

Alteration of Protease Expression Phenotype of Mouse Peritoneal Mast Cells by Changing the Microenvironment as Demonstrated by *in Situ* Hybridization Histochemistry

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Mouse mast cell protease (MMCP) mRNA expression was examined by *in situ* hybridization histochemistry. Peritoneal mast cells (PMCs) of WBB6F₁-+/+ mice expressed MMCP-2, MMCP-4, MMCP-5, and MMCP-6 mRNAs, but did not express MMCP-1 mRNA. When proliferation of PMCs was induced by culturing them in methylcellulose with T cell-derived cytokines, cells in mast cell colonies expressed MMCP-1 mRNA. These mast cells were transferred to a suspension culture to induce further proliferation. The phenotype of the resulting PMC-derived cultured mast cells was similar to that of bone marrow-derived cultured mast cells. When 10⁵ PMC-derived cultured mast cells or 10⁵ bone marrow-derived cultured mast cells were injected into the stomach wall of mast cell-deficient WBB6F₁-W/W^v mice, mast cells that appeared in the mucosa and muscularis propria were similar to mast cells in the stomach of intact WBB6F₁-+/+ mice, indicating the injected cells adapted to a new tissue environment. In contrast, when 10⁵ PMCs were injected into the stomach wall of WBB6F₁-W/W^v mice, the injected PMCs did not adapt to the mucosa. When 20 PMCs were injected, they proliferated and adapted to the mucosal environment. The present results suggest that PMCs adapt to new environments when proliferation occurs before redifferentiation. (*Am J Pathol* 1998, 153:931-936)

Six distinct serine proteases designated mouse mast cell proteases (MMCP) 1, 2, 4, 5, 6, and 7 and mouse mast cell carboxypeptidase A have been identified in the secretory granules of mouse mast cells by protein sequencing and/or by cDNA cloning.¹⁻⁷ *In situ* hybridization his-

tochemistry of mast cell proteases has been previously used for characterization of mast cell phenotypes.⁸⁻¹⁰ For example, MMCP-2 mRNA is expressed by mast cells located in the mucosa of the stomach of (WB × C57BL/6)F₁-+/+ (hereafter, WBB6F₁-+/+) mice but not by mast cells in the muscularis propria.¹⁰ On the other hand, mRNAs of MMCP-4 and MMCP-6 are not expressed by mast cells in the mucosa but are expressed by cells in the muscularis propria of WBB6F₁-+/+ mice.¹⁰

We investigated changes in protease expression phenotype of mast cells after transplantation of bone marrow-derived cultured mast cells (BMCMCs) or peritoneal mast cells (PMCs) of WBB6F₁-+/+ mice into tissues of mast cell-deficient WBB6F₁-W/W^v mice.¹⁰⁻¹³ When BMCMCs that expressed MMCP-2 mRNA were transplanted into the stomach wall, mast cells that appeared in the mucosa expressed MMCP-2 mRNA, but mast cells that appeared in the muscularis propria did not, indicating BMCMC adaptation to a new tissue environment. However, PMCs that also expressed MMCP-2 mRNA did not adapt to the muscularis propria. MMCP-2 mRNA continued to be expressed after settlement in the mucosa and muscularis propria of WBB6F₁-W/W^v mice. PMCs did not alter their MMCP-4 and MMCP-6 mRNA expression phenotypes after settlement in the mucosa and the muscularis propria of WBB6F₁-W/W^v mice. Although mast cells in the stomach mucosa of WBB6F₁-+/+ mice did not express MMCP-4 and MMCP-6 mRNAs, approximately one-half of mast cells in the stomach mucosa of WBB6F₁-W/W^v recipient mice expressed MMCP-4 and MMCP-6 mRNAs after transplantation of PMCs of WBB6F₁-+/+ mice.¹⁰ Although 10⁵ BMCMCs were proliferating before transplantation,¹² the injection of 10⁵ PMCs in this limited area appeared to inhibit their own proliferation.¹³

