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# Mast Cell Heterogeneity in the Gastrointestinal Tract

## *Variable Expression of Mouse Mast Cell Protease-1 (mMCP-1) in Intraepithelial Mucosal Mast Cells in Nematode-Infected and Normal BALB/c Mice*

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***Soluble granule chymases in rodent intestinal mucosal mast cells (IMMCs) may play an important role in altering epithelial permeability during immediate hypersensitivity reactions. Using a monoclonal antibody against the chymase mouse mast cell protease-1 (mMCP-1), we have shown that it is constitutively expressed in ≤20% of esterase-positive (esterase$^+$) IMMCs but not in esterase$^+$ gastric mucosal mast cells (GMMCs) in normal BALB/c mice. Intestinal infection with mouse- or rat-adapted strains of* Nippostrongylus brasiliensis *resulted in IMMC hyperplasia with 100% of esterase$^+$ IMMCs expressing mMCP-1. In contrast, there was a variable response in terms of numbers of GMMCs and of the proportion expressing mMCP-1. Esterase$^+$ mast cells in the gastric submucosa, muscularis, ear pinna, lung parenchyma, major airway submucosa, and peritoneal cavity did not express mMCP-1. The few airway esterase$^+$ mast cells expressing mMCP-1 were, like the great majority of IMMCs and GMMCs, located intraepithelially. In conclusion, mMCP-1 is predominantly expressed by intraepithelial mucosal mast cells but not in all sites; the immunological stimulus associated with intestinal nematodiasis substantially up-regulates mMCP-1 expression by mast cells in the jejunum but not in the stomach; IMMCs and GMMCs in BALB/c mice are phenotypically and possibly functionally distinct. (Am J Pathol 1997, 150:1661–1672)***

Mast cell secretory granule proteases (MCGPs) are selectively expressed in a tissue- and sometimes strain-specific fashion.[1,2] The functional significance of the tissue-specific expression of MCGPs is suggested from recent studies on rat mast cell protease-II (rMCP-II), which is predominantly expressed by intestinal mucosal mast cells (IMMCs).[3] Local release of this chymase, which is observed during nematode expulsion from the intestine,[4] is also associated with the rapid development of increased permeability to macromolecules with minimal disruption of epithelial architecture.[5,6] The intraepithelial location of a high proportion of the IMMCs in nematode-infected rodent jejunum means that locally released rMCP-II is ideally placed to alter paracellular permeability by acting on proteins in the epithelial junctional complex or receptors in the basolateral membrane.

The suggested homologue for rMCP-II in mouse intestine is mouse mast cell protease-1 (mMCP-1) with which it shares 74% amino acid sequence homology.[7] Like rMCP-II, mMCP-1 is predominantly expressed in IMMCs.[8–10] Analyses using RNA blotting,[11] polyclonal-antibody-based enzyme-linked immunosorbent assays (ELISAs), and immunohisto-

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chemistry have shown that mMCP-1 is also up-regulated in nematode-infected intestine.[10] These observations suggest that mMCP-1 is likely to be the functional homologue of rMCP-II, and it is therefore important to confirm that each protease is similarly distributed in their respective hosts.

The expression of MCGPs in the gastric mucosa is of particular interest because of the involvement of gastric mucosal mast cells (GMMCs) in stress-induced gastric ulcers,[12] in increased gastric mucosal permeability at weaning,[13] and in electrophysiological changes associated with type 1 hypersensitivity.[14] Importantly, GMMCs have been partially repopulated in mast-cell-deficient $W/W^v$ mice by intragastric injection of single peritoneal mast cells from +/+ littermates.[15] The latter result has been a key finding, supported by a number of recent *in vitro* and *in vivo* studies on the expression of MCGPs in the development of the hypothesis that mast cell phenotype is regulated by tissue microenvironment.[16] However, the question as to whether mouse GMMCs and IMMCs express the same proteases under some or all circumstances has not been addressed.

Here we report the development of a mMCP-1-specific monoclonal antibody (MAb) and its use in ELISA, immunohistochemistry, and Western blotting to quantify and immunolocalize mMCP-1 in different tissues. Our results show that mMCP-1 is expressed by a proportion of IMMCs but not by GMMCs in normal BALB/c mice. However, after nematode-induced hyperplasia of IMMCs and GMMCs, all of the IMMCs express mMCP-1 whereas expression of mMCP-1 is more variable in the gastric mucosa.

## Materials and Methods

### Monoclonal Antibody to MMCP-1

Wistar rats were immunized with three subcutaneous injections of purified mMCP-1[17] (9 to 10 μg/injection) given at monthly intervals. Complete Freunds adjuvant was used for the first inoculation and incomplete Freunds adjuvant was used subsequently. At varying intervals after the third inoculation, the rats were injected intraperitoneally with 25 μg of mMCP-1 in phosphate-buffered saline (PBS). Three days after challenge, rats were euthanized and spleen cells were harvested and fused with the mouse myeloma cell line NS0 using polyethylene glycol 4000 and a centrifugation method.[18] Supernatants from wells containing viable cells after hypoxanthine, aminopterin, and thymidine selection were screened for specific antibody by ELISA on microtiter plates coated with purified mMCP-1 at 1 μg/ml. Antibody-producing cells were cloned three times by limiting dilution to give three mMCP-1-specific monoclonal cell lines. Supernatant from one clone, RF6.1, gave optical densities on ELISA of >75% of a positive control (positive control was a 1:200 dilution of serum taken from the immune rat immediately before harvest of the spleen) and the secreted MAb RF6.1 was identified as a rat IgG1 (rat monoclonal isotyping kit, Serotec, Kidlington, UK).

