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Expression of Mast-Cell-Specific Proteases in Tissues of Mice Studied by *in Situ* Hybridization

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The protease mRNA expression phenotype of individual mast cells was studied by* in situ *hybridization. Mouse mast cell protease (MMCP)-2 mRNA was expressed by mast cells located in the mucosa of the stomach of WB-+/+ and (WB × C57BL/6)F_1-+/+ (hereafter WBB6F_1-+/+) mice but not by mast cells in the same tissue of C57BL/6-+/+ mice. Even in the stomach of WBB6F_1-+/+ mice, mast cells located in the muscularis propria did not express MMCP-2 mRNA. The mRNAs of MMCP-4 and mouse mast cell carboxypeptidase A were not expressed by mast cells in the stomach mucosa of untreated WBB6F_1-+/+ mice but were expressed after the infection of* Strongyloides venezuelensis. *We examined whether MMCP-2 mRNA expression varied by changing environments of mast cells. Cultured mast cells of WBB6F_1-+/+ mice that expressed MMCP-2 mRNA were transplanted into the stomach wall of genetically mast-cell-deficient WBB6F_1-W/W^v mice. Mast cells that appeared in the mucosa expressed the MMCP-2 mRNA, but mast cells that appeared in the muscularis propria did not, indicating the adaptation of cultured mast cells into a new environment. In contrast to cultured mast cells, peritoneal mast cells of WBB6F_1-+/+ mice that expressed MMCP-2 mRNA as well did not adapt to the muscularis propria of WBB6F_1-W/W^v mice. The MMCP-2 mRNA remained to be expressed after the settlement in either the mucosa or the muscularis propria. Furthermore, the peritoneal mast cells did not change the MMCP-4 and MMCP-6 mRNA expression phenotype after the settlement in either the mucosa or the muscularis propria of WBB6F_1-W/W^v mice. The present result indicated that both intracellular factors such as strain specificity and source of mast cells and extracellular factors such as tissue specificity and helminth infection influenced the protease expression phenotypes. (Am J Pathol 1997, 150:1373–1382)

Seven distinct serine proteases that are designated mouse mast cell protease (MMCP) 1 through 7 and mouse mast cell carboxypeptidase A (MC-CPA) have been identified in the secretory granules of different populations of mouse mast cells by protein sequencing and/or by cDNA cloning.[1–6] Factors that influence the expression of mRNA of these proteases have been studied by Northern blotting analysis.[1–6] As relatively large numbers of homogeneous mast cell populations are necessary for Northern blotting analysis, cultured mast cells (CMCs) are used as the material of most experiments.[1–6] Peritoneal mast cells (PMCs) can be obtained by pure suspensions, but many mice should be sacrificed to harvest sufficient numbers of pure PMCs for the Northern analysis. As MMCPs and MC-CPA appear to be expressed only by mast cells, tissue specimens containing mast cells may be used as the material for Northern analysis of these mast-cell-specific proteases.[7] However, there is a possibility that protease expression phenotypes of mast cells within a tissue may not be homogeneous.[8] In fact, histochemical and electron microscopic features of mast cells are different among mast cells within the dermis of rats and mice.[9,10] As we have shown that the *in situ* hybridization histochemistry is applicable for the analysis of protease expression phenotypes of mast

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cells,[8,11] we used this technique instead of the Northern blotting in the present study.

We induced changes in histochemical features of mast cells by transplanting CMCs or PMCs of WBB6F$_1$-+/+ mice into tissues of mast-cell-deficient WBB6F$_1$-*W/W^v* mice.[12] When CMCs were injected into the peritoneal cavity of WBB6F$_1$-*W/W^v* mice, berberine-sulfate-negative CMCs changed to berberine-sulfate-positive mast cells, suggesting that chondroitin-sulfate-containing CMCs may change to heparin-containing PMCs.[12] Chemical analysis of proteoglycan confirmed this change.[13] On the other hand, when berberine-sulfate-positive PMCs were injected into the stomach wall of WBB6F$_1$-*W/W^v* mice, mast cells that developed in the mucosa were berberine sulfate negative whereas mast cells that developed in the muscularis propria remained to be berberine sulfate positive.[14] This suggested that heparin-containing PMCs may change to chondroitin-sulfate-containing mucosal mast cells. In the present study, we examined whether protease expression phenotypes of CMCs and PMCs of WBB6F$_1$-+/+ mouse origin may change after the transplantation into the stomach wall of WBB6F$_1$-*W/W^v* mice.

Materials and Methods

Mice

WB-+/+ and C57BL/6-+/+ mice were raised in our laboratory, and (WB × C57BL/6) F$_1$-*W/W^v* and -+/+ mice (hereafter called WBB6F$_1$-*W/W^v* and -+/+ mice) were purchased from the Japan SLC (Hamamatsu, Japan). WBB6F$_1$-*W/W^v* mice are genetically deficient in mast cells.[15] Mice were used at 2 to 6 months of age.