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BMCMCs are undifferentiated and uncommitted cells that can adapt to new environments, whereas PMCs are differentiated and committed cells that can no longer adapt. BMCMCs proliferate in suspension culture containing T cell-derived cytokines, but naive PMCs do not. We examined whether proliferation of mast cells influenced their adaptability. Three approaches were used to induce proliferation: 1) generating cultured mast cells from PMCs (PCMCs) by plating PMCs in methylcellulose containing T cell-derived cytokines and then transferring them to suspension culture, 2) helminth infection, and 3) transplantation of a few (20) instead of many (10^5) PMCs into the stomach wall of WBB6F₁-W/W^v mice. All three procedures induced proliferation and phenotype changes. We used BMCMCs as the undifferentiated and uncommitted control and a large number of PMCs as the differentiated and committed control. In transplantation experiments, we used WBB6F₁-+/+ mice as a control of *in vivo* phenotype. We compared the MMCP-expression phenotype of transplanted mast cells of WBB6F₁-+/+ mouse origin with mast cells in tissues of WBB6F₁-+/+ mice.

Materials and Methods

Mice

WBB6F₁-+/+ and W/W^v were raised by crossing WB-W/+ females and C57BL/6-W^v/+ males at Japan SLC (Hamamatsu, Japan). The resulting WBB6F₁-+/+ and W/W^v mice were identified by their coat color. WBB6F₁-W/W^v mice have white hair and black eyes and are genetically deficient in mast cells.^{11,14,15} Mice were used at 2 to 4 months of age and killed by decapitation under ether anesthesia.

Establishment of BMCMCs

Pokeweed mitogen-stimulated spleen cell-conditioned medium (PWM-SCM) was prepared as described by Nakahata et al.¹⁶ To obtain BMCMCs, bone marrow cells were harvested from 2-month-old WBB6F₁-+/+ 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 bovine serum (Nippon Bio-Supply Center, Tokyo, Japan) were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. One-half of the medium was replaced every 7 days, and more than 95% of cells were BMCMCs 4 weeks after culture initiation.

Purification of PMCs and Establishment of PCMCs

Purification of PMCs was performed according to the method described by Yurt et al.¹⁷ In brief, peritoneal cells (6 to 10×10^7) were suspended in 1 ml of Tyrode's buffer, layered on 2 ml of 22.5% (w/v) metrizamide (den-

sity, 1.120 g/ml, Sigma Chemical Co., St. Louis, MO), and centrifuged at room temperature for 15 minutes at $400 \times g$. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode's buffer. Mast cells represented 70 to 80% of the nucleated cells in this preparation. To obtain PMC suspensions of $\geq 99\%$ purity, the procedure just described was repeated using the 70 to 80% pure mast cell suspensions. Cells were counted with a standard hemocytometer. Purified PMCs were identified by phase-contrast microscopy.

Clonal cell culture in methylcellulose (Sigma) was carried out according to the method described by Kanakura et al.¹⁸ One ml of a culture mixture containing 10^3 PMCs, α -MEM, 1% methylcellulose, 30% fetal bovine serum, 1% deionized bovine serum albumin (Sigma), 10^{-4} mol/L 2-mercaptoethanol, and 10% (v/v) PWM-SCM was plated in 35-mm tissue culture dishes. Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO₂ in air. Mast cell colonies were counted on day 14, and individual colonies were lifted from methylcellulose medium using a 3- μ l Eppendorf pipette under direct microscopic visualization and were collected in Eppendorf microcentrifuge tubes containing 0.5 ml of α -MEM. After washing two times with α -MEM, the cells were used for cytological examination and further amplified in suspension culture. The cells from each mast cell colony were seeded in single wells in 24-well microtiter plates (Corning Costar, Tokyo, Japan) containing 0.4 ml α -MEM supplemented with 10^{-4} mol/L 2-mercaptoethanol, 20% fetal bovine serum, 0.2% deionized bovine serum albumin, and 10% PWM-SCM. Culture plates were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Half of the medium was replaced every 7 days. When significant proliferation occurred in a well of the 24-well plate, the cells in the well were transferred to a well in a 6-well microtiter plate (Corning). Eight weeks after initiation of the suspension culture, we selected the wells in which PCMCs proliferated to $\geq 10^5$ cells. Cells contained in two or three wells were pooled and used for cytological examination and transplantation to the stomach wall.