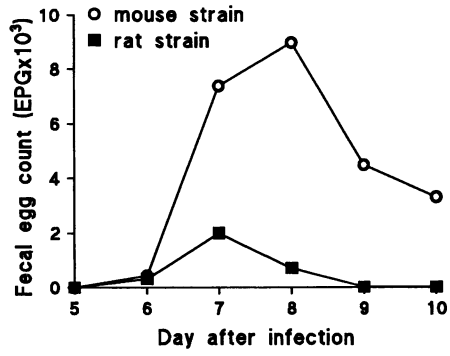


Figure 1. *Mean fecal egg counts from mice infected with either the rat- or mouse-adapted strain of* N. brasiliensis *(experiment 2).*

### Specificity of the mMCP-1 Monoclonal Antibody

Two identical immunoblots were prepared by separating purified mMCP-1 glycoforms A-E[17] (0.4 to 0.7 μg/lane), mMCP 4 (0.3 μg/lane), and purified mouse peritoneal cells ($3 \times 10^5$ cells/lane extracted and boiled in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis sample buffer containing 10% 2-mercaptoethanol) as described previously[17] by 12% SDS-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose. The blots were then probed using maximal MAb RF6.1 supernatant diluted 1:1 with PBS/0.5% Tween 20 or non-cross-absorbed rabbit anti-mMCP-1 serum diluted 1:50 with PBS/Tween 20. The blots were then developed using the appropriate conjugate (optimally diluted goat anti-rat IgG-horseradish peroxidase or goat anti-rabbit IgG-horseradish peroxidase) and diaminobenzidene substrate.

### *Infection with* Nippostrongylus brasiliensis

Two strains of the nematode parasite *N. brasiliensis* were maintained as described previously[19] and used to infect mice. The rat-adapted strain of the parasite caused a transient infection of the mice with no eggs detectable in the feces in experiment 1 and a low number of eggs detectable on days 6, 7, and

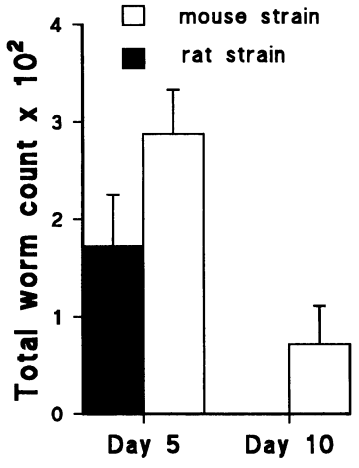


Figure 2. *Worm counts (mean ± SEM) from mice (n = 3/group) infected with the rat- or mouse-adapted strain of* N. brasiliensis.

8 in experiment 2 (Figure 1). The mouse-adapted strain (kindly supplied by Dr. J. Urban) produced a more prolonged infection resulting in measurable fecal egg counts from day 6 persisting to the end of experiment 2 (Figure 1). In support of these data, worms were detected in the intestines of mice (n = 3) 5 days after infection with either strain, but on day 10 there was a residual worm burden only in the mice infected with the mouse strain (Figure 2).

## *Tissue Collection and Preparation for mMCP-1 Assay and Histology*

Twelve-week-old BALB/c mice (Bantam and Kingman, Grimston, Hull, UK) were exsanguinated under terminal anesthesia either before (day 0, n = 5 experiments 1 and 2) or 10 days after infection (day 10, n = 5) with 400 to 500 third-stage *N. brasiliensis* larvae (L3; rat-adapted strain, experiments 1 and 2, and mouse-adapted strain, experiment 2). Two adjacent pieces of jejunum (2-cm length) from each mouse were fixed in 4% paraformaldehyde in PBS (4% PF/PBS)[20] or snap frozen by immersion in liquid nitrogen. A section of stomach taken from the greater curvature of the fundic region, an ear pinna, and one lung lobe were also fixed in 4% PF/PBS, and the remaining stomach, lung lobe, and ear pinna were snap frozen.

Tissues were fixed in 4% PF/PBS for 6 hours and transferred to 70% ethanol for storage before trimming, processing, and embedding in paraffin wax. Frozen tissue was stored at −50°C until homogenized. Frozen tissues were weighed and immediately homogenized in 1 ml of 20 mmol/L Tris/HCl, pH 7.5, 1 mol/L NaCl on ice.[10] The homogenate was then centrifuged at 12,000 × *g* for 30 minutes at 4°C. The supernatants were stored at −50°C until analyzed for mMCP-1 and soluble protein content. Soluble protein in homogenate supernatants was estimated using the bicinchoninic acid method (Pierce and Warriner, UK).

## *ELISA for mMCP-1*

mMCP-1 concentrations were assayed in serum samples using a commercially available ELISA kit (comELISA; Moredun Animal Health, Edinburgh, UK) based on a sandwich technique using a sheep anti-mMCP-1 polyclonal capture antibody and a rabbit anti-mMCP-1 polyclonal antibody for detection.[10,17] The recommended protocol was followed except that 3,3′,5,5′-tetramethylbenzidine (TMB; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used as the substrate. The results obtained for serum concentrations of mMCP-1 using the comELISA were compared with those obtained when the monoclonal anti-mMCP-1 was incorporated into the ELISA (MAbELISA) as follows. The same sheep anti-mMCP-1 was used as a capture antibody, and pooled MAb RF6.1 supernatant was used for detection of bound mMCP-1. Finally, goat anti-rat IgG horseradish peroxidase conjugate (Sigma Chemical Co., Poole, UK) was used diluted 1:2000 to detect the bound antibody (MAbELISA). Purified mMCP-1 was used as a standard for all ELISAs, and comparable standard curves were obtained from both the comELISA and MAbELISA techniques with the linear range between 0.25 and 4 ng/ml. Subsequently, the MAbELISA was used to measure mMCP-1 levels in tissue homogenates.