Establishment of CMCs and Purification of PMCs

Pokeweed-mitogen-stimulated spleen-cell-conditioned medium (PWM-SCM) was prepared as described by Nakahata et al.[16] To obtain CMCs, bone marrow cells were harvested from 2-month-old WB-+/+, C57BL/6-+/+, and WBB6F$_1$-+/+ mice. Culture flasks (Nunc, Roskilde, Denmark) containing 1 × 10^6/ml bone marrow cells in α-minimal essential medium (α-MEM; ICN Biomedicals, Costa Mesa, CA) supplemented with 10^{-4} mol/L 2-mercaptoethanol, 10% PWM-SCM and, 10% fetal calf serum (Nippon Bio-Supplement Center, Tokyo, Japan) were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. One-half of the medium was replaced every 7 days, and more than 95% of cells were CMCs 4 weeks after the initiation of the culture.[12] Purification of PMCs from mice were performed according to the method described by Yurt et al.[17]

Northern Blot Analysis

Total RNA was prepared from CMCs of WB-+/+, WBB6F$_1$-+/+, or C57BL/6-+/+ mouse origin. The cDNA probes for MMCP-2, MMCP-6, and MC-CPA were prepared in our laboratory.[8,18] Northern blot analysis was performed using MMCP-2, MMCP-6, MC-CPA, and mouse β-actin cDNAs labeled with α-[^{32}P]dCTP (DuPont/NEN Research Products, Boston, MA; 10 mCi/ml) as probes. After hybridization at 42°C, blots were washed to a final stringency of 0.2× standard saline citrate (150 mmol/L NaCl, 15 mmol/L trisodium citrate, pH 7.4) at 50°C and subjected to autoradiography.

Preparation of Probes for in Situ *Hybridization*

Total RNA extracted from CMCs of WBB6F$_1$-+/+ mice was used as a template, and the single-strand cDNA was synthesized with an antisense primer by reverse transcriptase (Takara Shuzou, Kyoto, Japan). cDNAs for MMCP-2, -4, and -6 and MC-CPA were amplified by a Perkin-Elmer Cetus (Norwalk, CT) DNA thermal cycler using *Taq* DNA polymerase (Takara Shuzou).[18] The products were subcloned into the *Eco*RV site of Bluescript KS (−) plasmid (Stratagene, La Jolla, CA), and the sequence was confirmed by model 373A DNA sequencer (Applied Biosystems, Foster City, CA) according to the method of Sanger et al.[19] This plasmid was either linealized with *Hin*dIII and transcribed with T7 RNA polymerase to generate an antisense probe or linealized with *Eco*RI and transcribed with T3 RNA polymerase to generate a sense probe.

In Situ *Hybridization*

CMCs or PMCs (10^5) were collected, washed with phosphate-buffered saline, and mixed with 2% agarose (FMC BioProducts, Rockland, ME). The mixture was fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) overnight and embedded in paraffin. Tissues were also fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) and embedded in paraffin. Serial sections (4 μm thick) were cut; sections of odd numbers were used to identify mast cells by staining alcian blue and

nuclear fast red, and sections of even numbers were used for *in situ* hybridization. Hybridization was carried out as described elsewhere with minor modifications.[20] Digoxigenin-labeled single-strand RNA probes were prepared using a DIG RNA labeling kit (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) according to the manufacturer's instructions.

Controls included 1) hybridization with the sense probe, 2) RNAse A treatment (20 μg/ml) before hybridization, and 3) use of neither an antisense RNA probe nor an anti-digoxigenin antibody.[20] None of the three controls showed any positive signals.

Proportion of Protease mRNA-Expressing Mast Cells

Number of alcian-blue-positive mast cells and numbers of MMCP-2, MMCP-4, MMCP-6, or MC-CPA mRNA-expressing cells were counted in the adjacent sections. Because in some cases numbers of mast cells counted in a pair of adjacent sections was few, data of many pairs of adjacent sections were pooled.

Infection of Helminth

A strain of *Strongyloides venezuelensis* used in this study was originally isolated from a wild brown rat in Okinawa, Japan, established as a laboratory strain,[21] and is now maintained in the Miyazaki Medical College with serial passages in Wistar rats.[22] Third-stage infective larvae (L3) were obtained from fecal culture by the filter paper method.[22] The degree of infection was monitored daily by egg excretion in feces (EPG) from five animals. WBB6F$_1$-+/+ mice were infected by subcutaneous injections of 1000 L3. Mice were killed on day 12, and the jejunum was removed and used for *in situ* hybridization histochemistry.

Transplantation of CMCs or PMCs

Recipient WBB6F$_1$-*W/W^v* mice were anesthetized with Nembutal; the peritoneal cavity was opened, and the stomach was exposed. CMCs or PMCs from WBB6F$_1$-+/+ or C57BL/6-+/+ mice were counted in a standard hemocytometer and were injected into the wall of the glandular stomach of WBB6F$_1$-*W/W^v* mice.[14] Cells (10^5) suspended in 0.1 ml of α-MEM were injected with a tuberculin syringe. Each mouse received two injections that were marked by tattooing both sides with India ink. WBB6F$_1$-*W/W^v* mice were killed 5 or 10 weeks after the injections. Injection sites that could be identified by the presence of India ink were removed. Serial sections were made, and one section was stained with alcian blue and nuclear fast red and another section was used for *in situ* hybridization.