Preparation of Probes for *In Situ* Hybridization

Total RNA was extracted from BMCMCs of WBB6F₁-+/+ mice. Single-strand cDNA was generated with a specific antisense primer for MMCP-2,⁸ MMCP-4,¹⁹ MMCP-5,²⁰ and MMCP-6^{8,19} by reverse transcriptase (Takara, Kyoto, Japan) from total RNA. Each cDNA was amplified by a Perkin-Elmer Cetus (Norwalk, CT) DNA thermal cycler using Taq DNA polymerase (Takara).¹⁹ PCR products were subcloned into the EcoRV site of the Bluescript KS(-) plasmid (Stratagene, La Jolla, CA), which contains T3 and T7 promoters to generate riboprobes, and the sequence was confirmed with a model 373A DNA sequencer (Applied Biosystems, Foster City, CA). The MMCP-1 plasmid was kindly donated by Dr. J. Wastling (Department of Veterinary Clinical Studies, University of Edinburgh, Edinburgh, United Kingdom). The plasmid was either linearized with *HincII* and transcribed with T7

Table 1. Proportion of Mast Cells That Expressed MMCP of Each Type in Various Mast Cell Populations of WBB6F₁-+/+ Mice

Mast cells	Proportion of protease mRNA-expressing cells to alcian-blue positive cells (%)				
	MMCP-1	MMCP-2	MMCP-4	MMCP-5	MMCP-6
PMCs	0	59	80	81	82
Mast cells in PMC-derived colonies*	52 [†]	75 [†]	74	73	79
PCMCs	68 [†]	83 [†]	68	69	78
BMCMCs	73 [†]	92 [†]	67	72	80

Ninety to 100 mast cells of each type were scored.

*One hundred mast cell colonies were pooled.

[†]*P* < 0.01, when compared with the value of PMCs by χ^2 test.

[‡]*P* < 0.05, when compared with the value of PMCs by χ^2 test.

RNA polymerase to generate an antisense probe or linearized with *EcoRI* and transcribed with T3 RNA polymerase to generate a sense probe.

In Situ Hybridization

BMCMCs, mast cells in PMC-derived colonies, PCMCs, and PMCs were each collected, washed with phosphate-buffered saline, and mixed with 2% agarose (FMC Bio-Products, Rockland, ME). The mixture was fixed with freshly prepared 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) overnight and then dehydrated and embedded in paraffin. The stomachs of WBB6F₁-*W/W^v* mice were removed 5 weeks after injection of mast cells, opened, and flattened onto a rubber plate. Fixation and embedding of the tissues was performed by the procedure used for the suspended cells. Serial sections (4 μ m thick) were cut, and odd-number sections were stained with alcian blue and nuclear fast red to identify mast cells and even-number sections were used for *in situ* hybridization. Hybridization was carried out as described previously with minor modifications.²¹ 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 μ g/ml) before hybridization, and 3) withholding of the antisense RNA probe and the anti-digoxigenin antibody.²¹ None of the three controls showed any positive signals.

Proportion of Protease mRNA-Expressing Mast Cells

The number of alcian blue-positive mast cells and that of MMCP-1, MMCP-2, MMCP-4, MMCP-5, or MMCP-6 mRNA-expressing cells were counted in adjacent sections. In cases in which the number of mast cells in adjacent sections were few, data from many pairs of adjacent sections were pooled.

Infection of Helminth

The strain of *Strongyloides venezuelensis* used in this study was originally isolated from a wild brown rat in Okinawa, Japan, established as a laboratory strain,²² and is now maintained in the Miyazaki Medical College

with serial passages in Wistar rats.²³ Third-stage infective larvae (L3) were obtained from fecal culture by the filter paper method.²³ The degree of infection was monitored daily by egg excretion in feces from five animals. WBB6F₁-+/+ mice were infected by subcutaneous injections of 1000 L3. Mice were killed on day 12, and the stomach was removed and used for *in situ* hybridization histochemistry.

