot

Purified RMCPI (250 ng), IMCP (140 ng) and extracts of purified SMC (>85% pure; $5 \times 10^4$ cell equivalents) were

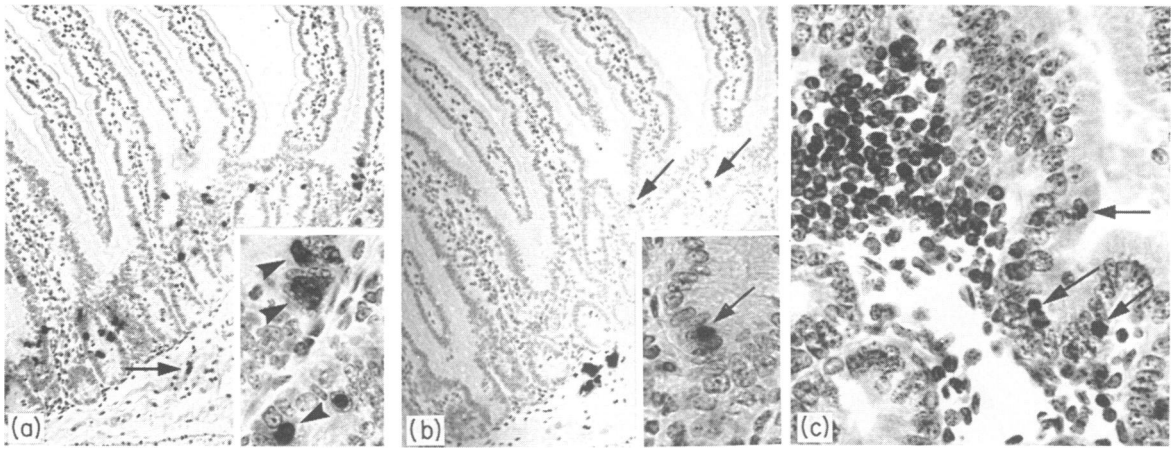


**Figure 3.** Distribution of mast cells in the jejunum of NIH mice. (a) Immunoperoxidase-labelled mast cells detected with rabbit anti-IMCP. The cells are located intra-epithelially and are stained as strongly as the submucosal cells (arrow) (×35). Inset. Higher magnification to show the diffuse cytoplasmic localization of reaction product of cells identified in (a) (arrowheads) (×85). (b) Section adjacent to (a) in which cells are labelled with rabbit anti-RMCPI. Mast cells in the submucosa are intensely stained, but only a small number of mast cells (arrows) in the mucosa are detected with this antibody (×35). Inset. A single, large peroxidase-labelled granule (arrow) is present intra-epithelially in one of the cells identified in (b) (×85). (c) Higher power photomicrograph of a section stained with toluidine blue. Several toluidine blue-stained cells (arrows) are located intra-epithelially (×85).

subjected to SDS–PAGE under reducing conditions (Fig. 1) and transferred to nitrocellulose. After probing with cross-absorbed rat or rabbit anti-IMCP antisera, bands of 26,000 and 25,000 MW molecular mass, respectively, were detected in the lanes containing RMCPI and SMC extract (Fig. 1), whereas a 28,000 MW band with a minor, superimposed, 28,500–29,000 MW band were detected in the lane containing IMCP. When probed with rabbit anti-RMCPI, RMCPI and a 25,000 MW band were detected in lanes 1 and 2, respectively, but no peroxidase reaction was observed in lane 3 containing IMCP (Fig. 1).

### Immunoperoxidase histochemistry of SMC

All mast cells isolated from peritoneal cavity were strongly stained with anti-RMCPI (Fig. 2a) but the peroxidase reaction was less intense with anti-IMCP although, again, all mast cells were stained (Fig. 2b). The few contaminating mononuclear cells (Fig. 2c) were unstained in the immunoperoxidase reaction (Fig. 2b).