Results

CMCs were obtained from mice of WB-+/+, C57BL/6-+/+, and WBB6F$_1$-+/+, and the expression of MMCP-2, MMCP-6, and MC-CPA was examined by Northern blotting. The expression of MMCP-6 and MC-CPA was comparable among CMCs of the examined mouse strains, but the expression of MMCP-2 was significantly greater in CMCs of WB-+/+ and WBB6F$_1$-+/+ mouse origin than in CMCs of C57BL/6-+/+ mouse origin (Figure 1A). We then examined the MMCP-2 mRNA expression in the stomach of WBB6F$_1$-+/+ and C57BL/6-+/+ mice by *in situ* hybridization. The MMCP-2 mRNA was expressed by mast cells located in the mucosa of WBB6F$_1$-+/+ mice but not by those of C57BL/6-+/+ mice (Figure 1B). Mast cells are present not only in the mucosa but also in the muscularis propria of the stomach. In contrast to the mast cells located in the mucosa, those of the muscularis propria did not express MMCP-2 mRNA even in the WBB6F$_1$-+/+ mice (data not shown). The expression of the MMCP-2 mRNA was influenced by both mouse strains and tissue environments.

Enhanced expression of mast cell proteases in the mucosa of the small intestine has been reported after the infection of helminths.[2,3,23] The enhancement may be attributable to the increase in number of mast cells in the mucosa. We used the *in situ* hybridization histochemistry to examine whether the expression of mast cell proteases changed after the infection in individual mast cell levels. WBB6F$_1$-+/+ mice were infected with *S. venezuelensis*, and the expression of MMCP-2, MMCP-4, MMCP-6, and MC-CPA was examined. Mast cells in the jejunal mucosa expressed only MMCP-2 mRNA before the infection (Figure 2A). The number of mast cells increased remarkably after the infection, and the increased mast cells expressed MMCP-2 (Figure 2B), MMCP-4 (Figure 2C), and MC-CPA (Figure 2D) mRNAs. As the number of mast cells in the jejunal mucosa of uninfected mice was small, there is a possibility that mast cells expressing MMCP-4 or MC-CPA mRNA could not be recognized. We examined the expression of these proteases in the mast cells in the stomach mucosa of uninfected animals, in which more