## *Esterase Histochemistry for Detection of Mast Cells*

Detection of mast cell granule esterase shows excellent correlation with toluidine blue staining of mast cells in mouse intestine and identifies all IMMCs.[21] This was the method of choice to allow optimal comparison of mast cell detection by histochemical and immunohistochemical techniques. Paraformaldehyde-fixed tissue sections (4-μm sections) were dewaxed in xylene, rehydrated, and then stained for esterase using Fast Garnet GBC salt (Sigma) and naphthol AS-D chloroacetate (Sigma)[22] and then mounted using Vectashield (Vector Laboratories, Bretton, UK).

### *Detection of mMCP-1 by Immunohistochemistry*

Preliminary studies showed an unacceptable level of nonspecific connective tissue staining when standard fixation in Carnoy's was used for immunodetection of mMCP-1 with MAb RF6.1; therefore, paraformaldehyde-fixed tissue was used as described above. Sections were dewaxed in xylene, washed in 100 and 95% ethanol, and then treated with 1% hydrogen peroxide in methanol for 20 minutes (3 ml of 30% $H_2O_2$ in 97 ml of methanol) to block endogenous peroxidase activity. The sections were incubated in PBS plus 0.5 mol/L NaCl with 0.5% Tween 80 (PBS/NaCl/T80; also used for all antibody dilutions) for 30 minutes before incubation for 1 hour with either MAb RF6.1 (maximal supernatant diluted 1:10) or rat IgG1 (10 $\mu$g/ml; Serotec) as a negative control. Finally, the sections were incubated with biotinylated anti-rat IgG (Vector) diluted 1:100 for 30 minutes before detection using an avidin-biotin complex technique (Vectastain *Elite* ABC kit, Vector). Specific binding was visualized using diaminobenzidine substrate (Vector), and the sections were counterstained with hematoxylin. Slides were washed three times in PBS for 5 minutes each between each step.

### *Enumeration of Mast Cells*

Adjacent tissue sections were stained for esterase or immunohistochemically for mMCP-1, and mast cells were counted at ×250 magnification; for ear pinna, lung, and stomach, the number of cells in a given number of graticule fields (0.24 $mm^2$) was counted. Esterase- or mMCP-1-positive mast cells in 20 villus crypt units were counted in the jejunum, and results are expressed as IMMCs/villus crypt unit.[23] Total mast cells were counted in 5 adjacent fields in longitudinal sections of ear (1.2-$mm^2$ area) and 20 sections of lung parenchyma (4.8-$mm^2$ area). Sections from major airways were examined, but mast cells were not counted. In sections of stomach, 5 adjacent fields (1.2-$mm^2$ area) in the glandular area were scanned with the edge of the graticule aligned along the luminal edge of the mucosa, and the percentage of intraepithelial and intra-lamina-propria cells positive for esterase and mMCP-1 was also estimated; identical areas of the adjacent sections were analyzed.

### *Statistics*

Minitab statistical software was used to compare data using the nonparametric Mann-Whitney and Kruskal-Wallis tests with a significance level of $P < 0.05$.

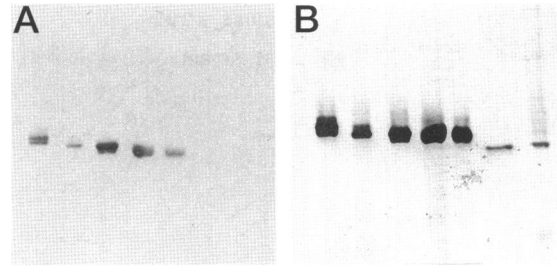


Figure 3. *Western blot analysis of mast cell proteases using monoclonal anti-mMCP-1 (RF6.1,* A*) or non-cross-absorbed rabbit anti-mMCP-1 serum* (B). *MAb RF6.1 recognizes only purified mMCP-1 glycoforms A to E* (lanes 1 *to* 5), *whereas the polyclonal rabbit serum recognizes mMCP-1* (lanes 1 *to* 5), *purified mMCP-4* (lane 6), *and six protease bands in peritoneal mast cell extracts* (lane 7).

## *Results*

### *Specificity of MAb RF6.1 for mMCP-1*

A number of MCGPs have been identified in the mouse by gene or protein expression.[2] The chymases, mMCP-1 to -5, are closely related serine proteases and may carry cross-reacting epitopes; therefore, the specificity of MAb RF6.1 was evaluated by Western blotting, immunohistochemistry, and ELISA. On Western blotting, MAb RF6.1 identified all five glycoforms of mMCP-1 (Figure 3A) but did not recognize any protein in lanes containing purified mMCP-4 or peritoneal cells (which are known to preferentially express mMCP-4, -5, and -6).[1] In contrast, the polyclonal rabbit anti-mMCP1 serum[8] identified not only purified mMCP-1 and mMCP-4 but also at least six protein bands in peritoneal mast cell extracts (Figure 3B).

The specificity of the MAb RF6.1 was also tested immunohistochemically on peritoneal cells and jejunum and ear pinna from normal BALB/c controls or from mice infected 10 days previously with *N. brasiliensis* (rat strain). IMMCs in the jejunum were stained, but peritoneal mast cells and connective tissue mast cells (CTMCs) in the ear pinna were not (Figure 4). mMCP-4 and -5 are strongly expressed and mMCP-2 (along with mMCP-7) is expressed at low levels in CTMCs in the ear of BALB/c mice[1]; therefore, the complete absence of immunostaining suggests that MAb RF6.1 does not cross-react with any of these chymases. In addition, in *N. brasiliensis*-infected mice in which the gene for mMCP-1 has been deleted,[24] MAb RF6.1 does not identify any cells in the jejunal mucosa (J. M. Wastling, J. Ure, J. Mason, P. Knight, A. Smith, E. M. Thornton, S. M. Wright, C. L. Seudamore, and H. R. P. Miller, unpub-