### Immunoperoxidase histochemistry

*Normal NIH jejunum.* Serial 5 μm Carnoy-fixed sections were stained with: (i) rabbit anti-IMCP, or (ii) rabbit anti-RMCPI followed by peroxidase conjugate, or (iii) toluidine blue; the numbers of intra-epithelial and lamina propria mast cells detected in the mucosa with each method were recorded separately (Table 1). Similar numbers of intra-epithelial mast cells were detected in each site with anti-IMCP and with toluidine blue (Table 1, Fig. 3a and c). By contrast, significantly fewer ($P < 0.05$) intra-epithelial mast cells were stained with anti-RMCPI (Table 1, Fig. 3b). All control sections reacted with normal rabbit immunoglobulin were unstained. Cells in lamina propria comprised less than 20% of the total intestinal mast cell population, but, when compared with intra-epithelial mast cells, a higher proportion of lamina propria mast cells was stained with anti-RMCPI (Table 1). The majority of intestinal intra-epithelial cells reacting with anti-IMCP did not have distinct granular morphology and the peroxidase reaction tended to be diffusely distributed in the cytoplasm (Fig. 3a). By contrast, cells stained with anti-RMCPI had large, well-defined peroxidase-labelled granules often located towards one pole of the cell (Fig. 3b). The majority of intra-epithelial IMCP-containing cells were located at the junction of the villus and crypt (Fig. 3a).

*Jejunum on Day 10 of infection with* T. spiralis. The results of staining serial sections from parasitized intestine with anti-IMCP, anti-RMCPI or toluidine blue are shown in Table 1. All controls, treated with normal rabbit immunoglobulin, remained unstained. Increases of five- to 10-fold in the populations of mast cells occurred at all sites. However, the pattern of staining was very similar to that of normal jejunum (Table 1) except that intra-epithelial cells were located at all levels in the villus (Fig. 4a and b). Again, the majority of cells detected with anti-IMCP had a diffuse cytoplasmic peroxidase staining (Fig. 4c), whereas those identified with anti-RMCPI had discrete and sometimes very large peroxidase-stained granules or globules (Fig. 4d).

### Immunofluorescence: normal and parasitized jejunum

Proximal and distal jejunum in both normal and *T. spiralis*-infected mice were populated with varying proportions of IMCP-containing mast cells which were unstained with anti-RMCPI (Table 2, Fig. 5a–d). The remaining cells were doubly labelled, fluorescing with both anti-IMCP and anti-RMCPI (Table 2, Fig. 5a–d). The pattern of staining and the proportions of each type at each site were similar to those obtained by the immunoperoxidase technique (Table 1), there being diffuse cytoplasmic staining with anti-IMCP and discrete granular staining with anti-RMCPI (Fig. 5a–d).

### Proteinase phenotypes at different levels in the normal GI mucosa

The total number of cells stained with (i) toluidine blue, (ii) rabbit anti-IMCP and (iii) rabbit anti-RMCPI were recorded in