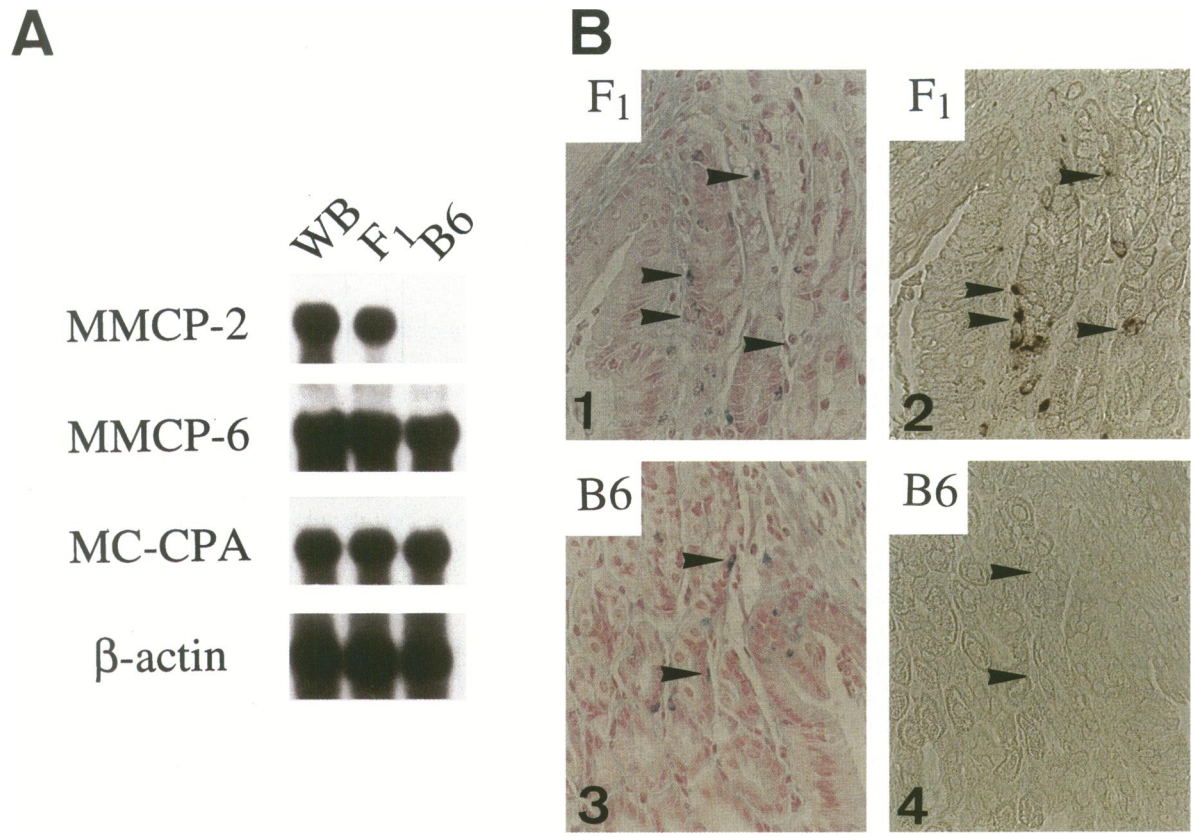

Figure 1. A: *Expression of MMCP-2, MMCP-6, and MC-CPA mRNA transcripts in CMCs of WB-+/+, WBB6F$_1$-+/+, and C57BL/6-+/+ mouse origin. Total RNA was extracted from CMCs of WB-+/+, WBB6F$_1$-+/+, and C57BL/6-+/+ mice, and 20 μg of total RNA was electrophoresed and hybridized with MMCP-2, MMCP-6, or MC-CPA probe.* **B:** *Expression of MMCP-2 mRNA in the stomach mucosa.* **1:** *Stomach mucosa of WBB6F$_1$-+/+ mice stained with alcian blue and nuclear fast red.* **2:** *An adjacent section of* **1**. *The expression of MMCP-2 mRNA is demonstrated by* in situ *hybridization.* **3:** *Stomach mucosa of C57BL/6-+/+ mice stained with alcian blue and nuclear fast red.* **4:** *An adjacent section of* **3**. *No mast cells expressed MMCP-2 mRNA.* **Arrowheads** *indicate the same mast cells in the paired photographs. Magnification, ×400.*

mast cells were detectable. Mast cells in the stomach mucosa of uninfected animals did not express MMCP-4 and MC-CPA mRNAs either (Table 1), suggesting that the expression of MMCP-4 and MC-CPA mRNAs was induced by the infection of *S. venezuelensis* in mast cells of the jejunal mucosa. The MMCP-6 mRNA was expressed neither before nor after the infection by mast cells in the jejunal mucosa of WBB6F$_1$-+/+ mice (Figure 2E). The expression of MMCP-2 mRNA was also examined in C57BL/6-+/+ mice after the infection. Although the number of mast cells remarkably increased in the jejunal mucosa of C57BL/6-+/+ mice after the infection, these mast cells did not express the MMCP-2 mRNA even after the infection (Figure 2F).

Mast cells in the mucosa of the stomach expressed the MMCP-2 mRNA in WBB6F$_1$-+/+ mice, but those of the muscularis propria did not. Both CMCs and PMCs of WBB6F$_1$-+/+ mouse origin expressed the MMCP-2 mRNA (Figure 3, A to D). We injected CMCs or PMCs of WBB6F$_1$-+/+ mouse origin into the stomach wall of genetically mast-cell-deficient WBB6F$_1$-*W/W^v* mice to examine whether the expression of MMCP-2 was influenced by the tissue environment, in which the injected mast cells settled. Although CMCs and PMCs were injected into the muscularis propria, mast cells appeared in both the mucosa and the muscularis propria. When CMCs were injected, the mast cells that appeared in the mucosa expressed the MMCP-2 mRNA (Figure 3, E and F), but the mast cells that appeared in the muscularis propria did not (Figure 3, G and H). On the other hand, when PMCs were injected, both the mast cells that appeared in the mucosa and those that appeared in the muscularis propria continued to express the MMCP-2 mRNA (Figure 3, I to L). We counted the number of alcian-blue-positive mast cells and MMCP-2 mRNA-expressing cells in the adjacent sections and calculated the proportion of MMCP-2 mRNA-expressing mast cells. At 5 weeks after the injection, the proportion of MMCP-2 mRNA-expressing mast cells was significantly higher in the muscularis propria of WBB6F$_1$-*W/W^v* mice injected with +/+ PMCs than in the muscularis propria of