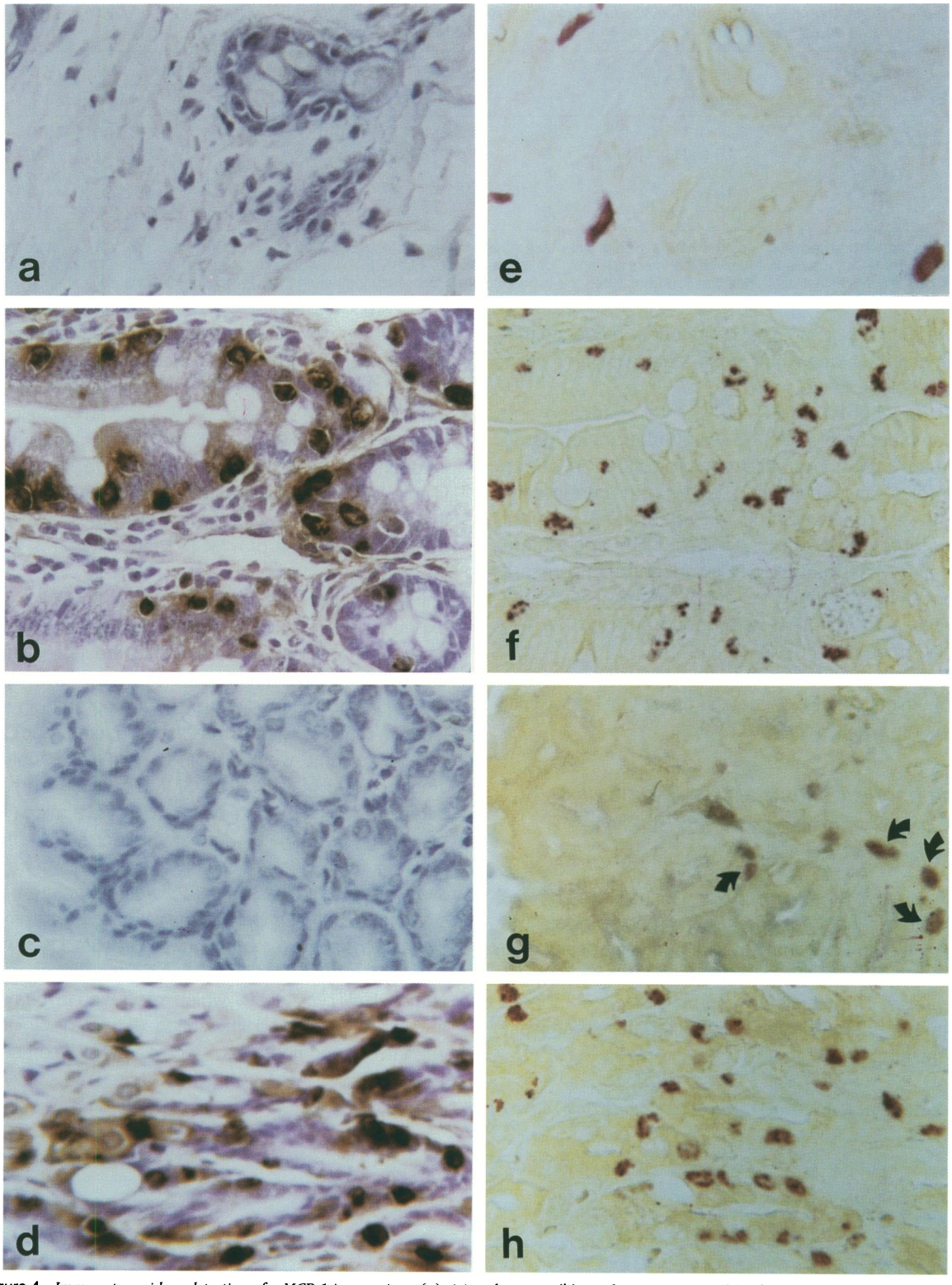


**Figure 4.** *Immunoperoxidase detection of mMCP-1 in ear pinna* (a), *jejunal mucosa* (b), *and gastric mucosa* (c) *of BALB/c mice, infected 10 days previously with the rat strain of* N. brasiliensis *and gastric mucosa* (d) *of BALB/c mice, infected 10 days previously with the mouse strain of* N. brasiliensis. *Adjacent sections of ear pinna* (e) *and parasitized jejunal* (f) *and gastric* (g *and* h) *mucosa were stained for esterase using Fast Garnet GBC salt and naphthol AS-D chloroacetate. Note that esterase*$^+$ *mast cells in ear pinna are mMCP-1*$^-$, *whereas the abundant intraepithelial esterase*$^+$ *mast cells in jejunum are mMCP-1*$^+$. *Staining of mast cells in the gastric mucosa is highly variable depending on individual animals and strain of parasite used. Esterase*$^+$ *mast cells* (arrows; g) *in the gastric mucosa of a mouse infected with the rat-strain of* N. brasiliensis *in which all are mMCP-1*$^-$ (c) *in contrast to intraepithelial esterase*$^+$ *mast cells* (h) *in gastric mucosa of a mouse infected with the mouse strain of* N. brasiliensis, *which are all mMCP-1*$^+$ (d). *Immunoperoxidase-labeled sections were counterstained with hematoxylin. Magnification,* ×550.