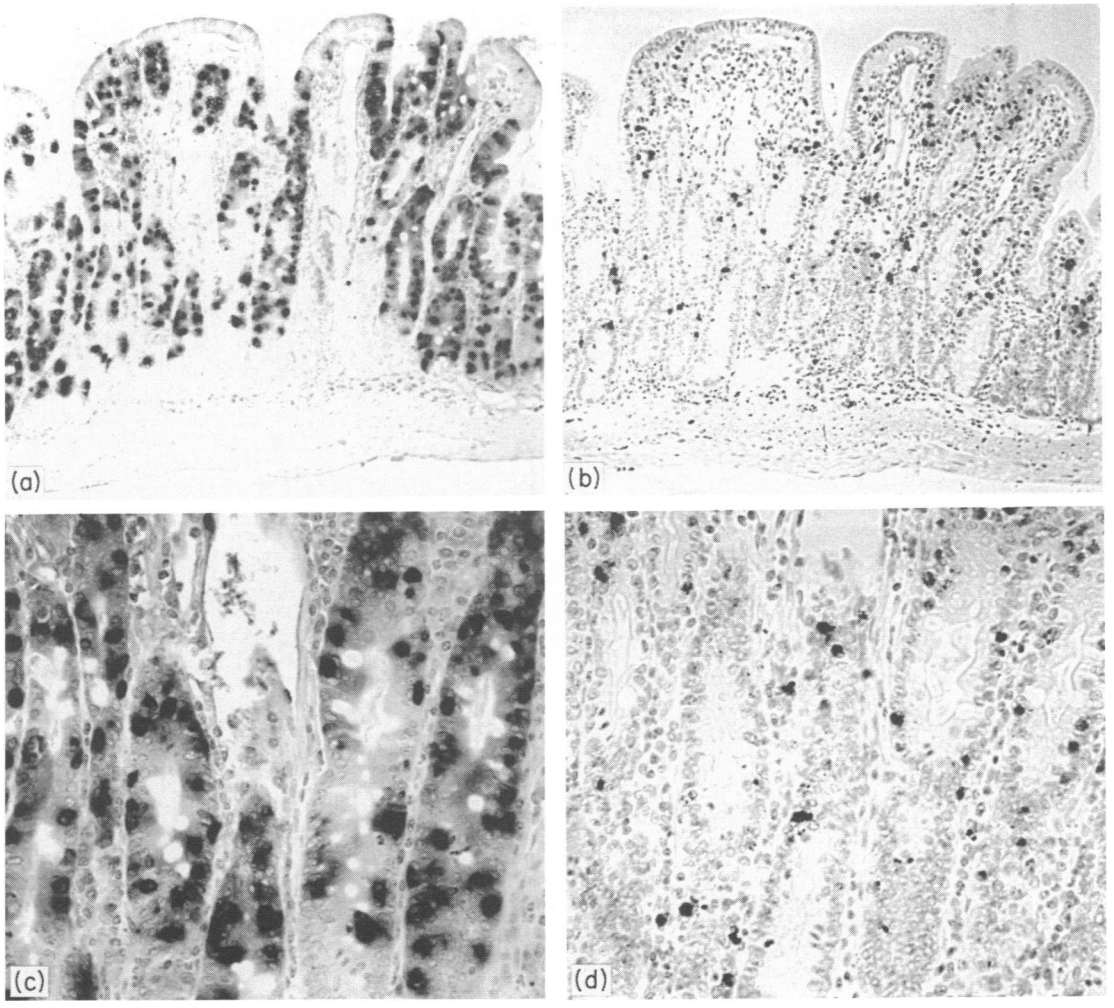


**Figure 4.** Proximal jejunum from a NIH mouse infected 10 days previously with 300 *T. spiralis* muscle larvae. (a) The numbers of mast cells stained with rat anti-IMCP are increased when compared with controls and the cells are distributed intra-epithelially at all levels in the mucosa (× 34). (b) Immunoperoxidase labelling with rabbit anti-RMCPI demonstrates the high proportion of labelled cells in this segment of jejunum (× 34). (c) High power photomicrograph of (a) in which the diffuse, ill-defined peroxidase reaction in the labelled cells is more readily visible (× 85). (d) Higher power view of (b). Note the discrete, granular or globular reaction with anti-RMCPI (× 85).

each of three serial sections from different regions of the GI tract of Swiss White mice. The proximal digestive tract, including tongue and gastric mucosa, was populated with cells that were stained with each of the peroxidase-labelled antibody conjugates and with toluidine blue (Fig. 6a–c, Table 3) and this was confirmed by paired fluorescence which showed that all cells detected in tongue with anti-IMCP were also labelled with anti-RMCPI. Similarly, in gastric mucosa, 88–96% of the mast cells were stained with both peroxidase conjugates and cells staining uniquely with anti-IMCP were rare (Table 3). It was notable, however, that the cells in the gastric mucosa, many of which were intra-epithelial, had discrete, granular immunoperoxidase staining with both antibody probes. In the small intestinal mucosa, the patterns of fluorescent and peroxidase staining were similar to that described for NIH mice, with the proportion of RMCPI-positive mast cells being greatest in the duodenum (Table 3). Again, rather diffuse immunoperoxidase staining occurred with anti-IMCP and discrete granular staining was noted with anti-RMCPI. The distribution of cells in the large intestinal mucosa varied according to the site examined, with 56%, 90% and 100% of the cells staining with anti-RMCPI in the caecum, colon, and rectum, respectively (Table 3). The mast cells in the more distal regions had discrete immunoperoxidase-stained granules, like those in gastric mucosa. This distribution of cell types was largely confirmed by paired immunofluorescence where a few cells labelled uniquely with anti-IMCP were detected in the mucosae of caecum and colon but not in the rectum.