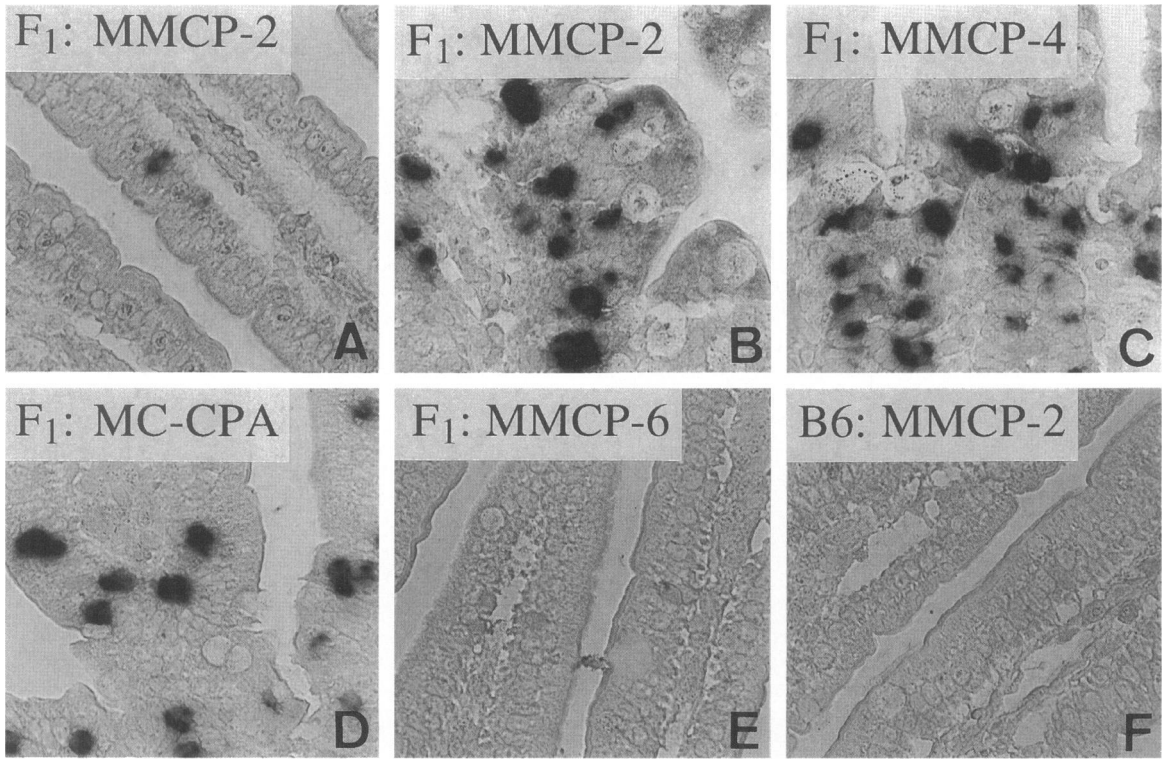

Figure 2. *Effect of the* Strongyloides venezuelensis *infection on the protease mRNA expression in WBB6F$_1$-+/+ and C57BL/6-+/+ mice.* **A:** *Expression of MMCP-2 mRNA by a mast cell in the jejunum of an untreated WBB6F$_1$-+/+ mouse.* **B:** *Increase of MMCP-2 mRNA-expressing mast cells in the jejunum of an infected WBB6F$_1$-+/+ mouse.* **C:** *Expression of MMCP-4 mRNA by the increased mast cells in the jejunum of a WBB6F$_1$-+/+ mouse.* **D:** *Expression of MC-CPA mRNA by the increased mast cells in the jejunum of an infected WBB6F$_1$-+/+ mouse.* **E:** *MMCP-6 mRNA was not expressed by the increased mast cells in the jejunum of an infected WBB6F$_1$-+/+ mouse.* **F:** *Jejunum of C57BL/6-+/+ mouse. The increased number of mast cells did not express MMCP-2 mRNA. Magnification, ×400.*

WBB6F$_1$-*W/W^v* mice injected with +/+ CMCs (Table 2). There is a possibility that 5 weeks may not be enough to induce changes in the phenotype of PMCs that reside in the muscularis propria of the stomach of WBB6F$_1$-*W/W^v* mice. Therefore, we killed several WBB6F$_1$-*W/W^v* mice 10 weeks after the injection of PMCs. The proportion of MMCP-2 mRNA-expressing mast cells remained to be high in the muscularis propria even 10 weeks after the injection of +/+ PMCs (Table 2). In one experiment,

Table 1. *Effect of* Strongyloides venesuelensis *Infection on Proportion of Mast Cells that Expressed MMCP-2, MMCP-4, MMCP-6, or MC-CPA mRNA in the Mucosa of the Jejunum of WBB6F$_1$-+/+ Mice*

Proteases	Infection	Tissue	Number of cells*		Percentage of cells expressing mRNA of each protease to alcian-blue+ cells
			Alcian-blue+	Protease mRNA+	
MMCP-2	No	Stomach†	154	145	94
	No	Jejunum	11	10	91
	Yes	Jejunum	205	195	95
MMCP-4	No	Stomach†	150	0	0
	No	Jejunum	10	0	0
	Yes	Jejunum	250	235	94‡
MMCP-6	No	Stomach†	138	0	0
	No	Jejunum	8	0	0
	Yes	Jejunum	233	0	0
MC-CPA	No	Stomach†	154	0	0
	No	Jejunum	9	0	0
	Yes	Jejunum	188	172	91‡

*Pooled data of two to three mice. As the number of mast cells in the jejunal mucosa of uninfected mice was small, approximately three sections were examined for each mouse.

†Data of stomach mucosa are shown because the number of mast cells in the jejunal mucosa of uninfected mice was small.

‡$P < 0.01$, when compared with the value observed in the mucosa of the jejunum or stomach of uninfected mice.