**Table 1.** *Comparison of Mouse Mucosal Mast Cell Protease (mMCP-1) Concentration in Mouse Serum Measured Using Two ELISA Techniques*

| Experiment | Day of infection | n | mMCP-1 (ng/ml) | |
|---|---|---|---|---|
| | | | comELISA | MAb ELISA |
| 1 | 0 | 5 | 127.4 (77–189) | 125.2 (85–194) |
| | 10 (rat) | 5 | 6490 (5065–8690) | 6885 (6105–7450) |
| 2 | 0 | 5 | | 90.3 (62.1–198.3) |
| | 10 (rat) | 5 | | 4682 (3640–11818) |
| | 10 (mouse) | 5 | | 9362 (2568–24493) |

Data are expressed as median (range). Serum was taken from mice before (day 0) or 10 days after infection with a rat-adapted (experiment 1) or mouse-adapted (experiment 2) strain of *N. brasiliensis*. ELISA techniques were used as described in Materials and Methods. No significant difference was found between the two techniques for measuring serum mMCP-1 concentrations.

lished observations), providing further evidence that the antibody does not cross-react with any other mast cell protease.

The suitability of MAb RF6.1 for detection and quantification of mMCP-1 was further investigated by comparing protease concentrations in serum samples assayed using either the commercial ELISA kit (comELISA) or an adaptation of the kit incorporating MAb RF6.1 (MAbELISA; see Materials and Methods). mMCP-1 concentrations in sera from mice on day 0 or 10 of infection were not significantly different when compared by the two ELISA methods (Table 1).

Taken together, the Western blotting, immunohistochemical, and ELISA data suggest that MAb RF6.1 is highly specific for mMCP-1.

## *Immunohistochemical Localization of mMCP-1: Comparison with Esterase Staining*

The precise tissue distribution of mMCP-1 in the gastrointestinal tract and lungs of normal and parasite-infected BALB/c mice was analyzed using MAb RF6.1 to detect the intracellular localization of the protease. The results were quantitatively compared with mast cell distribution identified histochemically by esterase staining.

When the numbers of esterase$^+$ mast cells in the gastrointestinal tract were compared with the number of mMCP-1$^+$ mast cells in normal BALB/c mice, approximately 4 to 20% of IMMCs and an occasional intraepithelial GMMC (<10% in 2 of 10 mice) but no esterase$^+$ submucosal mast cells were mMCP-1$^+$ (Table 2). These results are entirely consistent with the ELISA data (see below) and suggest that the constitutive release of mMCP-1 into plasma is from the IMMC population.

Infection with the rat strain of *N. brasiliensis* in experiment 1 resulted in comparable increases in esterase$^+$/mMCP-1$^+$ mast cells in the jejunal mucosa (Table 2), but a more variable picture was seen in the gastric mucosa. An eightfold increase in esterase$^+$ mast cells occurred in the gastric mucosa, but the majority were mMCP-1$^-$ (Table 2). Only a small number of mMCP-1$^+$ GMMCs, which were all intraepithelial, were detected in two of five mice (Table 2). To investigate whether this apparent difference in mMCP-1 expression in different mucosal sites in the gastrointestinal tract was due to the transient nature of infection with the rat-adapted strain of *N. brasiliensis*, the experiment was repeated and was compared with an infection using a mouse-adapted strain of *N. brasiliensis*, which produced a heavier and more prolonged infestation (Figure 1). In this second experiment, the increase in esterase$^+$/

**Table 2.** *Median (Range) Mast Cell Counts from Adjacent Tissue Sections Stained for Esterase or mMCP-1*

| Experiment | Tissue | n | Esterase$^+$ | | | mMCP-1$^+$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | Day 0 | Day 10 (rat) | Day 10 (mouse) | Day 0 | Day 10 (rat) | Day 10 (mouse) |
| 1 | Jejunum | 5 | 0.95 (0.45–1.8) | 9.75* (5.1–10.9) | | 0.2 (0.1–0.45) | 9.1* (6.35–10.5) | |
| | Stomach | 5 | 6.7 (4.2–10.8) | 55* (31.7–102.5) | | 0 (0–5) | 0 (0–7.5) | |
| | Ear | 5 | 41.7 (30.0–45.8) | 31.7 (23.3–33.3) | | 0 (0) | 0 (0–0.8) | |
| | Lung | 5 | 0 (0–0.4) | 0.6 (0–0.8) | | 0 (0–0.4) | 0.2 (0–1.3) | |
| 2 | Jejunum | 5 | 0.35 (0–1.35) | 8.1 (6.5–11.8) | 9.3 (5.7–13.65) | 0 (0–0.1) | 5.75 (3.35–12) | 6.45 (3.7–13.95) |
| | Stomach | 5 | 53.3 (24.2–76.7) | 55.8 (12.75–81.7) | 87.5 (60.8–183.3) | 0 | 0 (0–138.3) | 5.8 (0–47.5) |
| | Ear | 5 | 45.8 (25.8–70.8) | 30 (28.3–59.2) | 47.5 (23.3–75) | 0 | 0 | 0 |
| | Lung | 5 | 0 | 0.21 (0–0.42) | 0 (0–0.63) | 0 | 0 (0–0.42) | 0 |

Counts are expressed as mast cells/mm$^2$ except for jejunum where figures represent IMMC/vcu. Sections were stained with naphthol AS-D chloroacetate (esterase$^+$) or using immunoperoxidase with the monoclonal RF6.1 anti-mMCP-1 antibody (mMCP-1$^+$). Tissues were taken from mice before (day 0) or 10 days after infection with rat-adapted *N. brasiliensis* (experiment 1) or mouse-adapted *N. brasiliensis* (experiment 2).

