Mast Cells that Reside at Different Locations in the Jejunum of Mice Infected with *Trichinella spiralis* Exhibit Sequential Changes in their Granule Ultrastructure and Chymase Phenotype

Daniel S. Friend,*§ Namit Ghildyal,‡‖ K. Frank Austen,‡‖ Michael F. Gurish,‡‖ Ryoji Matsumoto,‡‖ and Richard L. Stevens‡‖

*Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; ‡Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115; §Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115; and ‖Division of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115

Abstract. Whether or not a nontransformed, mature mouse mast cell (MC) or its committed progenitor can change its granule protease phenotype during inflammatory responses, has not been determined. To address this issue, the granule morphology and protease content of the MC in the jejunum of BALB/c mice exposed to *Trichinella spiralis* were assessed during the course of the infection. Within 1 wk after helminth infection of the mice, increased numbers of MC appeared in the crypts at the base of the villi, and by wk 2 the number of MC throughout the villi increased by ~25-fold. Shortly after the peak of the mastocytosis, the intraepithelial population of MC disappeared, followed by a progressive loss of lamina propria MC. The presence of stellate-shaped granules containing crystalline structures in intraepithelial MC at the height of infection and the retention of such granules with fragmented crystals in lamina propria MC during resolution of the mastocytosis suggest that MC migrate during the various phases of the inflammation. As assessed by immunohistochemical analyses of serial sections, predominant chymase phenotypes were observed at the height of the infection in the muscle that expressed mouse MC protease (mMCP) 5 without mMCP-1 or mMCP-2 and in the epithelium that expressed mMCP-1 and mMCP-2 without mMCP-5. Accompanying these two MC populations were transitional forms in the submucosa that expressed mMCP-2 and mMCP-5 without mMCP-1 and in the lamina propria that expressed mMCP-2 alone. These data suggest that jejunal MC sequentially express mMCP-2, cease expressing mMCP-5, and finally express mMCP-1 as the cells progressively appear in the submucosa, lamina propria, and epithelium, respectively. In the recovery phase of the disease, MC sequentially cease expressing mMCP-1, express mMCP-5, and finally cease expressing mMCP-2 as they present at the tips of the villi, the base of the villi, and the submucosa, respectively. That MC can reversibly alter their protease phenotypes suggests that a static nomenclature with fixed functional implications is inadequate to describe MC populations during an inflammatory process within a particular tissue.

MICE, rats, and other mammals that have been infected with a helminth such as *Trichinella spiralis* or *Nippostrongylus brasiliensis* experience a transient, but pronounced, T cell–dependent mastocytosis in their intestinal mucosa (Wells, 1962; Murray et al., 1968; Miller and Jarrett, 1971; Ruitenberg and Elgersma, 1976; Mayrhofer, 1979; Ruitenberg et al., 1979). The mast cells (MC)[1] that increase in number in the lamina propria and between the epithelial cells of the mucosa have less histamine and tend to have fewer, but larger granules than the MC that reside in muscle, peritoneal cavity, and skin (Murray et al., 1968; Enerbäck and Lundin, 1974; Befus et al., 1982; Crowle and Phillips, 1983). In contrast to the MC that reside at other tissue locations, MC in the mucosa epithelium are poorly stained by safranin (Enerbäck, 1966; Murray et al., 1968), presumably because their serglycin proteoglycans contain predominately chondroitin sulfate diB/E glycosaminoglycans rather than heparin glycosaminoglycans (Stevens et al., 1986). Based primarily on morphologic criteria, Murray et al. (1968) proposed that the lamina propria MC and intraepithelial MC in the jejunum of *Nippostrongylus brasiliensis*–infected rats are developmentally related.

Address all correspondence to Dr. D.S. Friend, Harvard Medical School, Seeley G. Mudd Building, Rm. 528, 250 Longwood Ave., Boston, MA 02115. Tel.: (617) 432-4976; Fax: (617) 432-0979.

1. *Abbreviations used in this paper*: MC, mast cell; mMCP, mouse MC protease.

Besides serglycin proteoglycans, MC store in their granules different types of proteases that are enzymatically active at neutral pH. cDNAs that encode four chymase-like serine proteases (designated mouse MC protease [mMCP] 1, mMCP-2, mMCP-4, and mMCP-5) (Miller et al., 1988; Le Trong et al., 1989; Serafin et al., 1990, 1991; McNeil et al., 1991; Huang et al., 1991; Chu et al., 1992; Ghildyal et al., 1992*b*) whose genes reside at a complex on chromosome 14 (Gurish et al., 1993) have been used to identify different populations of MC in tissues. As assessed by RNA blot analysis, 1–2 wks after mice have been infected with *Trichinella spiralis* or *Nippostrongylus brasiliensis*, the intestine contains high steady-state levels of the transcripts that encode mMCP-1 and mMCP-2 but not mMCP-4 or mMCP-5 (Serafin et al., 1990; Ghildyal et al., 1992*b*, 1993). Because the MC in the mucosa of helminth-infected mice also contain mMCP-1 (Newlands et al., 1987; Miller et al., 1988; Le Trong et al., 1989) and/or mMCP-2 proteins (Ghildyal et al., 1993), they are phenotypically different from the populations of MC that reside in the spleen (Gurish et al., 1995) and peritoneal cavity (Miller et al., 1988; Le Trong et al., 1989; Reynolds et al., 1990; Serafin et al., 1990, 1991; Ghildyal et al., 1992*b*; McNeil et al., 1991, 1992) of normal, noninfected BALB/c mice.

The v-*abl*–immortalized V3 MC line undergoes tissue-dependent changes in its granule protease expression after adoptive transfer into BALB/c mice (Gurish et al., 1995) and the interleukin 3–dependent, mouse bone marrow–derived MC undergoes changes in its granule protease expression in culture in response to particular combinations of cytokines (Ghildyal et al., 1992*a*, *b*; Gurish et al., 1992; Eklund et al., 1993). Whereas the MC in the skin and jejunum of *Nippostrongylus brasiliensis*–infected rats selectively express RMCP-I and RMCP-II, respectively, the MC in the lungs of these animals express both chymases (Huntley et al., 1993; Arizono et al., 1993). While it is now clear that the mouse is not the only species having MC that undergo tissue-dependent changes in protease expression, no investigation has yet addressed whether or not a non-transformed, mature mouse MC or its committed progenitor can undergo a dynamic change in its protease content in a specific tissue site during an inflammatory response. Morphologic, histochemical, immunohistochemical, and ultrastructural analyses were therefore conducted on MC during the mastocytosis that develops and then subsides in the jejunum of *Trichinella spiralis*–infected BALB/c mice. We now show that jejunal MC can sequentially and reversibly alter their granule morphology and chymase expression in response to the onset and subsidence of inflammation caused by helminth infection.

Materials and Methods

Derivation of an mMCP-1–specific Antibody

Peptide A (Asn-Phe-Gln-Val-Cys-Val-Gly-Ser-Ser-Thr-Lys-Leu-Lys-Thr-Ala-Tyr-Met-Gly), which corresponds to residues 163–180 of mMCP-1 (Fig. 1 *A*), is not present in any other mMCP or in any other mouse protein listed in the Protein Identification Resource database of the National Biomedical Foundation. A model of the three-dimensional structure of mMCP-1 predicted that this peptide would protrude from the surface of the folded enzyme (Šali et al., 1993). Peptide A, peptide B (Gly-Ser-Ser-Thr-Lys-Leu-Lys-Thr-Ala-Tyr-Met), and peptide C (Gly-Ser-Pro-Thr-Thr-Leu-Lys-Ser-Ile-Gly-Gln) were synthesized by Quality Controlled Biochemicals (Hopkinton, MA). Peptide B corresponds to residues 169–179 in mMCP-1. Relative to the other MC chymases (Fig. 1 *A*), peptide B matches the least conserved sequence in peptide A. Peptide C corresponds to residues 169–179 in the most homologous chymase, mMCP-2.