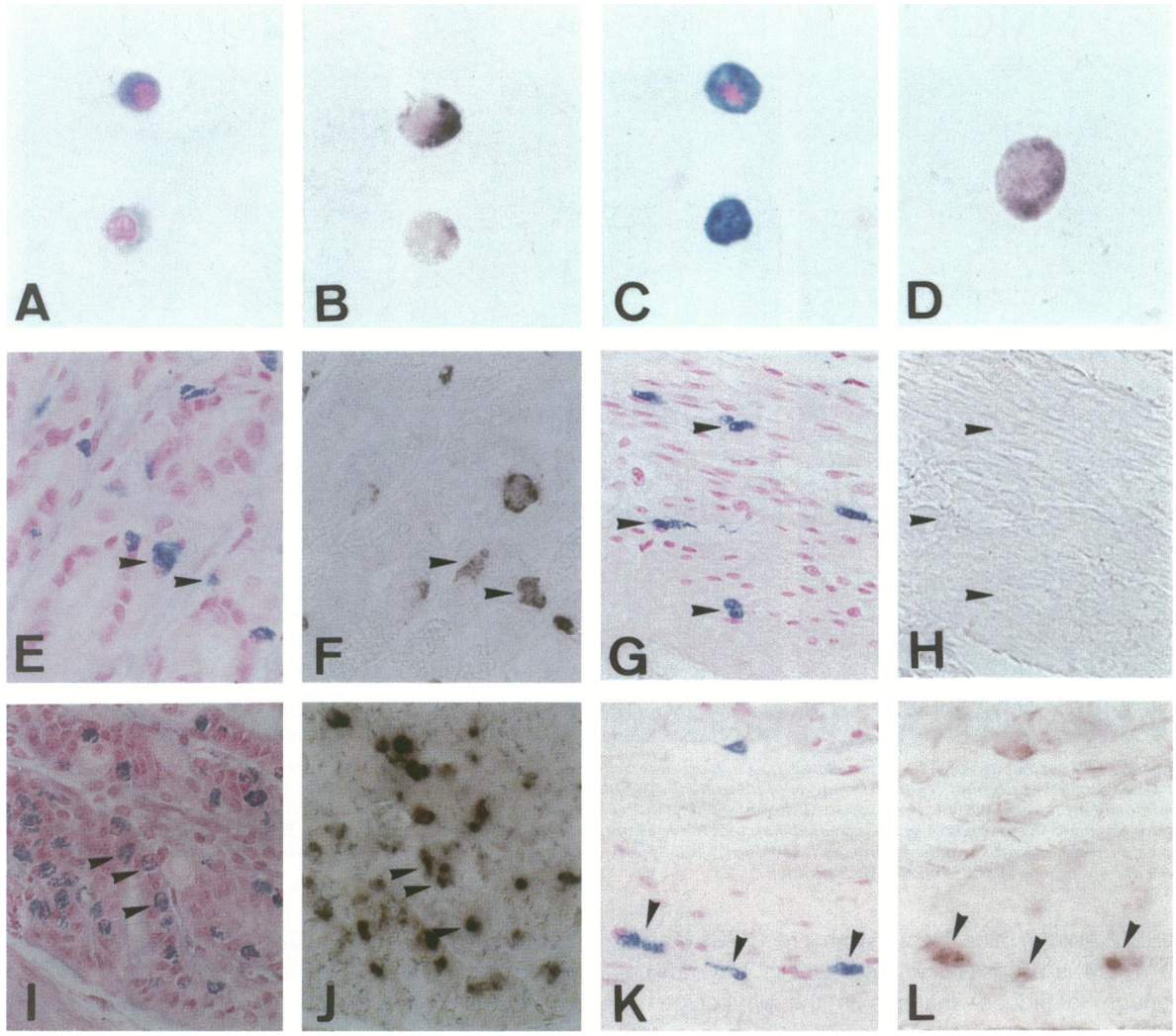

Figure 3. *Expression of MMCP-2 mRNA demonstrated by* in situ *hybridization.* **A:** *CMCs of WBB6F$_1$-+/+ mice stained with alcian blue and nuclear fast red.* **B:** *Expression of MMCP-2 mRNA by CMCs of WBB6F$_1$-+/+ mouse origin.* **C:** *PMCs obtained from WBB6F$_1$-+/+ mice stained with alcian blue and nuclear fast red.* **D:** *Expression of MMCP-2 mRNA by PMCs from WBB6F$_1$-+/+ mice.* **E:** *Development of mast cells in the stomach mucosa of a WBB6F$_1$-W/W^v mouse after the transplantation of CMCs derived from WBB6F$_1$-+/+ mice, demonstrated by staining with alcian blue and nuclear fast red.* **F:** *An adjacent section of* **E**. *Expression of MMCP-2 mRNA by the CMC-derived mast cells was demonstrated by* in situ *hybridization.* **G:** *Development of mast cells in the muscularis propria of a WBB6F$_1$-W/W^v* mouse after the transplantation of CMCs derived from WBB6F$_1$-+/+ mice, stained with alcian blue and nuclear fast red. **H:** *An adjacent section of* **G**. *MMCP-2 mRNA was not expressed by the CMC-derived mast cells.* **I:** *Development of mast cells in the stomach mucosa of a WBB6F$_1$-W/W^v* mouse after the transplantation of PMCs from WBB6F$_1$-+/+ mice, stained with alcian blue and nuclear fast red. **J:** *An adjacent section of* **I**. *MMCP-2 was expressed by the PMC-derived mast cells.* **K:** *Development of mast cells in the muscularis propria of a WBB6F$_1$-W/W^v* mouse after the transplantation of PMCs from WBB6F$_1$-+/+ mice, stained with alcian blue and nuclear fast red. **L:** *An adjacent section of* **K**. *In contrast with the CMC-derived mast cells shown in* **L**, *the PMC-derived mast cells remained to express MMCP-2 mRNA even after the settlement in the muscularis propria.* **Arrowheads** *indicate the same mast cells in the paired photographs. Magnification, ×1000 (***A** *to* **D**) *and ×400 (***E** *to* **L**).

CMCs of C57BL/6-+/+ mouse origin were injected into the muscularis propria of WBB6F$_1$-*W/W^v* mice. Although mast cells appeared in both the mucosa and the muscularis propria, both mast cells did not express the MMCP-2 mRNA at all (data not shown).