Transplantation into the Stomach Wall

Recipient WBB6F₁-*W/W^v* mice were anesthetized with Nembutal, the peritoneal cavity was opened, and the stomach was exposed. BMCMCs, PCMCs or PMCs from WBB6F₁-+/+ mice were counted with a standard hemocytometer and injected into the wall of the glandular stomach of WBB6F₁-*W/W^v* mice.^{10,24} Cells (10⁵ or 20) suspended in 0.1 ml α -MEM were injected with a tuberculin syringe. Each mouse received two injections recorded by tattooing with India ink. WBB6F₁-*W/W^v* mice were killed 5 weeks after injections. Injection sites 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 the next section was used for *in situ* hybridization. Numbers of mast cells were counted with a microscope. When we injected 20 PMCs, the section whose adjacent section contained \geq 50 alcian blue-positive mast cells was used for *in situ* hybridization.

Results

PMCs expressed MMCP-2, MMCP-4, MMCP-5, and MMCP-6 mRNAs but did not express MMCP-1 mRNA. We plated 10³ PMCs in methylcellulose containing PWM-SCM to induce proliferation, and approximately 25% of PMCs formed colonies. Half of the mast cells in large mast cell colonies (\geq 64 cells) expressed MMCP-1 mRNA (Table 1 and Figure 1). Large colonies were transferred to suspension culture for expansion. Approximately 40% of large colonies generated \geq 10⁵ mast cells after 8 weeks of growth. The phenotype of the resulting PCMCs was similar to that of mast cells in PMC-derived colonies and BMCMCs (Table 1).

MMCP-1 mRNA was not expressed by mast cells in the stomach mucosa of intact mice. Because MMCP-1 mRNA appeared to be expressed after stimulation by T-cell-derived cytokines, we investigated the effect of

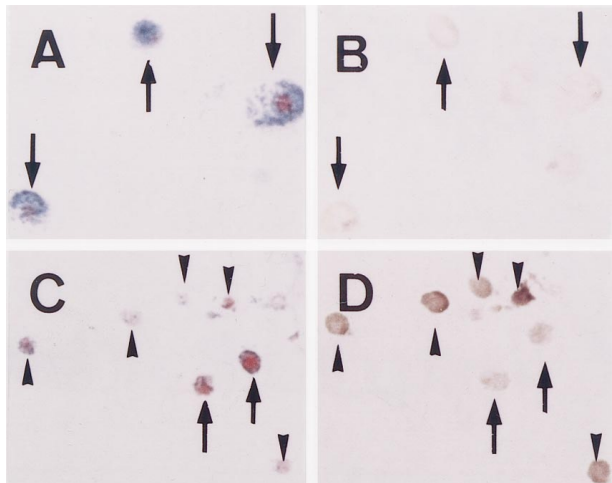


Figure 1. Expression of MMCP-1 mRNA demonstrated by *in situ* hybridization. **A:** PMCs of WBB6F₁-+/+ mice stained with alcian blue and nuclear fast red. **B:** Section adjacent to that shown in **A**. **Arrows** in **A** and **B** show the same cells that do not express MMCP-1 mRNA. **C:** Mast cells in PMC-derived colonies of WBB6F₁-+/+ mouse origin stained with alcian blue and nuclear fast red. Mast cells in PMC-derived colonies are significantly smaller than PMCs. Moreover, alcian blue-positive granules are significantly fewer in the former than in the latter. **D:** Section adjacent to that shown in **C**. **Arrows** and **arrowheads** in **C** and **D** show the same cells. **Arrows** show the cells that do not express MMCP-1 mRNA, and **arrowheads** show the cells that express MMCP-1 mRNA. Magnification, ×500.

helminth infection on the expression of MMCP-1 mRNA. When WBB6F₁-+/+ mice were infected with *S. venezuelensis*, mast cells in the stomach mucosa remarkably increased in number, and more than half of the mast cells expressed MMCP-1 mRNA (Figure 2).