As described previously for the derivation of peptide antibodies to mMCP-2 (Ghildyal et al., 1993) and mMCP-5 (McNeil et al., 1992), ~500 μg of peptide A (coupled to octavalent lysine) was suspended in 0.5 ml of

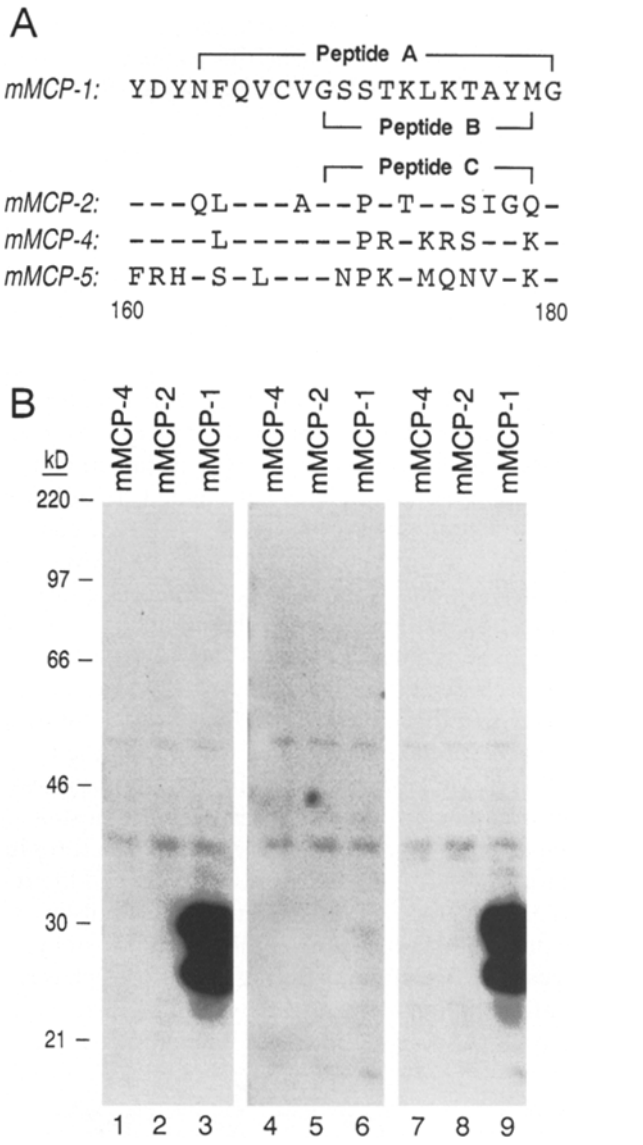


Figure 1. Generation of rabbit anti–mMCP-1 Ig. (*A*) Comparison of the deduced amino acid sequences of the relevant homologous regions in mMCP-1, mMCP-2, mMCP-4, and mMCP-5. Dashes indicate identical amino acids. The immunizing peptide (peptide A) and the two peptides (peptides B and C) used in the specificity study are highlighted. (*B*) SDS-PAGE/immunoblot analysis of lysates of High Five insect cells infected with recombinant baculovirus constructs containing cDNAs that encode mMCP-1 (lanes *3*, *6*, and *9*), mMCP-2 (lanes *2*, *5*, and *8*), or mMCP-4 (lanes *1*, *4*, and *7*). All lanes contain abundant amounts of a recombinant protein (data not shown). The depicted immunoblots were probed with anti–mMCP-1 Ig in the absence of any synthetic peptide (lanes *1–3*), in the presence of peptide B, which corresponds to residues 169–179 of mMCP-1 (lanes *4–6*), or in presence of peptide C, which corresponds to residues 169–179 of mMCP-2 (lanes *7–9*).

PBS. 0.5 ml of Titermax synthetic adjuvant (CytRx Corp., Norcross, GA) was added and a New Zealand white rabbit was injected with the resulting emulsion im at multiple sites. The immunized rabbit received booster injections (~250 μg) 4, 8, and 12 wks later, and then its serum was collected at biweekly intervals. As assessed by ELISA, the rabbit produced a high titer of antibodies that recognized the immunizing peptide. After affinity purification with a 4-ml column containing ~10 mg of peptide A coupled to Affi-Gel 10 (Bio-Rad, Hercules CA), the antibodies reacted in an ELISA at a >5,000-fold dilution.

In terms of its primary amino acid sequence, mMCP-1 (Le Trong et al., 1989) is more homologous to mMCP-2 (Serafin et al., 1990) and mMCP-4 (Serafin et al., 1991) than to mMCP-5 (McNeil et al., 1991). To confirm the specificity of the anti–mMCP-1 peptide antibody, pro–mMCP-1, pro–mMCP-2, and pro–mMCP-4 were expressed separately in High Five insect cells, as described for pro–mMCP-7 (Matsumoto et al., 1995). Each full-length mMCP cDNA was liberated from its plasmid and inserted in the correct orientation into the multiple cloning site of pVL1393 (PharMingen, San Diego, CA) downstream of the promoter of the polyhedrin gene. Plasmid DNA (~2 μg), purified by CsCl density gradient centrifugation, was mixed with 0.5 μg of linearized BaculoGold™ DNA (PharMingen) and calcium phosphate in a total volume of 1 ml. The resulting DNA solution was briefly incubated at room temperature and added to a tissue culture dish containing Grace's insect medium and adherent insect cells that were in their log phase of growth. After a 4-h incubation at 27°C, the medium was replaced with insect culture medium (Invitrogen, San Diego, CA) supplemented with 10% heat-inactivated FCS. The infected insect cells were cultured for 7 d at 27°C, and a plaque assay was used to select a single virus clone. After this clone was amplified, ≥3×10^7 recombinant virus particles were added to each 10-cm tissue culture dish containing 6×10^6 High Five cells in their log phase of growth. 3–4 d later, the collected insect cells and supernatants were separated from one another by centrifugation at ~300 *g*. The infected High Five cells were then cultured in the presence of protein-free medium (Insect Xpress; BioWhittaker, Walkersville, MD). All infected insect cells produced large amounts of recombinant protein, which remained cell associated.

SDS-PAGE/immunoblot analysis revealed that anti–mMCP-1 Ig recognized a diffuse band of ~28 kD in the lysates of High Five insect cells that were induced to express recombinant pro–mMCP-1 but not recombinant pro–mMCP-2 or pro–mMCP-4 (Fig. 1 *B*). In some experiments, affinity-purified antibody was preincubated for 1 h at 37°C and then incubated overnight at 4°C with 1 mg/ml of peptide B or peptide C. The antibody did not recognize recombinant pro–mMCP-1 if the reaction was carried out in the presence of peptide B (Fig. 1 *B*). Thus, the primary epitope recognized by the antiserum corresponds to the least conserved COOH-terminal domain in the peptide. Because peptide C did not inhibit the SDS-PAGE/immunoblot analysis, it was concluded that the rabbit antibody was specific for mMCP-1. In subsequent immunohistochemical analyses of the three populations of baculovirus-infected insect cells, anti–mMCP-1 Ig recognized only those High Five cells that had been induced to express pro–mMCP-1 (data not shown).

Histochemistry, Enzyme Cytochemistry, and Immunohistochemistry of the MC in the Jejunum of Noninfected and Trichinella spiralis–Infected BALB/c Mice

BALB/c mice were each infected orally with 500 freshly isolated, stage-3 *Trichinella spiralis* larvae, as previously described (Ghildyal et al., 1992*b*, 1993). At various times after infection, the mice were killed and 2-cm lengths of jejunum were removed and fixed for analysis. For histologic examination, serial 1.5-μm-thick glycolmethacrylate-embedded sections of jejunal tissue from noninfected and from *Trichinella spiralis*–infected BALB/c mice were placed on coverslips or slides, air dried, and incubated sequentially with double-strength hematoxylin for 2 min, 1% aqueous eosin Y for 15 min, azure II for 1 min, and ethylene glycol monomethyl ether for 5 s (Beckstead et al., 1981). Alternately, sections were stained for 20 s in a 5% ethanolic solution of methylene blue (Matin et al., 1992) or toluidine blue (Befus et al., 1982).

Unlike human MC, all mouse MC that have been analyzed contain large amounts of at least one chymase in their granules. Thus, even when tissue sections are fixed, dehydrated, and embedded, MC are readily recognized in mouse tissues because of their pronounced chloroacetate esterase activity. Because intraepithelial MC in rodents do not stain intensely with cationic dyes when intestinal tissue is fixed in aldehydes (Enerbäck, 1966), a modification (Beckstead et al., 1981) of the chloroacetate esterase procedure of Leder (1979) was used to facilitate the morphometric analysis of MC in sections of jejunum. In this enzyme cytochemistry procedure, fixed sections of intestine from noninfected and helminth-infected mice were incubated at 30°C for 1 h with a solution containing naphthol AS-D chloroacetate. The tissue preparations were rinsed and counterstained with hematoxylin. In numerous control experiments carried out on serial sections of jejunum, all toluidine blue$^+$ (data not shown)/methylene blue$^+$ MC in the mucosa exhibited an intense, intracellular-localized, red reaction product when incubated with the chloroacetate esterase substrate (Fig. 2). In the jejunum of noninfected (data not shown) and *Trichinella spiralis*–infected (Fig. 2) BALB/c mice, no other cell type exhibited a chloroacetate esterase activity comparable to that of a mature MC. Polymorphonuclear leukocytes also contain chloroacetate esterase activity. However, relative to MC, these cells stain less intensely, are smaller in size, and are fewer in number in the intestine. They are not seen if the ×20 objective magnification is used to quantitate cells that contain