*Significantly different from day 0; $P = 0.01$.

mMCP-1$^+$ mast cells in the jejunum was consistent with experiment 1 regardless of the strain of parasite used (Table 2). However, there was still a variable response in the stomach. The number of esterase$^+$/mMCP-1$^-$ mast cells in the control mice (day 0) was similar to that seen after infection in experiment 1, and there was no increase in esterase$^+$ cells after infection with the rat strain of the parasite, but again two of five mice had some mMCP-1$^+$ GMMCs. In the mice infected with the mouse strain of the parasite there was a tendency toward greater mMCP-1 expression. These mice showed a slight (not statistically significant) increase in esterase$^+$ cells, and in four of five mice there were some esterase$^+$/mMCP-1$^+$ GMMCs. In an additional experiment using the mouse strain of *N. brasiliensis*, 100% of the esterase$^+$ GMMCs (median and range of counts, 77 and 49.9 to 155/mm$^2$) were found to be mMCP-1$^+$ (median and range of counts, 72.8 and 33.2 to 156/mm$^2$; Figure 4), suggesting that there can be considerable variability between batches of mice and infections. In this additional experiment, very high fecal egg counts (30,000 eggs per gram on day 7) were noted, and it is therefore possible that in this particular instance mMCP-1 expression is related to both persistence and level of infection. In all of the mice studied, none of the esterase$^+$ submucosal mast cells expressed mMCP-1 protein, emphasizing that this protease is expressed exclusively at mucosal sites.

Esterase$^+$ mast cells in the ear pinna of all mice studied were uniformly mMCP-1$^-$ (Figure 4). Esterase$^+$ mast cells in the lungs were grouped around the major airways and were again mMCP-1$^-$ (Figure 5). The only mMCP-1$^+$/esterase$^+$ cells found in the lungs were either intraepithelial (Figure 5) or, in one infected mouse, associated with a small parasitic granuloma.

### *mMCP-1 Distribution in Normal and Nematode-Infected Mice: ELISA Measurement*

Protease concentrations in tissue homogenates from normal mice and from mice infected with the mouse- and rat-adapted strains of *N. brasiliensis* were also compared using the MAb RF6.1-adapted ELISA. In uninfected animals, mMCP-1 was present in the intestine (Table 3) and in serum (Table 1), confirming previous observations that mMCP-1, like rMCP-II,[6] is secreted constitutively in normal animals (reviewed by Miller et al[3]). On day 10 after infection with either strain of *N. brasiliensis*, mMCP-1 concentrations had significantly increased ($P = 0.01$) in serum (Table 1) and increased levels of mMCP-1 were detected in all tissues assayed (Table 3), in agreement with previous studies using *Trichinella spiralis*.[10] Increases in mMCP-1 concentration were most apparent in the serum and jejunum where there was a 20- to 279-fold increase in the median concentration (Table 3). mMCP-1 levels in the gastric homogenates from normal (day 0) mice were 6 (experiment 1) to 13 (experiment 2) times lower on a per wet weight basis than in the jejunum. After infection with either strain of *N. brasiliensis*, mMCP-1 levels in the stomach were significantly ($P = 0.01$) increased, but in contrast to the jejunum the concentrations were only 5 to 14 times that seen in controls. This modest increase in mMCP-1, when compared with that seen in the jejunum, may in part be attributable to the increased level of plasma mMCP-1 (Table 1) at this time because mast cells positive for mMCP-1 were virtually absent from the gastric mucosa (Table 2). Western blotting of homogenates from infected and uninfected mice also confirmed this pattern, with little or no observable mMCP-1 on day 0 but clearly visible immunoreactive bands in jejunal but not gastric homogenates on day 10 (experiment 1; Figure 6).

Concentrations of mMCP-1 were very low in homogenates of ear pinna and lung from control mice but were slightly increased after infection with either strain of *N. brasiliensis* (Table 3). These increases may, similarly, be due to increased levels of mMCP-1 in plasma, as reported previously,[10] and this is supported by the absence of mMCP-1$^+$ cells in these tissues (Table 2).

## *Discussion*

Mast cell heterogeneity has historically been defined on the basis of proteoglycan and protease composition of the cytoplasmic granules and, using these criteria, IMMCs are phenotypically distinct from CTMCs in a number of species.[25] In the mouse, expression of proteases has previously been identified by RNA blotting and through the use of polyclonal anti-peptide and anti-protein antibodies.[1,17] In this study, we have, for the first time, used a MAb (RF6.1) that is highly specific for mature mMCP-1 protein. It has clearly demonstrated the highly restricted localization of mMCP-1 immunoreactivity to intraepithelial mast cells in the jejunum, gastric mucosa, and major airways and its complete absence from mast cells in the submucosa, peritoneum, and ear pinna. The results are consistent with RNA blotting[1] and with previous ELISA studies[10] and provide