Approximately one-half of PMCs of WBB6F$_1$-+/+ mice expressed MMCP-4 and MMCP-6 mRNAs. Then we examined whether +/+ PMCs continued to express MMCP-4 and MMCP-6 mRNAs after the settlement in the stomach of WBB6F$_1$-*W/W^v* mice. At 5 weeks after the injection of +/+ PMCs into the stomach wall of WBB6F$_1$-*W/W^v* mice, the recipient mice were killed and the proportion of MMCP-4 mRNA-expressing cells to alcian-blue-positive mast cells and the proportion of MMCP-6 mRNA-expressing cells were obtained. Although mast cells in the stomach mucosa of WBB6F$_1$-+/+ mice did not express MMCP-4 and MMCP-6 mRNAs, approximately one-

Table 2. *Proportion of MMCP-2 mRNA-Expressing Cells in Various Mast Cell Populations*

Mast cells	Number of cells		Percentage of cells expressing MMCP-2 mRNA to alcian-blue+ cells
	Alcian-blue+	MMCP-2 mRNA+	
Mast cells in +/+ mucosa*	140	124	89
Mast cells in +/+ muscle*	120	0	0
+/+ CMCs	205	193	95
+/+ CMCs in W/W^v mucosa 5 weeks after injection†‡	78	68	87
+/+ CMCs in W/W^v muscle 5 weeks after injection†‡	247	2	1§
+/+ PMCs	202	175	87
+/+ PMCs in W/W^v mucosa 5 weeks after injection‡‖	35	28	80
+/+ PMCs in W/W^v muscle 5 weeks after injection‡‖	230	166	72¶
+/+ PMCs in W/W^v mucosa 10 weeks after injection‡‖	41	30	73
+/+ PMCs in W/W^v muscle 10 weeks after injection‡‖	141	99	70¶

*Mast cells in the mucosa and those of the muscularis propria of $WBB6F_1$-+/+ mice, respectively.
†CMCs of $WBB6F_1$-+/+ mouse origin were injected into the stomach wall of $WBB6F_1$-W/W^v mice.
‡Pooled data of two to three injection sites.
§$P < 0.01$, when compared with the value of +/+ CMCs or with the value of mast cells that appeared in the mucosa of $WBB6F_1$-W/W^v mice after injection of +/+ CMCs.
‖PMCs of $WBB6F_1$-+/+ mouse origin were injected into the stomach wall of $WBB6F_1$-W/W^v mice.
¶$P < 0.01$, when compared with the value of mast cells in the muscularis propria of $WBB6F_1$-+/+ mice or with the value of mast cells that appeared in the muscularis propria of $WBB6F_1$-W/W^v mice after injection of +/+ CMCs.

half of mast cells in the stomach mucosa of the $WBB6F_1$-W/W^v recipient mice expressed MMCP-4 and MMCP-6 (Tables 3 and 4).

Discussion

The present results showed that several factors influence the protease expression phenotype of mast cells in tissues of mice. The poor expression of MMCP-2 in the ear of BALB/c-+/+ mice has been reported by Stevens et al.[24,25] The present results showed the poor expression of MMCP-2 mRNA in the stomach mucosa, stomach muscle, and CMCs of C57BL/6-+/+ mice. Whether the same genetic mechanisms regulate the poor MMCP-2 expression in both BALB/c and C57BL/6 mice should be examined. The poor MMCP-2 expression was also observed after the transplantation of C57BL/6-+/+ CMCs into the stomach mucosa of $WBB6F_1$-W/W^v mice. As the CMCs derived from $WBB6F_1$-W/W^v mice did express MMCP-2,[25] the poor MMCP-2 expression in mast cells of C57BL/6-+/+ mouse origin did not appear to be influenced by the tissue environment of $WBB6F_1$-W/W^v mice.

The physiological importance of the MMCP-2 mRNA expression in the gastrointestinal mucosa has not been reported to our knowledge. However, Goyal et al[26] described that the response to *Trichinella spiralis* was different among strains of mice. The rapidity and level of the mast cell response in the C57BL/10 strain were lower than the values observed in the NIH strain. The mRNA expression of MMCP-2 has not been reported in C57BL/10 mice, and the mast cell response of C57BL/6 mice to *T. spiralis* has not been compared with that of NIH mice. If C57BL/6 mice show the poor mast cell response and if NIH mice express the normal level of MMCP-2, there is a possibility that MMCP-2 may be involved in the mast cell response to *T. spiralis*.

Tissue environments also influenced the expression of MMCP-2 mRNA. When CMCs of $WBB6F_1$-

Table 3. *Proportion of MMCP-4 mRNA-Expressing Cells in Various Mast Cell Populations*

Mast cells	Number of cells		Percentage of cells expressing MMCP-4 mRNA to alcian-blue+ cells
	Alcian-blue+	MMCP-4 mRNA+	
Mast cells in +/+ mucosa*	110	0	0
Mast cells in +/+ muscle*	62	26	42
+/+ PMCs	179	79	44
+/+ PMCs in W/W^v mucosa 5 weeks after injection†‡	47	21	44§
+/+ PMCs in W/W^v muscle 5 weeks after injection†‡	192	80	42

*Mast cells in the mucosa and those of the muscularis propria of $WBB6F_1$-+/+ mice, respectively.
†PMCs of $WBB6F_1$-+/+ mouse origin were injected into the stomach wall of $WBB6F_1$-W/W^v mice.
‡Pooled data of two to three injection sites.
§$P < 0.01$, when compared with the value of mast cells in the mucosa of $WBB6F_1$-+/+ mice.