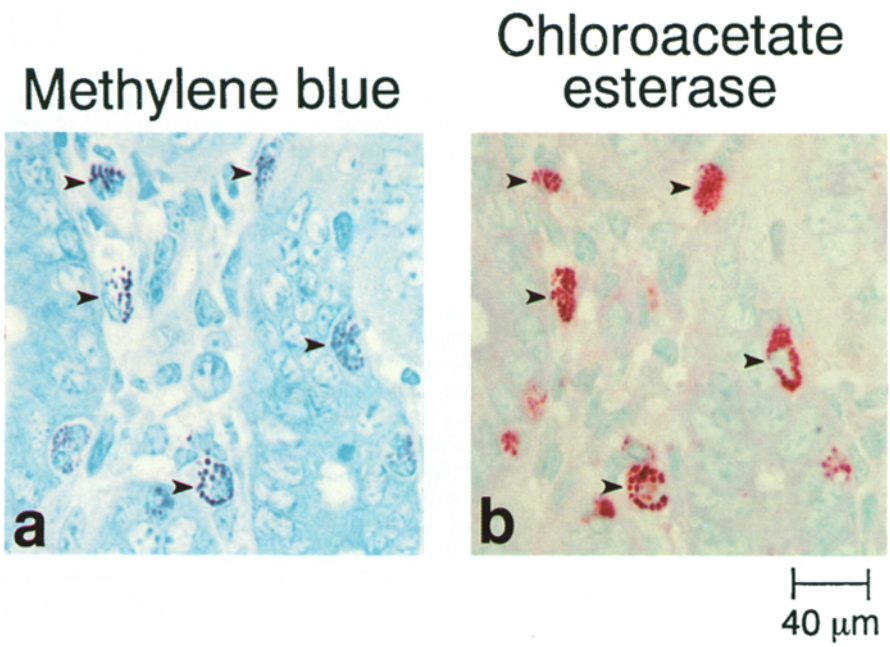


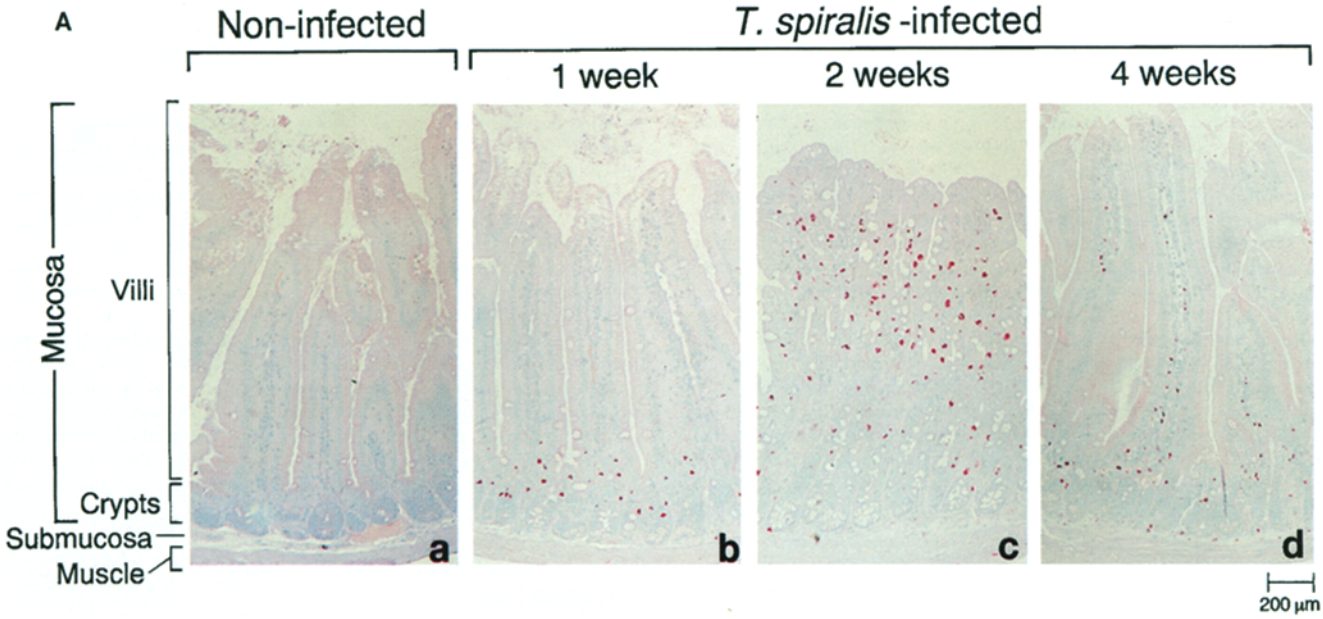
Figure 2. Histochemistry and enzyme cytochemistry of the jejunum of a *Trichinella spiralis*–infected BALB/c mouse. Serial sections of the jejunum of a helminth-infected BALB/c mouse at wk 2 were incubated with methylene blue (*a*) or the chloroacetate esterase substrate (*b*). Arrows indicate methylene blue$^+$/chloroacetate esterase $^+$MC.

chloroacetate esterase activity. Thus, for quantitating MC in the jejunum, areas were traced from paraformaldehyde-fixed, glycolmethacrylate–embedded blocks of tissue, sectioned at 1.5-μm thickness, and subjected to the chloroacetate esterase cytochemistry procedure. Using a drawing tube attachment to an optical microscope (E. Leitz, Inc., Rockleigh, NJ), the areas traced were then measured on a computer-controlled digitizing board using a digitizing program (Sigma Scan; Jandel Scientific, San Rafael, CA). MC were quantitated with a Dialux-20 optical microscope possessing a ×20 objective; the results were expressed as the number of MC/mm^2. In some instances, the numbers obtained were confirmed by high magnification examination of replicate sections stained with methylene blue or toluidine blue.

Sections of jejunum from noninfected and *Trichinella spiralis*–infected mice were stained with immunoalkaline phosphatase, as previously described (Boenisch et al., 1989; McNeil et al., 1992; Ghildyal et al., 1993). Briefly, collected tissues were fixed for 4 h at room temperature in 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.6. Alternately, selected samples were fixed in Carnoy's solution. Preparations were washed twice with PBS containing 2% DMSO and then were suspended in 50 mM NH_4Cl overnight at 4°C. The specimens were dehydrated and embedded in accordance with the JB-4 embedding kit instructions from Polysciences Inc (Niles, IL). Sections were cut on a microtome (Reichert-Jung Supracut; Leica Inc., Deerfield, IL) with glass knives and picked up on glass slides. The slides were incubated sequentially for 15 min at 37°C in 2 mM $CaCl_2$ containing 0.025% trypsin, for 15 min at room temperature in PBS containing 0.05% Tween-20 and 0.1% BSA, for 30 min at 37°C in PBS containing 0.05% Tween-20 and 4% normal goat serum, and then overnight at 4°C in 4% normal goat serum containing purified rabbit anti–mMCP-1 Ig, anti–mMCP-2 Ig (Ghildyal et al., 1993), or anti–mMCP-5 Ig (McNeil et al., 1992). The mMCP-2–specific and mMCP-5–specific antibodies were previously obtained against synthetic peptides that correspond to residues 56–71 and residues 146–162 in the respective protease. Samples were washed, incubated for 40 min at room temperature in buffer containing biotin-labeled goat anti–rabbit IgG, washed twice in 0.1% BSA and 0.05% Tween-20 in PBS, incubated for 40 min at room temperature in reagent (Vectastain ABC-AP; Vector Laboratories, Burlingame, CA), and then incubated for 15 min in the dark at room temperature in an alkaline phosphatase substrate solution. Controls consisted of sections of tissue from mice treated with nonimmune IgG (Endogen, Boston, MA) or without primary antibody. Tissue sections were counterstained with Gill's hematoxylin in 20% ethylene glycol, and then coverslips with ImmuMount (Shandon, Pittsburgh, PA) were applied.