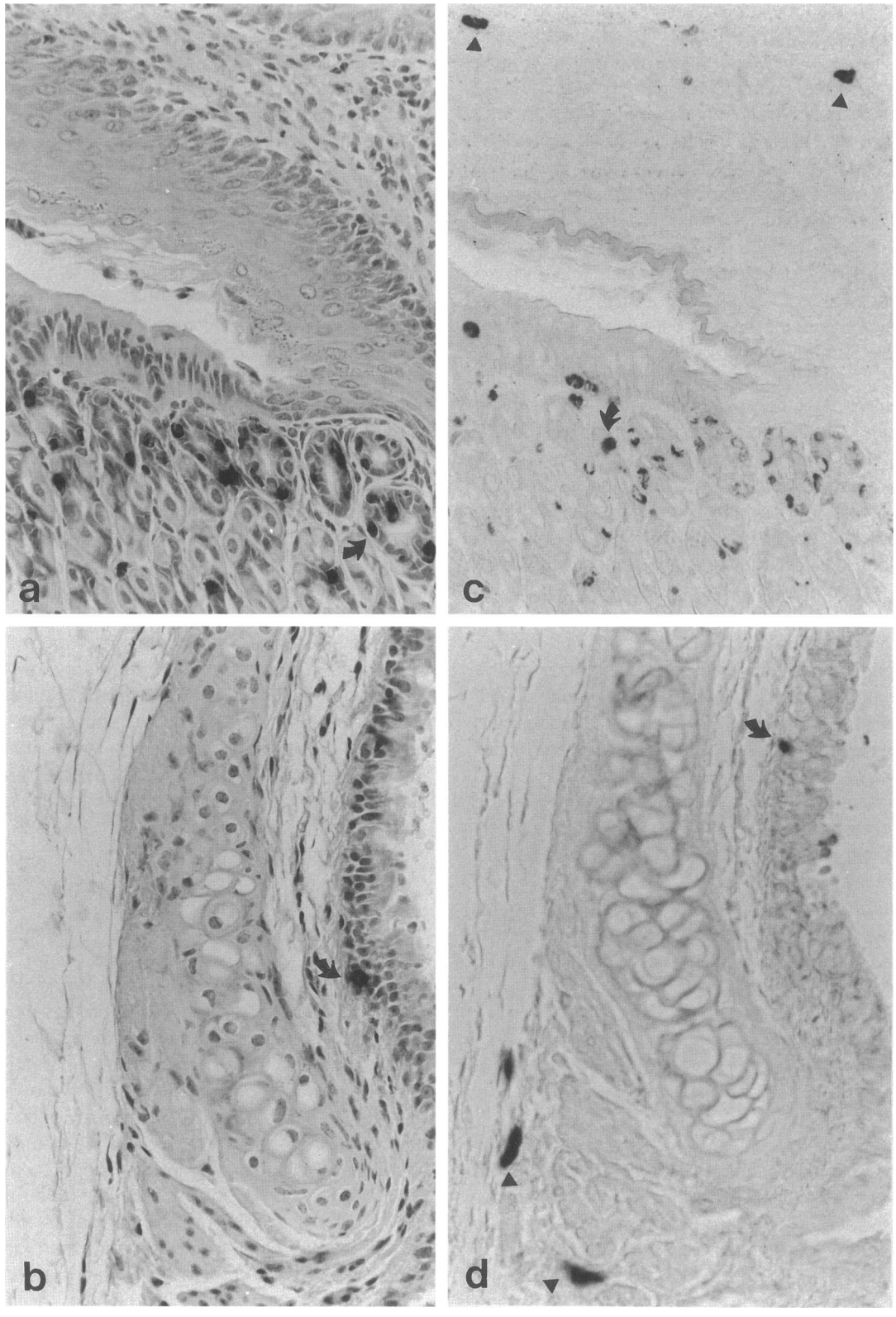


Figure 5. *Immunoperoxidase labeling demonstrating the intraepithelial expression of mMCP-1 by mast cells in the glandular part of the stomach* (**a**) *and a main stem bronchus* (**b**) *from a BALB/c mouse infected with the mouse strain of* N. brasiliensis. *Abundant mMCP-1$^+$ GMMCs* (**arrow**) *are located intraepithelially in the glandular tissue of the stomach* (**a**), *which is confirmed by esterase staining* (**arrow**) *in* **c**. *In the section of bronchus, an mMCP-1$^+$ mast cell is located intraepithelially* (**arrow** *in* **b**), *as is an esterase$^+$ mast cell* (**arrow** *in* **d**) *in an adjacent section. Note that esterase$^+$ mast cells* (**arrowheads**) *are present in the submucosa of the nonglandular part of the stomach* (**c**) *and in the submucosa of the bronchus* (**d**), *but none is detected immunohistochemically* (**a** *and* **b**). *Immunoperoxidase-labeled sections were counterstained with hematoxylin. Magnification,* ×550.

**Table 3.** *Comparison of Mouse Mast Cell Protease-1 (mMCP-1) Concentrations Measured by ELISA in Different Tissue Homogenates*

| Experiment | Tissue | n | mMCP-1 (ng/mg soluble protein) | | |
|---|---|---|---|---|---|
| | | | Day 0 | Day 10 (rat) | Day 10 (mouse) |
| 1 | Jejunum | 5 | 56.5 (41.9–85) | 15740* (12972–25643) | |
| | Stomach | 5 | 9.4 (4.9–19.3) | 134.5* (34.6–419.4) | |
| | Ear | 5 | 0.27 (0.19–3.34) | 18.3* (15.4–23.2) | |
| | Lung | 5 | 0.22 (0.14–0.42) | 16.8* (12.7–46.3) | |
| 2 | Jejunum | 5 | 223.8 (55.7–982.8) | 4649* (4287–7169) | 12380* (4914–19018) |
| | Stomach | 5 | 16.7 (6.2–39.2) | 97.5† (31.8–899.8) | 246.6* (71.2–1917) |
| | Ear | 5 | ND | 8.6 (2.2–9.8) | 16.5 (4.3–62.8) |
| | Lung | 5 | ND | 10.4 (2.4–13.7) | 18.4 (4.6–35.5) |

Data are expressed as median (range). Tissues were taken from mice before (day 0) or 10 days after infection with rat-adapted (experiments 1 and 2) or mouse-adapted (experiment 2) *N. brasiliensis*.
Significantly different from day 0; $^{*}P = 0.01$; $^{\dagger}P = 0.02$.