The protease mRNA expression phenotype of mast cells differed between the mucosa and muscularis propria of the stomach of WBB6F₁-+/+ mice.¹⁰ Mast cells in the mucosa expressed MMCP-2 mRNA but did not express MMCP-4, MMCP-5, or MMCP-6 mRNAs. On the contrary, mast cells in the muscularis propria did not express MMCP-2 mRNA but did express MMCP-4, MMCP-5, and MMCP-6 mRNAs. In previous experiments, we injected 10⁵ PCMCs into the stomach wall of mast cell-deficient WBB6F₁-W/W^Y mice¹⁰ and found that the protease expression phenotype of PCMCs did not adapt to the new environment. MMCP-2 mRNA continued to be expressed in the muscularis propria, and MMCP-4 and MMCP-6 mRNA continued to be expressed in the mucosa. In the present experiment, we injected 20 PCMCs into the stomach wall of WBB6F₁-W/W^Y mice. Mast cells that appeared in the muscularis propria continued to express MMCP-2 mRNA, but the proportion of MMCP-2 mRNA-expressing cells was significantly decreased (Table 2). The expression of MMCP-4, MMCP-5, and MMCP-6 mRNAs was abolished in the mucosa (Table 2).

We then transplanted 10⁵ PCMCs or 10⁵ BMCMCs derived from WBB6F₁-+/+ mice into the stomach wall of WBB6F₁-W/W^Y mice. MMCP-1 mRNA continued to be expressed in mast cells in the mucosa but not in mast cells in the muscularis propria (Table 2). The proportion of MMCP-1 mRNA-expressing mast cells in the mucosa was lower than that of PCMCs and BMCMCs (Tables 1 and 2). Expression of MMCP-2 mRNA continued in mast

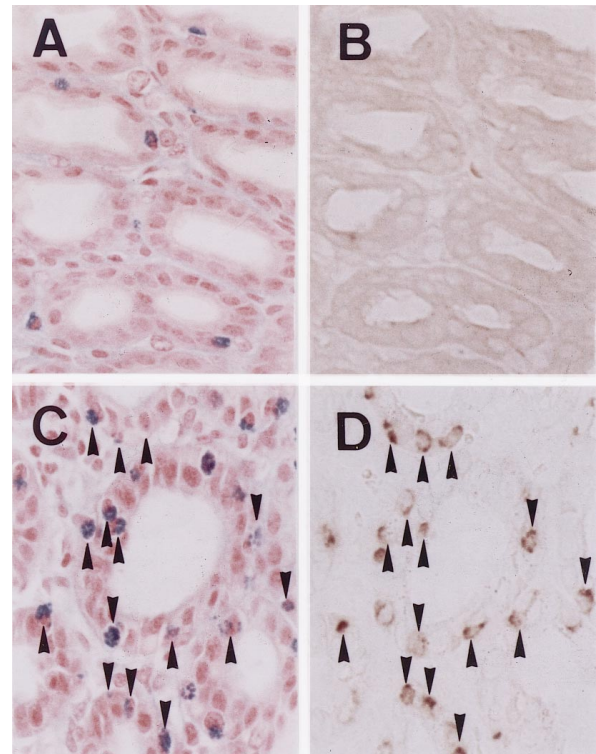


Figure 2. Effect of *S. venezuelensis* infection on the MMCP-1 mRNA expression in the stomach mucosa of WBB6F₁-+/+ mice, demonstrated by *in situ* hybridization. **A:** Stomach mucosa of nontreated mouse stained with alcian blue and nuclear fast red. **B:** Section adjacent to that shown in **A**; no MMCP-1 mRNA-expressing cells were observed. **C:** Stomach mucosa of the infected mouse stained with alcian blue and nuclear fast red. **D:** Section adjacent to that shown in **C**. Many mast cells expressing MMCP-1 mRNA were observed. **Arrowheads** indicate the same mast cells in the paired photographs. Magnification, ×375.