Electron Microscopy

Electron microscopy was performed on ultrathin sections, according to



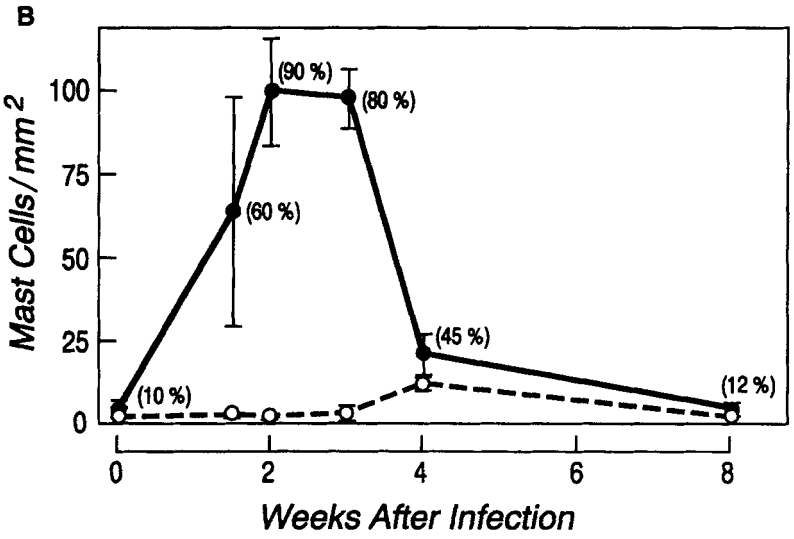


Figure 3. Enzyme cytochemistry and morphometric analysis of the MC in the jejunum of noninfected and *Trichinella spiralis*–infected BALB/c mice. (*A*) Cross sections of jejunum from a noninfected mouse (*a*) and from *Trichinella spiralis*–infected mice at 1 (*b*), 2 (*c*), and 4 (*d*) wks after infection were incubated with the chloroacetate esterase substrate. Much of the crypt region of the jejunum consists of epithelium and many of the MC at the wk-1 time point reside in the epithelium portion of the crypt. (*B*) MC per mm^2 in the submucosa and mucosa (lamina propria MC + intraepithelial MC) of noninfected mice and mice 1–8 wks after infection with *Trichinella spiralis*. The "n" values for 0, 1.5, 2, 3, 4, and 8 wks are 6, 4, 7, 2, 5, and 2, respectively. The bars represent the SD for data obtained at 0, 1.5, 2, and 4 wks and the SE for data obtained at 3 and 8 wks. The relative percentages of intraepithelial MC in the mucosa at each time point are indicated. ●, mucosa; ○, submucosa.

standard procedures (Friend and Heuser, 1981). Samples of jejunum (1 mm^3) were immersed in 2.5% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate. Each tissue sample was fixed for 4–6 h at room temperature, washed overnight in pH 7.4 buffer containing 0.1 M sodium cacodylate and 2% sucrose, and then postfixed for 2 h at 4°C in an acetate/Veronal buffer, pH 7.4, containing 1% OsO_4. The OsO_4-postfixed tissues were dehydrated in graded ethanol solutions and embedded in Epon. Sections were cut with diamond knives on the Reichert microtome, picked up on copper grids, stained with uranyl acetate and alkaline lead, and examined on an electron microscope (100CX; JEOL USA Inc., Peabody, MA) equipped with a eucentric goniometer stage. For analysis of the crystalline granules of an MC, selected sections were tilted in the y-axis ± 10°; photographs were taken at each setting.

Results

Time-dependent Change in the Number of MC in the Intestinal Mucosa of Trichinella spiralis–infected Mice

As assessed by histochemistry and by enzyme cytochemistry, only one or two MC were generally present in a 1-mm-wide section of jejunum of a noninfected BALB/c mouse (Table I; Fig. 3 *A*). After BALB/c mice were infected with *Trichinella spiralis* larvae, MC appeared in submucosa by 3 d and remained confined to the crypt area of the mucosa for the first 7 d. MC were detected in the crypts 2 wks after infection but also were numerous in the epithelium of the villi. Relative to MC in noninfected mice, the number of MC in the mucosa increased >25-fold during the height of helminth infection at 2–3 wks (Fig. 3, *A* and *B*). Accompanying this rise in MC was a marked increase in the number of goblet cells and mucuslike material in the jejunal epithelium (data not shown). Mucosal MC in the process of mitosis were occasionally found during the developmental phase but not the recovery phase of the mastocytosis (data not shown). At 4 wks, MC were located in the crypts and the lower halves of the villi (Fig. 3 *A*). However by 8 wks, the number of mucosal MC was not significantly different from that of noninfected mice (Fig. 3 *B*).

Characterization of Intestinal MC during the Development and at the Height of the Mastocytosis

The MC located in the muscle and submucosa 1–2 wks after helminth infection contained large numbers of granules that were spherical in shape and ~1 μm in diameter (Fig. 4, *a* and *d*). At this stage of the disease, lamina propria MC also contained spherical granules, but generally they were fewer in number and the majority were ~2.5 μm in diameter (Fig. 4, *b* and *e*). No crystalline granules were identified in the MC present in the muscle, submucosa, or lamina propria at the height of the mastocytosis. Intraepithelial MC contained predominantly large sized granules that were few in number (Fig. 4, *c* and *f*). However, unlike lamina propria MC, every one of the sixty intraepithelial MC examined at the ultrastructural level possessed at least one granule that exhibited an irregular stellate shape due to the presence of crystals. The crystals were detected in tissue sections prepared either for enzyme cytochemistry (Fig. 4 *c*) or electron microscopy (Fig. 4, *f–h*). At even higher magnification, the lattices of the crystals in intraepithelial MC granules were found to possess a prominent spacing of 8.3 nm. Superimposed at a 78° diagonal angle was a second prominent lattice spacing of 5.4 nm. Depending on how the crystal was tilted, the 5.4 or 8.3 nm spacing became more prominent. Thus, both spacings are part of the same unit structure.

In the jejunum of noninfected BALB/c mice, the sparse MC in the submucosa and muscle expressed mMCP-5 alone and the sparse MC in the epithelium expressed both mMCP-1 and mMCP-2 (Table I). No MC were detected at either site which expressed mMCP-1 alone, mMCP-2 alone,

*Table I. Quantitation of MC Exhibiting Specific mMCP Phenotypes in Cross Sections of the Jejunum of Noninfected and Trichinella spiralis–infected Mice**

Location and stage of infection	MC number examined	mMCP-1 alone	mMCP-1 mMCP-2	mMCP-2 alone	mMCP-2 mMCP-5	mMCP-5 alone
Noninfected						
Mucosa epithelium	15	0	5.0 ± 3.6	0	0	0
Mucosa lamina propria	0	0	0	0	0	0
Submucosa	15	0	0	0	0	5.0 ± 1.4
Infected 1 wk						
Mucosa epithelium	253	0	81 ± 29	4.0 ± 2.9	0	0
Mucosa lamina propria	25	0	0.33 ± 0.47	6.7 ± 5.0	1.0 ± 0.82	0.33 ± 0.47
Submucosa	31	0	0	0	1.0 ± 0.82	9.3 ± 4.1
Infected 2 wk						
Mucosa epithelium	735	0	230 ± 37	13 ± 3.3	0	0
Mucosa lamina propria	61	0	0.33 ± 0.05	15 ± 4.1	3.0 ± 1.4	2.3 ± 0.47
Submucosa	34	0	0	0.33 ± 0.47	0.66 ± 0.47	10 ± 0.94
Infected 4 wk						
Mucosa epithelium	172	0	49 ± 6.7	8.3 ± 1.7	0	0
Mucosa lamina propria	146	0	0.30 ± 0.50	27 ± 6.5	13 ± 0.82	8.7 ± 2.1
Submucosa	106	0	0	2.0 ± 1.6	4.7 ± 2.5	29 ± 6.2

*Serial cross sections (~2 μm) of jejunum were sequentially stained with anti–mMCP-1 Ig, anti–mMCP-2 Ig, and anti–mMCP-5 Ig, and then the five distinct protease phenotypes were quantitated. The depicted results are the mean ± SD of three mice examined at each time point. All epithelial MC found in the crypts express both mMCP-1 and mMCP-2, whereas some of the epithelial MC in the villi express only mMCP-2. At wk 4, lamina propria MC that only express mMCP-2 tend to reside in the tips of the villi, whereas those thay only express mMCP-5 tend to be located in the crypts.