supporting evidence that this chymase is likely to be active at epithelial barriers. However, in contrast to previous studies using polyclonal antibodies,[9,17] the present results show that not all IMMCs in normal BALB/c mice store detectable quantities of mMCP-1 in their granules; nor is this protease detectable in the great majority of GMMCs. Some of the differences between this and previous studies can be ascribed to differences in antibody specificity and/or fixation technique. For example, comparison of mMCP-1 concentrations in tissue homogenates from experiment 1 using the commercial ELISA kit based on the polyclonal antibody *versus* the adaptation using the MAb clearly indicated that the polyclonal antibody overestimated mMCP-1 concentrations in the jejunum (110 *versus* 56 ng/ml) and stomach (194 *versus* 9.4 ng/ml) of normal mice and in the stomach of infected mice (3261 *versus* 134 ng/ml). These data highlight the increased specificity of MAb RF6.1 when compared with the polyclonal antibody. Our original study[9] using polyclonal anti-mMCP-1 on Carnoy-fixed tissues was not able to distinguish mMCP-1 from the other mouse chymases, although in a subsequent study[17] using affinity-purified cross-absorbed polyclonal antibodies, the specificity was greatly improved. However, MAb RF6.1 not only demonstrates a more highly restricted distribution of mMCP-1 by immunohistochemistry but its specificity is further confirmed in *N. brasiliensis*-infected, mMCP-1 knockout mice[24] in which no immunoreactivity is detected in the intestine by immunohistochemistry or by ELISA in intestinal homogenates or serum (J. M. Wastling, J. Ure, J. Mason, P. Knight, A. Smith, E. M. Thornton, S. M. Wright, C. L. Scudamore, and H. R. P. Miller, unpublished observations).

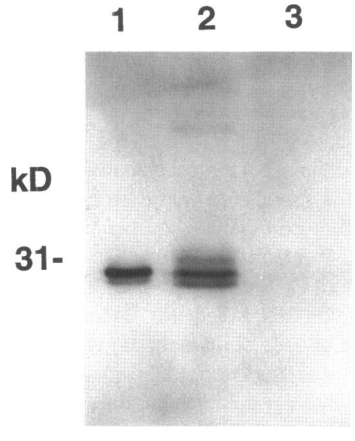


**Figure 6.** *Western blot analysis using MAb RF6.1 of purified mMCP-1* (lane 1; *0.24 μg of mMCP-1*) *and jejunal* (lane 2; *50 μg of soluble protein*) *and gastric* (lane 3; *50 μg of soluble protein*) *homogenates taken from a mouse 10 days after infection with the rat-adapted strain of* N. brasiliensis. *Note the high level of expression of mMCP-1 in the jejunal compared with the gastric homogenate (distinct band clearly visible in* lane 3 *of original blot).*

The current experiments also demonstrated that GMMCs can be recruited to the mucosa during intestinal nematode infection and remain mMCP-1$^-$. Taken together, the results suggest that MMC populations, based on their protease content, can be subdivided into additional phenotypes. This is in agreement with a recent publication[26] describing the presence of mMCP-1$^+$/mMCP-2$^+$/mMCP-5$^-$ and mMCP-1$^-$/mMCP-2$^+$/mMCP-5$^+$ IMMCs in BALB/c mice 2 and 4 weeks after infection with *T. spiralis*.

Although the existence of heterogeneity within mast cell populations is not disputed, the origin of the different populations is not understood. Mast cells are known to be derived from pluripotent bone marrow stem cells,[27,28] but the stage of differentiation and site at which their phenotype is committed is unknown. Mature mast cells are not found in the circulation, although circulating mast cell precursors in fetal blood do contain mRNA transcripts for mMCP-4 and carboxypeptidase A.[29] This would suggest that CTMCs may have a committed precursor population during fetal development. However, it

is also likely that final differentiation is triggered by the local tissue microenvironment. This still leaves the question of whether the ultimate phenotype is defined in the bone marrow (and that the predestined cells enter specific tissues due to homing molecules on the cell surface[30]) or once the cell has entered a tissue and been exposed to local growth factors and cytokines. Evidence for the latter view is supported by a number of *in vivo* and *in vitro* studies. *In vivo*, the repopulation of W/W$^v$ mast-cell-deficient mice with mouse bone marrow cells suggested that mast cells could differentiate in an animal lacking the ability to produce its own normal mast cell population.[31,32] More specifically, the ability of CTMCs to transdifferentiate into MMCs in the gastric mucosa when injected into W/W$^v$ mice has also been demonstrated.[15,33] Recently, careful studies involving the transplantation of readily identifiable v-*abl*-immortalized immature mast cells into BALB/c mice have shown that the grafted cells localize to the spleen, liver, and intestine where protease expression becomes modified dependent on the tissue.[34] *In vitro* studies have shown that the transcription of protease genes in cultured mouse BMMCs can be modulated by the presence of fibroblasts, cytokines (IL-3, -4, -9, and -10) and kit ligand (reviewed by Springman and Serafin[16]). In particular, mMCP-1 transcription in mBMMCs grown in the presence of IL-3 is up-regulated by IL-9[35] and IL-10,[11,36] and the control of chymase expression, at least *in vitro*, is dependent on post-transcriptional regulation.[37] In the current study, the pattern of mMCP-1 protein expression in tissues differed in individual animals. In addition, there was a tendency for more mMCP-1$^+$ GMMCs in mice infected with the mouse strain of the parasite, and it is possible that alterations in the time course of a parasite infection could alter the recruitment pattern of mast cells as it is known that with bone marrow reconstitution of W/W$^v$ mice the repopulation of different organs occurs at different times.[38] It also has to be noted, as has been pointed out for human IMMCs,[39] that the apparent absence of a particular chymase in a given population of mast cells may be due to different function or stage of maturation. The current results, however, indicate that in normal BALB/c mice GMMCs and IMMCs are phenotypically distinct and that mMCP-1 probably does not play a functional role in the normal gastric mucosa. Given that there are strain differences in the expression of MCGPs in mice,[1] it will be interesting to determine whether other mouse strains in which GMMC function has been studied[40] vary in their gastric expression of mMCP-1.

This study has demonstrated that both mMCP-1$^+$ and mMCP-1$^-$ MMCs can be found in the gastrointestinal tract of BALB/c mice and that the localization of mMCP-1$^+$ cells may be altered in different parasite infections. Additional studies are required to follow the establishment of MMC populations with varied protease phenotypes during the course of parasite infections and to determine the relationship of these changes *in vivo* to other factors such as cytokine production.

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