cells in the mucosa and muscularis propria, but the proportion of MMCP-2 mRNA-expressing cells in the muscularis propria was significantly lower when 10⁵ PCMCs were injected than when 10⁵ BMCMCs were injected (Table 2). The expression of MMCP-4, MMCP-5, and MMCP-6 mRNAs was abolished in mast cells in the mucosa of WBB6F₁-W/W^Y mice after the injection of 10⁵ PCMCs. Injection of 10⁵ PCMCs had results similar to those of injection of 10⁵ BMCMCs.

We counted the number of mast cells in the mucosa and the muscularis propria of the stomach of WBB6F₁-W/W^Y mice 5 weeks after injection of 20 PCMCs. The results shown in Table 3 indicate the proliferation of injected PCMCs after settlement.

Discussion

Expression of MMCP-1 mRNA was observed in mast cells proliferating in the gastric mucosa of *S. venezuelensis*-infected mice. In contrast, mast cells in the mucosa of intact mice did not express MMCP-1 mRNA. This is consistent with the results of Scudamore et al,²⁵ which show that MMCP-1 expression is induced in mast cells in the gastric and intestinal mucosa by infection with *Nippostrongyloides brasiliensis*. Because the gastric mucosa is not infested with *S. venezuelensis*, MMCP-1 mRNA ex-

Table 2. Proportion of Mast Cells that Expressed MMCP of Each Type in the Stomach of WBB6F₁-W/W^v Mice after Transplantation of Various Mast Cell Populations of WBB6F₁-+/+ Mouse Origin

Mast cells	No. of injected cells	Proportion of protease mRNA-expressing cells to alcian-blue positive cells (%)									
		MMCP-1		MMCP-2		MMCP-4		MMCP-5		MMCP-6	
		Mucosa*	Muscularis	Mucosa*	Muscularis	Mucosa*	Muscularis	Mucosa*	Muscularis	Mucosa*	Muscularis
Mast cells in +/+ mice	0	0	0	89	0 [†]	0 [†]	42	0 [†]	50	0 [†]	51
BMCMCs [‡]	10 ⁵	10 ^{†§}	0	93	3 [†]	0 [†]	46	0 [†]	58	0 [†]	59
PCMCs [‡]	10 ⁵	6 [¶]	0	87	10 ^{†§}	0 [†]	41	0 [†]	53	0 [†]	52
PMCs [‡]	20	0	0	78	32 ^{†§}	0 [†]	43	0 [†]	49	0 [†]	52
PMCs [‡]	10 ⁵	0	0	83	74 [§]	45 [§]	43	53 [§]	54	56 [§]	54

*Pooled data of two or three injection sites when BMCMCs, PCMCs, 20 PMCs, or 10⁵ PMCs were injected. Eighty-five to 104 mast cells were scored.

[†]P < 0.01, when compared with the value of mast cells that appeared in the stomach of WBB6F₁-W/W^v mice after injection of 10⁵ PMCs of WBB6F₁-+/+ mice.

[‡]Results obtained after injection of mast cells of each type into the stomach wall of WBB6F₁-W/W^v mice.

[§]P < 0.01, when compared with the value of mast cells in the stomach of intact WBB6F₁-+/+ mice.

[¶]P < 0.05, when compared with the value of mast cells that appeared in the stomach of WBB6F₁-W/W^v mice after injection of 10⁵ PMCs of WBB6F₁-+/+ mice.

^{||}P < 0.05, when compared with the value of mast cells in the stomach of intact WBB6F₁-+/+ mice.

pression appeared to be induced by diffusible factors. In fact, Finkelman et al²⁶ reported that the expression of interleukin (IL)-3, IL-4, IL-5, IL-9, and IL-10 mRNAs increases in mesenteric lymph nodes and Peyer's patches after infection with *N. brasiliensis*. Addition of IL-9 and IL-10 to culture medium has been shown to increase the expression of MMCP-1 mRNA in BMCMCs.^{27,28} IL-9 and IL-10 may play a role in MMCP-1 mRNA induction in both mucosal mast cells of parasite-infected mice and BMCMCs.