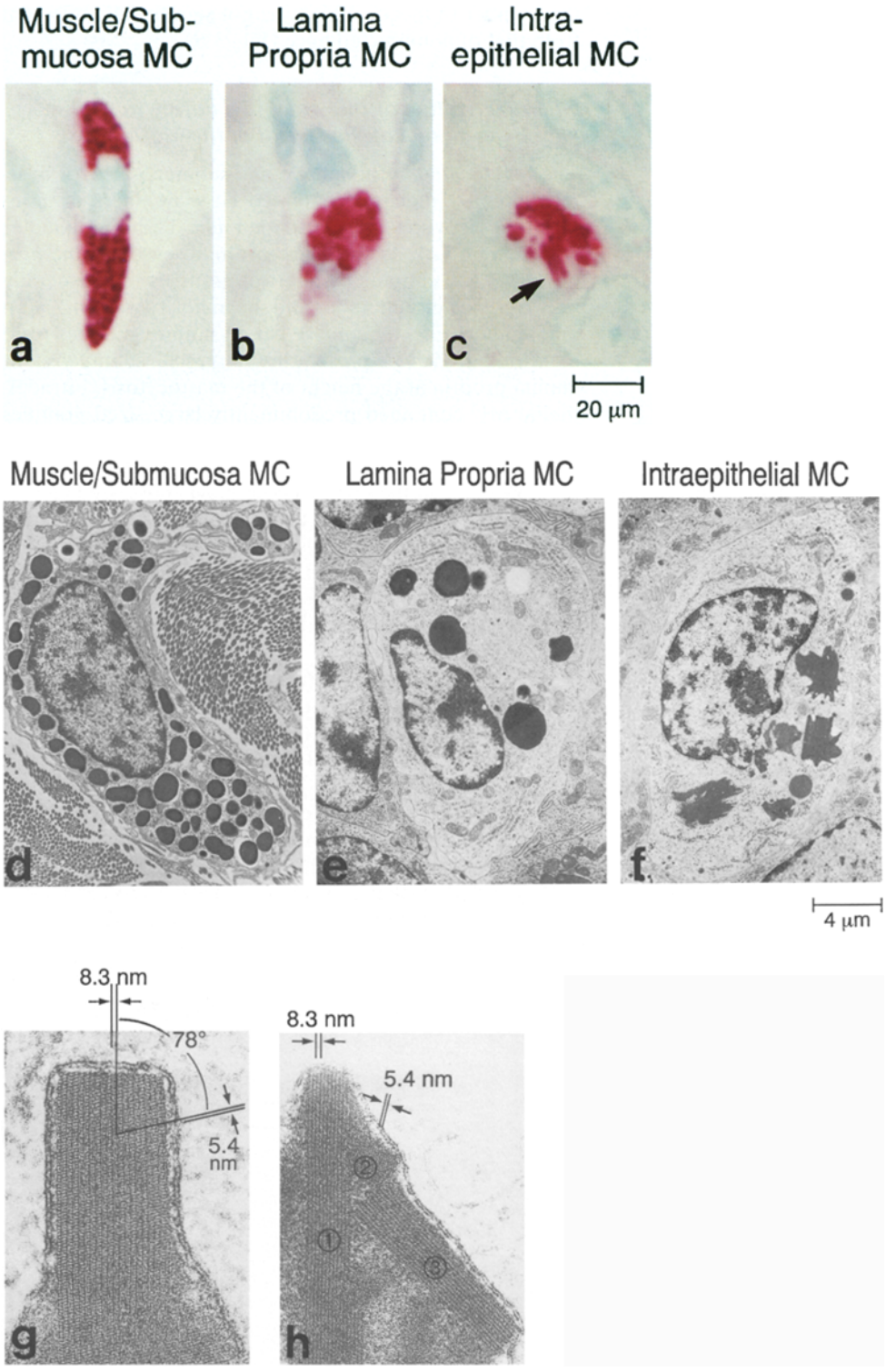


Figure 4. Enzyme cytochemistry and ultrastructure of the MC that reside in different locations in the jejunum of BALB/c mice 2 wks after infection with *Trichinella spiralis*. In *a*, *b*, and *c* are MC that contain chloroacetate esterase activity in the muscle/submucosa, lamina propria, and epithelium, respectively. The arrow in *c* indicates an irregular shaped granule in an intraepithelial MC. In *d*, *e*, and *f* are electron micrographs of representative MC that reside in the muscle/submucosa, lamina propria, and epithelium, respectively, at the same magnification. In panels *g* and *h* at higher magnification are electron micrographs of the stellate crystals in two intraepithelial MC. The circled numbers in *h* mark three randomly oriented, distinct crystals in the same stellate granule.

or which coexpressed mMCP-2 and mMCP-5. 1 wk after *Trichinella spiralis* infection, when the majority of MC are in the crypts, those in the epithelium tended to coexpress mMCP-1 and mMCP-2, while those in the lamina propria tended to express just mMCP-2 (Table I). The majority of the MC in the submucosa continued to express only mMCP-5, but now MC were occasionally found at this location and in the lamina propria that coexpressed mMCP-2 and mMCP-5. Thus, the presence of MC in the lamina propria and epithelium that express only mMCP-2 and MC in the lamina propria and submucosa that coexpress mMCP-2 and mMCP-5 are the two most marked phenotypic changes associated with the helminth-induced mastocytosis. Although there are considerably more MC in the jejunum at wk 2, the MC that presented at the varied locations possessed chymase phenotypes similar to that obtained at wk 1 (Table I). Most of the intraepithelial MC in the villi (Fig. 5, *a–c*) and crypts (Fig. 5, *g–i*) 2 wk after *Trichinella spiralis*

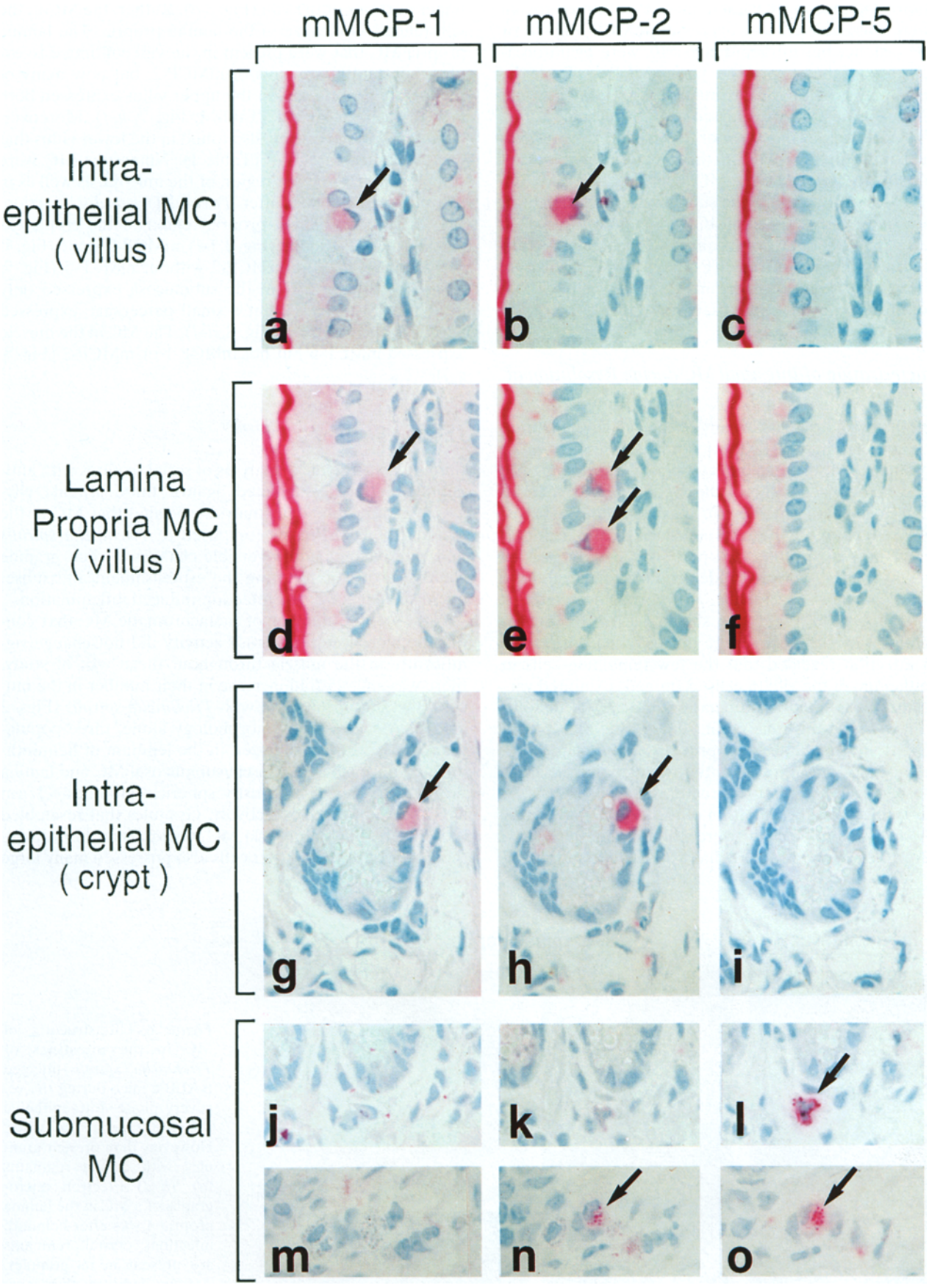


Figure 5. Immunohistochemistry of the MC in the intestinal villi at the height of the mastocytosis that occurs after *Trichinella spiralis* infection. 2 wks after infection, 1.5-μm serial sections of jejunum (*a–c*, *d–f*, *g–i*, *j–l*, and *m–o*) were stained with anti–mMCP-1 Ig (*a*, *d*, *g*, *j*, and *m*), anti–mMCP-2 Ig (*b*, *e*, *h*, *k*, and *n*), or anti–mMCP-5 Ig (*c*, *f*, *i*, *l*, and *o*). Arrows indicate immunoreactive MC. The red reaction product on the brush borders of the villi (*a–f*) is due to the endogenous intestinal alkaline phosphatase. The presence of this product indicates that the color substrate was active in these immunohistochemical reactions.