Because the area of the section and mast cell density varied for each tissue, mast cell counts were transformed to $\log_{10}$ before the numbers of cells detected in each tissue, respectively, by staining with toluidine blue, anti-RMCPI and anti-IMCP were compared by simple regression analysis. For toluidine blue ($x$) against anti-IMCP ($y$) the regression equation for all the tissues

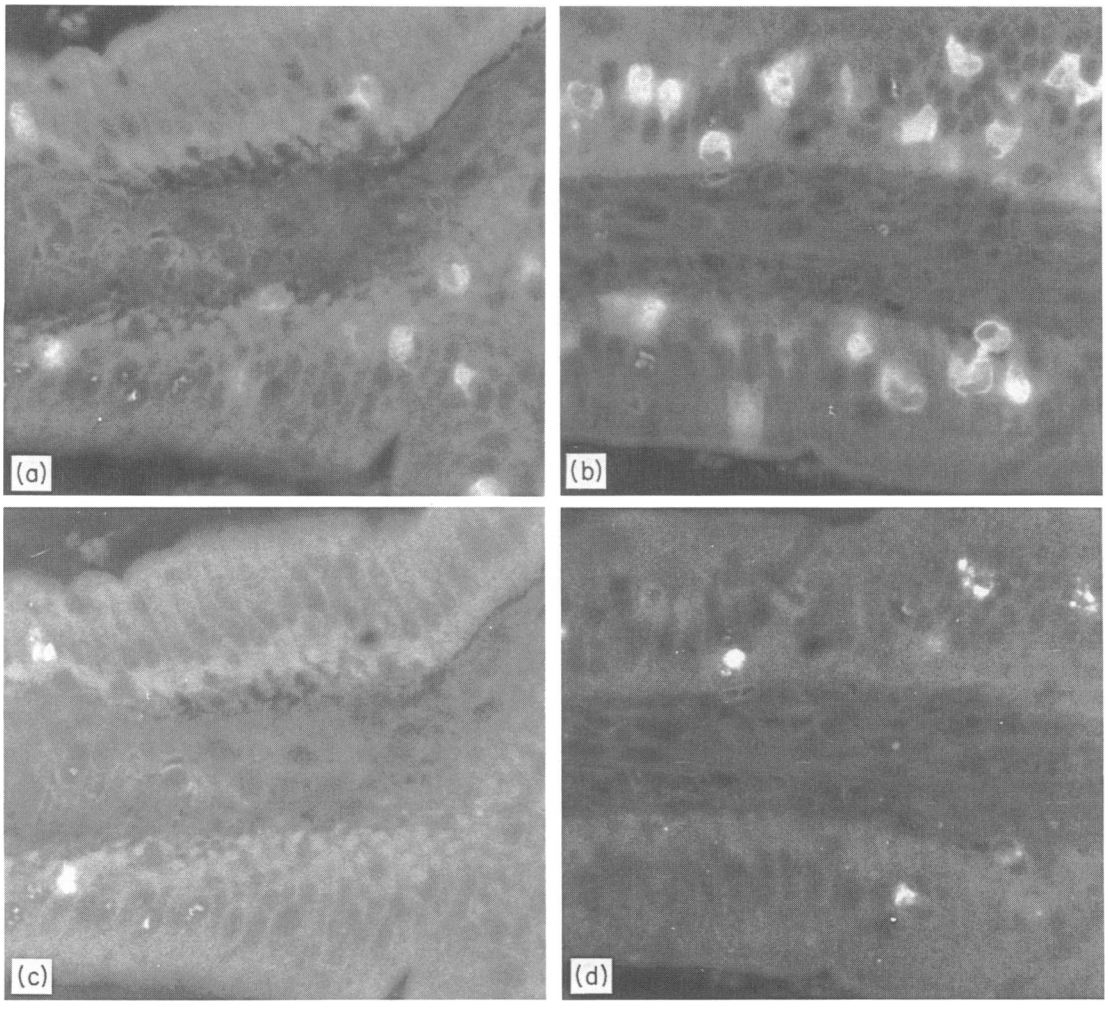


**Figure 5.** Paired fluorescence with rat anti-IMCP/FITC conjugate and rabbit anti-RMCPI/TRITC conjugate. (a) Normal jejunum in which IMCP-containing cells are labelled with FITC (× 127·5). (b) IMCP-containing cells in jejunum on Day 10 infection. Note that there is diffuse cytoplasmic fluorescence (× 127·5). (c) Normal proximal jejunum as in (a) but demonstrating TRITC fluorescence. The few labelled cells have a discrete granular fluorescence (× 127·5). (d) parasitized proximal jejunum as in (b). Only a proportion of cells fluoresce with TRITC and again they have a discrete granular fluorescence (× 127·5).