Table 4. *Proportion of MMCP-6 mRNA-Expressing Cells in Various Mast Cell Populations*

Mast cells	Number of cells		Percentage of cells expressing MMCP-6 mRNA to alcian-blue+ cells
	Alcian-blue+	MMCP-6 mRNA+	
Mast cells in +/+ mucosa*	105	0	0
Mast cells in +/+ muscle*	116	59	51
+/+ PMCs	179	91	51
+/+ PMCs in *W/W^v* mucosa 5 weeks after injection†‡	40	23	57§
+/+ PMCs in *W/W^v* muscle 5 weeks after injection†‡	246	131	53

*Mast cells in the mucosa and those of the muscularis propria of WBB6F$_1$-+/+ mice, respectively.
†PMCs of WBB6F$_1$-+/+ mouse origin were injected into the stomach wall of WBB6F$_1$-*W/W^v* mice.
‡Pooled data of two to three injection sites.
§$P < 0.01$, when compared with the value of mast cells in the mucosa of WBB6F$_1$-+/+ mice.

+/+ mice were injected into the stomach wall of WBB6F$_1$-*W/W^v* mice, mast cells developed in both the muscularis propria and the mucosa.[15] The injected CMCs and the mast cells that appeared in the mucosa expressed MMCP-2 mRNA, but the mast cells that appeared in the muscularis propria did not, suggesting that the MMCP-2 mRNA expression was suppressed in the muscularis propria by certain tissue factors. By the transplantation of CMCs into the stomach wall of WBB6F$_1$-*W/W^v* mice, both the expression of MMCP-2 and the type of proteoglycan changed.[12] The MMCP-2 expression continued in the mucosa but stopped in the muscularis propria. Mast cells in the muscularis propria were stained with berberine sulfate, suggesting the content of heparin, but those of the mucosa were not stained with berberine sulfate as observed in the injected CMCs, suggesting the content of chondroitin sulfate.[12] In both cases, the injected CMCs showed the phenotype of mast cells located in the stomach of WBB6F$_1$-+/+ mice (MMCP-2 positive and berberine sulfate negative in the mucosa and MMCP-2 negative and berberine sulfate positive in the muscularis propria).

In contrast with CMCs the phenotype of which adapted to the new tissue environment after the transplantation to WBB6F$_1$-*W/W^v* mice, PMCs of WBB6F$_1$-+/+ mice remained to express MMCP-2 mRNA in both the mucosa and the muscularis propria of WBB6F$_1$-*W/W^v* mice even 10 weeks after the injection. This suggested the presence of different regulation mechanisms of MMCP-2 mRNA between CMCs and PMCs. The MMCP-2 mRNA expression of PMCs did not appear to be suppressed by tissue factors of the stomach muscle that could suppress the MMCP-2 expression in CMCs. The mRNA expression of MMCP-4 and MMCP-6 by +/+ PMCs was not suppressed by the transplantation into the stomach mucosa of WBB6F$_1$-*W/W^v* mice either. The present result is not consistent with the change of staining characteristics observed after the transplantation of PMCs of WBB6F$_1$-+/+ mice into the stomach wall of WBB6F$_1$-*W/W^v* mice. Berberine-sulfate-positive PMCs retained its original phenotype in the muscularis propria but became berberine sulfate negative in the mucosa.[14] The present result suggests that the protease expression phenotype and the type of proteoglycan phenotype may be regulated by different mechanisms.

Infection of *S. venezuelensis* induced the expression of MMCP-4 and MC-CPA in mast cells localized in the jejunal mucosa of WBB6F$_1$-+/+ mice. Finkelman et al[27] reported the expression of interleukin (IL)-3, IL-4, IL-5, IL-9, and IL-10 genes in mesenteric lymph nodes and Peyer's patches increased after the infection of *Nippostrongyloides brasiliensis*. Friend et al[28] reported that the protease phenotype of mast cells changed during the course of *T. spiralis* infection. However, the physiological significance of this change is not clear. As addition of stem cell factor and IL-9 in the culture medium increased the expression of MMCP-4 in CMCs,[29,30] the cytokine for which production was induced by the helminth infection led in turn to the expression of MMCP-4 mRNA. This is also consistent with the present result that the expression of MMCP-6 mRNA was not detectable after the helminth infection. Production of IL-3 followed the helminth infection,[26] and IL-3 has been reported to suppress the MMCP-6 mRNA expression.[29,31]

The present result showed that various factors can influence the protease expression phenotype of mast cells. These factors can divide into two categories: intracellular and extracellular factors. The strain specificity has not been well defined but appear to be intracellular in nature. The poor expression of MMCP-6 in the mutant *mi/mi* mice was attributed to the abnormality of the transcription factor encoded by the mouse *mi* locus.[7,8,31,33] GATA-binding transcription factors are also known to influence the expression of MC-CPA.[34] Transcription factors

are apparently intracellular factors. Soluble cytokines such as IL-3, IL-4, and IL-9, cell-bound cytokines such as stem cell factor, and extracellular matrices are extracellular factors. The *in situ* hybridization histochemistry is considered to be a suitable method to study the protease expression phenotypes of mast cells in tissues.

Several potential properties of mast cell proteases have been reported. The human mast cell tryptase degrades fibrinogen,[35] activates prostromelysin,[36] and stimulates the proliferation of fibroblasts.[37] On the other hand, the human mast cell chymase degrades basement membrane,[38] activates the IL-1β precursor,[39] and generates angiotensin II.[40] Although the present study did not directly demonstrate any physiological roles of mast cell proteases, the dynamic changes in the mRNA expression of mast cell proteases may implicate their physiological importance.

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