infection contained mMCP-1 and mMCP-2. Intraepithelial MC were not detected that expressed mMCP-5 alone or mMCP-1 alone, but intraepithelial MC were again occasionally found that expressed just mMCP-2 (Table I). Some lamina propria MC expressed both mMCP-1 and mMCP-2 but most only expressed mMCP-2 (Table I; Fig. 5, *d–f*). Moreover, at this stage of the mastocytosis, ~15% of the lamina propria MC in the crypts also possessed granules that contained both mMCP-2 protein and mMCP-5 protein. Most of the MC in the submucosa at the height of the infection expressed just mMCP-5 (Table I; Fig. 5, *j–l*). However, a few submucosa-localized MC possessed granules that contained both mMCP-2 and mMCP-5 (Fig. 5, *m–o*). The MC that reside in the muscle expressed mMCP-5, but not mMCP-1 or mMCP-2 (data not shown).

Characterization of Intestinal MC during Resolution of the Mastocytosis

During the recovery phase, many of the MC in the lamina propria and submucosa of the jejunum possessed only a few granules. Moreover, the cytoplasm became lightly metachromatic when some of these cells were stained by toluidine blue (data not shown), suggesting that many of the MC remaining in the intestine during the resolution phase of the mastocytosis had partially degranulated or had begun to turn over their older granules intracellularly (data not shown). Occasionally, an MC was detected undergoing apoptosis (data not shown). Electron microscopic analysis of the MC in the mucosa 4 wks after helminth infection revealed that the few remaining cells in the epithelium of the villi (8 cells examined) exhibited various stages of granule fragmentation and crystal dissolution (Fig. 6 *a*). At this time point, some of the MC (6 cells examined) in the lamina propria also contained fragmented crystals and a few slightly irregular-shaped granules possessing ~8-nm lattice structures (Fig. 6, *b–d*).

Four weeks after the initiation of the *Trichinella spiralis* infection, intraepithelial MC were still found in the crypts. However, very few intraepithelial MC were found in the upper portions of the villi (Fig. 3 *d*). Rather, the MC in the villi tended to localize in the lamina propria. The lamina propria MC that were present in the villi continued to express substantial amounts of mMCP-2, but now many of the lamina propria MC in the upper villus expressed both mMCP-2 and mMCP-5 (Table I; Fig. 7, *a–f*). Moreover, lamina propria MC were also found in the lower villus that expressed just mMCP-5 (Table I). Numerous MC were still present in the crypt region of the mucosa, as well as in the submucosa 4 wks after *Trichinella spiralis* infection. The MC in the lamina propria region of the crypts expressed mMCP-5 alone (Fig. 7, *j–l*), mMCP-2 alone (Fig. 7, *g–i*), or mMCP-1 and mMCP-2 without mMCP-5 (Fig. 7, *g–i*). Most of the MC in the submucosa expressed only mMCP-5 (Fig. 8, *a–c*), but a small percentage expressed mMCP-2 and mMCP-5 (Fig. 8, *d–f*). The MC in the muscle expressed mMCP-5 but not mMCP-1 or mMCP-2 (Fig. 8, *g–i*).

Discussion

Immunohistochemical analyses of serial sections with antipeptide antibodies directed against three granule chymases and morphologic analysis revealed that MC in the jejunum of BALB/c mice exposed to *Trichinella spiralis* undergo dynamic and reversible changes in their granule chymase expression during the establishment and subsequent subsidence of the infection-induced inflammation.

Although the number of metachromatic MC that contained chloroacetate esterase activity did not change significantly in the muscle throughout the 8 wks of study, there was an >25-fold increase in their number in the mucosa 2 wks after infection with *Trichinella spiralis* (Figs. 2 and 3). As assessed by morphology alone, three populations of MC were recognized in the jejunum of helminth-infected mice (Fig. 4). Muscle/submucosal MC and lamina propria MC possessed mostly spherical granules ~1 μm and 2.5 μm in size, respectively. Granules that resembled those in the lamina propria MC were present in intraepithelial MC, but these latter cells also possessed many large

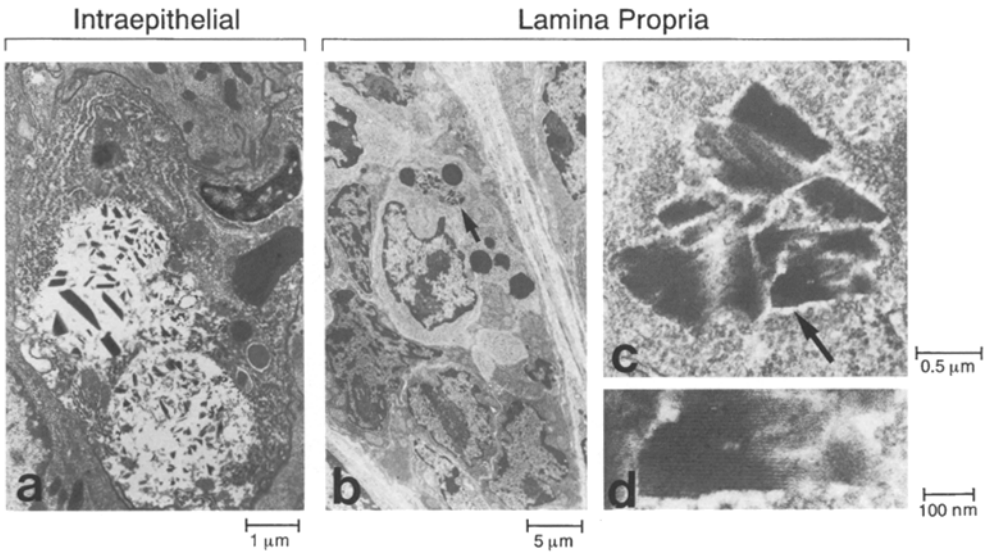


Figure 6. Ultrastructure of MC in the intestines of *Trichinella spiralis*–infected BALB/c mice during the recovery phase. Intraepithelial MC are rare in the villus. However, they present granules with crystal remnants (*a*). (*b–d*) Electron micrographs of a MC in the lamina propria 4 wks after helminth infection; crystal remnants are present in its granules. Arrows indicate the same granule at incremental magnifications.