shown in Table 3 was $x = 0{\cdot}0742 + 0{\cdot}955\ y$, $r = 0{\cdot}988$, and for toluidine blue ($x$) against anti-RMCPI ($y$) the equation was $x = 0{\cdot}589 + 0{\cdot}775\ y$, $r = 0{\cdot}829$. These results confirm the observations in Table 3 that toluidine blue staining correlated more closely with anti-IMCP than with anti-RMCPI in the detection of gastrointestinal mast cells.

## DISCUSSION

Previous studies of granule serine proteinases in gastrointestinal mast cells in rat (Gibson *et al.*, 1987) and man (Irani *et al.*, 1986) have demonstrated heterogeneity amongst cells where none had previously been recognized using differential fixation techniques and GAG histochemistry (Enerback, 1987). In the current study, advantage was taken of the fact that antibodies raised against IMCP apparently detected all mast cells (Newlands *et al.*, 1987), whereas those directed against RMCPI were of a more restricted specificity. The results establish that in the mouse, like the rat, gastrointestinal mast cells are a heterogeneous population.

The specificities of the antibodies used in this study were characterized by Western blot analysis. Antisera raised against IMCP in rats or rabbits cross-reacted with a 25,000 MW polypeptide in extracts of purified SMC. By contrast, anti-RMCPI was uniquely specific for the 25,000 MW polypeptide in SMC. This 25,000 MW protein, which is readily extracted from SMC only with high concentrations of salt (G. F. J. Newlands and H. R. P. Miller, unpublished data), may be the insoluble murine equivalent of RMCPI and is possibly the same as the 25,000 MW chymotrypsin-like enzyme identified in a murine mastocytoma cell line (Vensel, Komender & Barnard, 1971) whereas IMCP, because of its high solubility and cross-reactivity with anti-RMCPII antibodies (Newlands *et al.*, 1987), is more akin to RMCPII. Interestingly, the Western blot data show that SMC do not contain IMCP in detectable quantities.

By comparison with the rat, where RMCPI-containing cells are rare (< 10%) in gastric mucosa and large intestine and

**Table 2.** Use of paired immunofluorescence to determine the distribution of proteinases in jejunal mast cells from normal and *T. spiralis*-infected NIH mice

| | | Cells/villus–crypt unit ± SE | |
|---|---|---|---|
| | | FITC* | FITC+TRITC† |
| Normal | | | |
| Proximal | Intra-epithelial | 3·3±0·8 | 0·7±0·4 |
| | Lamina propria | 0·2±0·1 | 0·4±0·1 |
| Distal | Intra-epithelial | 3·2±0·5 | 0·1±0·1 |
| | Lamina propria | 0·2±0·1 | 0·1±0·4 |
| Infected | | | |
| Proximal | Intra-epithelial | 30·9±3·8 | 18·4±3·9 |
| | Lamina propria | 0·5±0·1 | 5·7±1·2 |
| Distal | Intra-epithelial | 38·4±6·9 | 0·3±0·3 |
| | Lamina propria | 1·3±0·3 | 0·6±0·6 |

* Single FITC fluorescence detects IMCP.

† Double FITC and TRITC fluorescence detects RMCPI-like antigen.

The observations were from the same five animals in each group shown in Table 1 but the tissue sites examined were not the same.

**Table 3.** Relative percentages of the total toluidine blue-stained mast cell population which, in adjacent sections, stain with anti-IMCP and with anti-RMCPI

| Organ | *n* | % IMCP | % RMCPI |
|---|---|---|---|
| Tongue | 5 | 88·3±3 | 99·8±4 |
| Stomach | 5 | 95·8±5 | 88·2±5 |
| Duodenum | 5 | 85·7±6 | 64·9±11 |
| Jejunum | 4 | 103·9±8 | 22·7±5* |
| Ileum | 4 | 99·3±9 | 38·2±17·5* |
| Caecum | 4 | 109·2±3 | 56·2±11* |
| Colon | 4 | 102 ±9 | 90 ±11 |
| Rectum | 4 | 86·6±13 | 104·8±15 |

* Percentage of mast cells detected with anti-RMCPI significantly ($P < 0.05$) lower than the percentage detected with anti-IMCP (Student's *t*-test).