PMCs did not express MMCP-1 mRNA, but one-half of PCMCs did express it. Approximately 10% of PCMCs formed large mast cell colonies in methylcellulose, and ≥10⁵ PCMCs developed from 40% of the large mast cell colonies. Roughly 4% of PCMCs augmented up to 10⁵ PCMCs and appeared to change the MMCP expression phenotype after proliferation. It is possible that mast cells with extensive proliferation potential may change the MMCP expression phenotype.

PCMCs were derived from PMCs, whereas BMCMCs originated from mast cell precursors in bone marrow. Despite differences of origin, MMCP expression pheno-

type of PCMCs and BMCMCs were similar. The MMCP expression phenotype appeared to be dependent on the microenvironment rather than on mast cell origin. It is believed that cytokines contained in the medium are the most important environmental factor. When 10⁵ PCMCs, BMCMCs, or PMCs were transplanted into the stomach wall of WBB6F₁-W/W^v mice, PCMCs behaved similarly to BMCMCs, not to PMCs. The proportion of MMCP-2 mRNA⁺ cells markedly decreased in the muscularis propria, and MMCP-4 mRNA⁺, MMCP-5 mRNA⁺ and MMCP-6 mRNA⁺ cells disappeared in the mucosa. After proliferation in culture, PMCs appeared to adapt to the new tissue environments.

When PMCs were injected into the stomach wall of mast cell-deficient WBB6F₁-W/W^v mice, the MMCP expression phenotype of mast cells appeared to be influenced by the number of PMCs injected. The proportion of MMCP-2 mRNA⁺ cells in the muscularis propria was significantly lower after the injection of 20 PMCs than after the injection of 10⁵ PMCs. Although MMCP-4 mRNA⁺, MMCP-5 mRNA⁺, or MMCP-6 mRNA⁺ mast cells remained in the mucosa of WBB6F₁-W/W^v mice after injection of 10⁵ PMCs, such mast cell types were not detectable after injection of 20 PMCs. Given that MMCP-2 mRNA⁺ mast cells were not detectable in the muscularis propria of WBB6F₁-+/+ mice, and that MMCP-4 mRNA⁺, MMCP-5 mRNA⁺, or MMCP-6 mRNA⁺ mast cells were not detectable in the mucosa of WBB6F₁-+/+ mice, PMCs appeared to adapt to the new tissue environment after transplantation of 20 but not after transplantation of 10⁵ cells. Because at least a part of the 20 PMCs proliferated after injection into the stomach wall of WBB6F₁-W/W^v mice, PMCs appeared to change the MMCP expression phenotype after proliferation. This is consistent with the results of Sonoda et al,²⁴ which demonstrate that chondroitin sulfate-containing mucosal mast cells develop after the injection of heparin-containing PMCs in the mucosa of WBB6F₁-W/W^v mice. Glycosaminoglycan content is not a direct result of a single gene action. Moreover, the presence of heparin by individual mast cells was indirectly shown by staining with berberine

Table 3. Number of Mast Cells Observed in the Stomach of WBB6F₁-W/W^v Mice after the Transplantation of 20 PMCs of WBB6F₁-+/+ Mice

Injection sites	No. of mast cells per injection site	
	Mucosa	Muscularis
1*	310	940
2*	980	1020
3	0	0
4	7	3
5	119	13
6	140	20
7	0	13
8	210	650
9*	760	1440
10	0	20
11	3	2
12	24	0

Twenty peritoneal mast cells were injected into the stomach wall of 6 WBB6F₁-W/W^v mice. Each mouse received two injections.

*Used to obtain results shown in Table 2.

sulfate. *In situ* hybridization histochemistry of MMCPs is a better method to clarify the change of mast cell phenotypes after the introduction into new tissue environment.

Taken together, PMCs may change the MMCP expression phenotype after proliferation either in culture or in tissues.

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