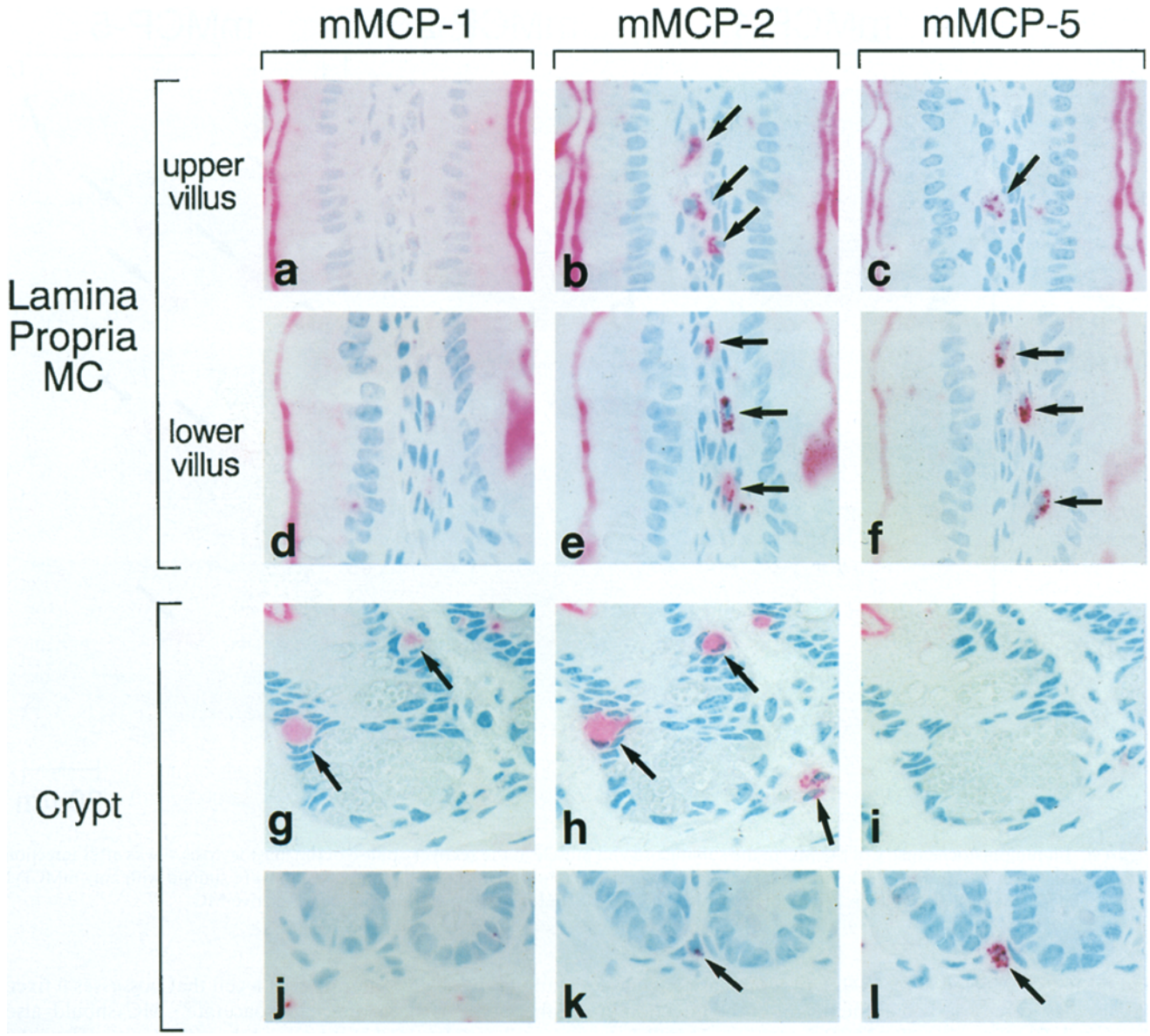


Figure 7. Immunohistochemistry of the MC in the lamina propria and crypts of the jejunum at the recovery phase of the mastocytosis that occurs after *Trichinella spiralis* infection. 4 wks after infection, 1.5-μm-thick serial sections of the upper villus region of the lamina propria (*a–c*), the lower villus region of the lamina propria (*d–f*), and the crypts (*g–i* and *j–l*) were stained with anti–mMCP-1 Ig (*a*, *d*, *g*, and *j*), anti–mMCP-2 Ig (*b*, *e*, *h*, and *k*), or anti–mMCP-5 Ig (*c*, *f*, *i*, and *l*). Arrows indicate immunoreactive MC.

sized granules with stellate crystals. Although no other MC type in the BALB/c mouse contains crystalline structures in its granule, Crowle and Phillips (1983) also found irregularly shaped granules in some of the jejunal MC in the mucosa of *Nippostrongylus brasiliensis*–infected Beige (bg/bg) mice and their normal C57BL/6 mouse littermates. Likewise, Murray et al. (1968) found irregular shaped granules with paracrystalline structures in the intraepithelial MC of *Nippostrongylus brasiliensis*–infected rats. The unique crystallization of the preformed mediators in the BALB/c mouse intraepithelial MC, therefore, is not unique to this mouse strain nor is it dependent on the type of helminth that infects the intestine. The lattices of the granule crystals in a BALB/c mouse intraepithelial MC possess a prominent spacing of 8.3 nm. Superimposed in the same unit structure at a 78° diagonal angle is a second prominent lattice spacing of 5.4 nm. The crystalline structures (e.g., scrolls, gratings, and lattices) observed in human MC differ in that they have 15- and 7.5-nm spacings (Caulfield et al., 1980). Murray et al. (1968) proposed that the lamina propria MC and intraepithelial MC in the jejunum of *Nippostrongylus brasiliensis*–infected rats are developmentally related. If they also are developmentally related in the *Trichinella spiralis*–infected BALB/c mouse, the crystalline structures observed in latter population of MC probably are a consequence of direct contact of these MC with the epithelium or the basement membrane.

With the use of a newly derived anti-peptide antibody specific for mMCP-1 (Fig. 1) and previously generated anti-peptide antibodies to mMCP-2 (Ghildyal et al., 1993)

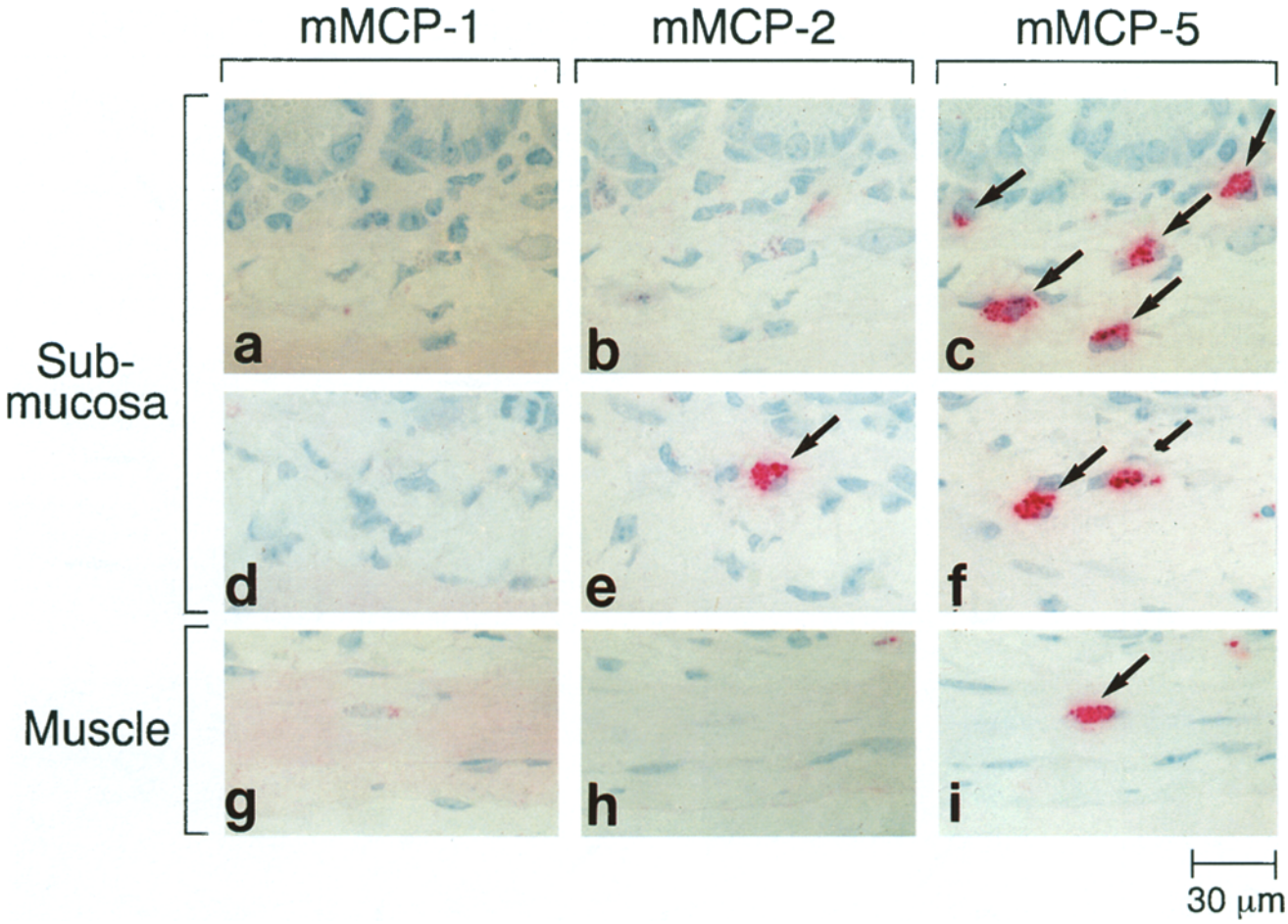


Figure 8. Immunohistochemistry of the MC in the submucosa and muscle at the recovery phase of the mastocytosis. 4 wks after infection with *Trichinella spiralis*, 1.5-μm-thick serial sections of the submucosa (*a–c* and *d–f*) and the muscle (*g–i*) were stained with anti–mMCP-1 Ig (*a*, *d*, and *g*), anti–mMCP-2 Ig (*b*, *e*, and *h*), or anti–mMCP-5 Ig (*c*, *f*, and *i*). Arrows indicate immunoreactive MC.