The number of mast cells per section was counted for each of the slides stained with toluidine blue, anti-RMCPI and anti-IMCP. The above percentage values are expressed as group mean ± SE.

virtually absent from intestinal mucosa (Gibson *et al.*, 1987), RMCPI-positive mast cells were abundant in both stomach and large intestine of the mouse. At this stage it is not certain whether such cells contain the 25,000 MW polypeptide present in SMC or whether there are other RMCPI-like proteases in the mucosal cells. It is probably significant that RMCPI-positive granules were invariably sharply delineated with relatively little diffusion, except in the vicinity of the most densely granulated cells. This lack of diffusion might be expected of a relatively insoluble RMCPI-like enzyme. By contrast, the pattern of intracellular staining of intestinal mast cells with anti-IMCP was often diffuse with very poor definition of granules and cell boundaries. IMCP, like RMCPII, is a highly soluble enzyme (Newlands *et al.*, 1987) and similar, ill-defined, diffuse mast cell staining with anti-RMCPII has previously been observed in Carnoy-fixed rat intestine (Woodbury, Gruzenski & Lagunoff, 1978). However, the staining of SMC and mast cells in tongue, stomach, and large intestine with anti-IMCP was also, to a large extent, discrete and granular, which would suggest that the putative RMCPI-like proteinase rather than IMCP was being detected in these cells.

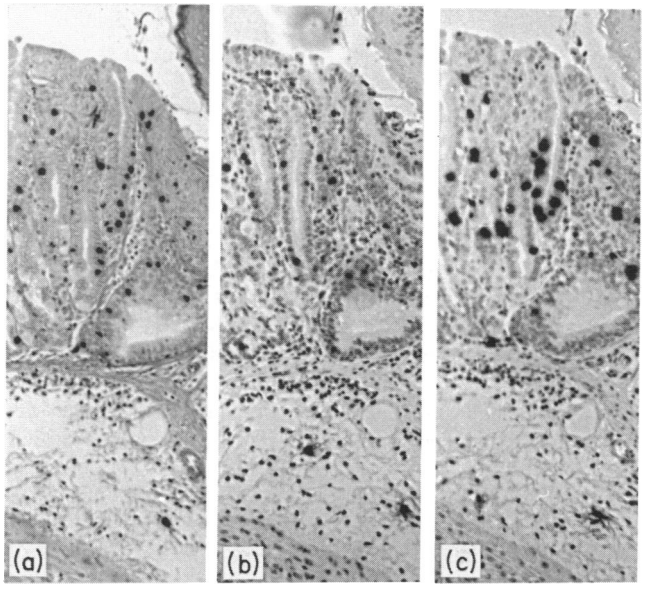


**Figure 6.** Gastric mucosa stained (a) with toluidine blue, (b) with anti-IMCP and (c) anti-RMCPI. The immunoperoxidase staining intensity is greatest with anti-RMCPI although the mast cell density is similar in each of the three adjacent sections (×40).

Substantial recruitment of intra-epithelial intestinal mast cells was observed following infection with *T. spiralis* and this is in agreement with numerous previous reports (reviewed by Miller, 1984). The pattern of antibody staining, however, revealed that the population of recruited cells was heterogeneous in its expression of granule proteinases. The heterogeneity was similar to that observed in naive, non-infected mice, there being a greater proportion of cells in proximal jejunum detected with anti-RMCPI than in the more distal jejunum. It is not certain, however, whether the pattern of staining represents a transitory phase in the development of the response or whether the distribution of proteinase phenotypes is predestined and tissue- or organ-specific. Indeed, the proteinase content of intra-epithelial as opposed to lamina propria mast cells (Tables 1 and 2) suggest that the microenvironment may regulate the expression of these important granule enzymes. Answers to these questions will require a more detailed and extensive study of the development of the response against *T. spiralis*.

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