and mMCP-5 (McNeil et al., 1992), predominant chymase phenotypes were observed at the height of the infection in the muscle that expressed mMCP-5 without mMCP-1 or mMCP-2 and in the epithelium that expressed mMCP-1 and mMCP-2 without mMCP-5 (Fig. 5). Accompanying these two MC populations, however, were transitional cells in the submucosa and lamina propria that expressed mMCP-2 and mMCP-5 without mMCP-1, and in the lamina propria that expressed mMCP-2 alone. The protease phenotype data could be explained, in part, by a local proliferation of varied cell types that are each irreversibly programmed to express particular protease phenotypes. However, the diversity of chymase expression in the jejunal MC of *Trichinella spiralis*–infected BALB/c mice, including the appearance of phenotypes not appreciated in noninfected mice, suggests that this possibility is unlikely. The presence of MC in the same section of the lamina propria 2 wks after infection that express mMCP-2 with or without mMCP-1 and in the same section of the submucosa that express mMCP-5 with or without mMCP-2 (Fig. 5; Table I) suggests that the specific microenvironment may require some time interval to provide a particular chymase phenotype. As depicted in Fig. 3 *A*, increased numbers of MC are first seen in the submucosa/crypt area of the jejunum. If local proliferation of a cell that possesses a fixed phenotype is occurring, chloroacetate$^+$ MC should also have been detected in the tips of the villi at wk 1. Thus, the scenario that best describes the obtained data is that MC-committed progenitors undergo varied rates of in situ differentiation as they traverse the jejunum. In such an interpretation of infection/inflammation-dependent modulation, a MC that resides initially in the muscle/submucosa region of the jejunum has the ability to move into the lamina propria and then into the epithelium. It is unlikely that all epithelial MC found in the tips of the villi at the height of helminth infection are derived from progenitors that initially resided in the submucosa. Some epithelial MC could be derived from progenitors that originally resided in, or homed to, the lamina propria. Because MC in the process of mitosis were occasionally found, it is possible that migrating MC are proliferating at the same time they are changing their phenotypes during the inductive phase of the mastocytosis. However, if a submucosal MC eventually makes its way into the epithelium, it sequentially expresses mMCP-2, ceases expressing mMCP-5, and finally expresses mMCP-1. The concept that a nontransformed MC can change its phenotype in the jejunum is in agreement with data obtained from the V3 mastocytosis mouse (Gur-

ish et al., 1995). The interpretation also is consistent with the data obtained from the recovery phase of the mastocytosis observed in the *Trichinella spiralis*–infected mouse.

Intraepithelial MC disappeared shortly after the peak of the mastocytosis. This was followed by a progressive loss of mature lamina propria MC from the upper regions of the villi. Some of the MC appeared to be undergoing apoptosis in the jejunum during the recovery phase of the mastocytosis. However, the presence of MC in the lamina propria 4 wks after infection that had remnants of crystals in its granules (Fig. 6 *b*) suggested that some of the MC in the epithelium at 2 wks not only had moved into the lamina propria but also had begun to metabolize their granule constituents. During this recovery phase, the lamina propria MC continued to express mMCP-2, but generally in association with mMCP-5 rather than mMCP-1 (Fig. 7; Table I). Many of the MC in the submucosa expressed mMCP-2 and mMCP-5, whereas the remainder expressed only mMCP-5, like the MC in the muscle (Fig. 8; Table I). These findings suggest that during the recovery phase of the infection, MC sequentially cease expressing mMCP-1, express mMCP-5, and finally cease expressing mMCP-2 as they present in the tips of the villi, the base of the villi, and the submucosa, respectively. The changes in chymase expression in the various populations of MC are depicted schematically in Fig. 9.

If MC in the submucosa 4 wks after infection are indeed derived from those in the lamina propria and epithelium at 2 wks, it remains to be determined how they are able to selectively metabolize their mMCP-1 and mMCP-2. Many of the MC in the jejunum at 4 wks exhibit some of the histologic and morphologic features of degranulated and/or activated MC (Fig. 6). Although particular proteases could be lost through selective exocytosis if they were sequestered in different granules, a more likely explanation for the change in chymase expression is that factors in the microenvironment instruct the newly arrived MC to selectively stop expressing a particular chymase. This regulation could occur at the level of gene transcription but recent in vitro studies on nontransformed BALB/c mouse bone marrow–derived mouse MC suggest that it probably occurs at the level of mRNA stability. Treatment of these MC with interleukin-10 causes them to rapidly express high steady-state levels of mMCP-2 mRNA (Ghildyal et al., 1993), primarily by inducing the expression of a *trans*-acting factor that prevents the degradation of the mMCP-2 transcript (Xia et al., 1996). Presumably, the intracellular level of an immunoreactive chymase gradually falls because the cell continues to degranulate or continues to internally metabolize its older granules.

Different cationic dyes and fixation techniques have been developed by Enerbäck (1966) and others to distinguish granules in rat and mouse MC that contain heparin glycosaminoglycans from those that do not. Because MC with different stainable granules have not been detected in a single tissue microenvironment, it was concluded that if a mature MC is able to change its granule phenotype it probably dedifferentiates to an unrecognizable agranular cell before acquiring its new phenotype (Kitamura et al., 1984). Adoptive transfer studies with the v-*abl*–immortalized V3 MC line seemed to support this concept. V3 MC express mMCP-5 but not mMCP-1 or mMCP-2. 6 d after V3 MC were adoptively transferred into BALB/c mice, agranular cells were detected in the lamina propria which contained immunoreactive *abl* protein but no immunoreactive mMCP (Gurish et al., 1995). By 2 wks, most of the V3 MC in this location expressed mMCP-1 and mMCP-2 but not mMCP-5. The immunohistochemical recognition of MC in the lamina propria in the recovery phase of the *Trichinella spiralis*–induced mastocytosis that contain both mMCP-2 and mMCP-5 or just mMCP-2 (Fig. 7) now suggests that a mature MC need not become an agranular, undifferentiated cell before it begins to acquire its new phenotype. Whatever the mechanism by which jejunum MC modulate their chymase expression, it is now clear that conclusions about the phenotype, and thereby function, of

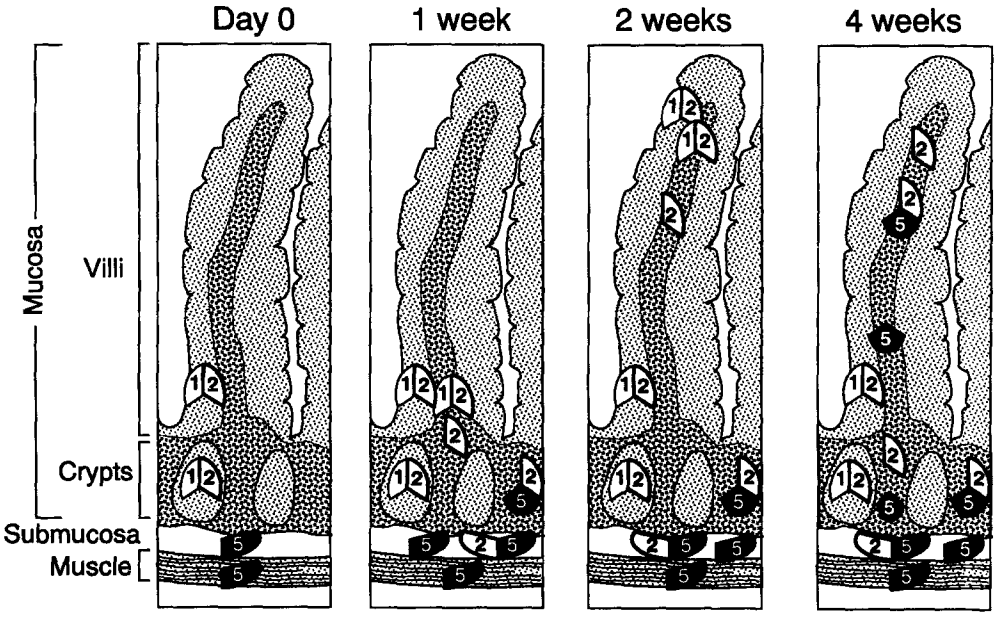


Figure 9. Schematic representation of the chymase phenotypes of the MC that reside at different locations in the jejunum of noninfected and helminth-infected BALB/c mice. The numbers 1, 2, and 5 in the diagrams refer to mMCP-1, mMCP-2, and mMCP-5, respectively. The intent of this figure is to show schematically the types of MC that reside at different sites in the jejunum during helminth infection. The figure does not adequately portray the quantitative relationships of the different types of MC which are presented in Table I. ▩, lamina propria; ▨, epithelium.

a MC cannot be made solely on its tissue location during an inflammatory response.

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