

Mouse Bone Marrow-derived Mast Cells (mBMMC) Obtained In Vitro from Mice That Are Mast Cell-deficient In Vivo Express the Same Panel of Granule Proteases as mBMMC and Serosal Mast Cells from Their Normal Littermates

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Summary

The ear, skin, and purified serosal mast cells of WBB6F₁/J-+/+ (WB-+/+) and WCB6F₁/J-+/+ (WC-+/+) mice contain high steady-state levels of the transcripts that encode mouse mast cell protease (mMCP) 2, mMCP-4, mMCP-5, mMCP-6, and mouse mast cell carboxypeptidase A (mMC-CPA). In contrast, no mast cell protease transcripts are present in abundance in the ear and skin of WBB6F₁/J-*W/W^v* (*W/W^v*) and WCB6F₁/J-*Sl/Sl^d* (*Sl/Sl^d*) mice which are mast cell-deficient in vivo due to defects in their *c-kit* and *c-kit* ligand genes, respectively. We now report that the immature bone marrow-derived mast cells (mBMMC) obtained in vitro with recombinant interleukin 3 (rIL-3) or WEHI-3 cell conditioned medium from WB-+/+, WC-+/+, *W/W^v*, and *Sl/Sl^d* mice all contain high steady-state levels of the mMCP-2, mMCP-4, mMCP-5, mMCP-6, and mMC-CPA transcripts. As assessed immunohistochemically, mMCP-2 protein and mMCP-5 protein are also present in the granules of mBMMC from WB-+/+, WC-+/+, and *W/W^v* mice. That *Sl/Sl^d* and *W/W^v* mBMMC contain high steady-state levels of five granule protease transcripts expressed by the mature serosal, ear, and skin mast cells of their normal +/+ littermates suggests that *c-kit*-mediated signal transduction is not essential for inducing transcription of these protease genes. Because rIL-4 inhibits the rIL-10-induced expression of mMCP-1 and mMCP-2 in BALB/cJ mBMMC, the ability of rIL-4 to influence protease mRNA levels in WC-+/+ mBMMC and *W/W^v* mBMMC was investigated. Although rIL-10 induced expression of the mMCP-1 transcript in WC-+/+ and *W/W^v* mBMMC, rIL-4 was not able to suppress the steady-state levels of the mMCP-1 transcript or any other protease transcript in these cultured mast cells. Thus, not only do BALB/cJ mBMMC express fewer granule proteases than mBMMC from mast cell-deficient strains and their normal littermates but the subsequent induction of late-expressed proteases in BALB/cJ mBMMC is more tightly regulated by IL-3 and IL-4.

When bone marrow cells from the BALB/cJ mouse are cultured in the presence of recombinant (r)IL-3 or conditioned medium containing IL-3, immature mouse bone marrow-derived mast cells (mBMMC)¹ are obtained (1, 2) that have high steady-state levels of the transcripts that en-

code mouse mast cell protease (mMCP) 5 (3), mMCP-6 (4), and mouse mast cell carboxypeptidase A (mMC-CPA) (5). BALB/cJ mBMMC exposed to recombinant *c-kit* ligand (rKL), a fibroblast-derived cytokine (6), have high steady-state levels of the mMCP-4 transcript in addition to the other protease transcripts (7). BALB/cJ mBMMC exposed concomitantly to rKL and either rIL-9 or rIL-10 contain high steady-state levels of the mMCP-1 and mMCP-2 transcripts in addition to the mMCP-4 transcript (8). However, the ability of rKL, rIL-9, or rIL-10 to induce BALB/cJ mBMMC to accumulate high steady-state levels of the mMCP-1, mMCP-2, and

¹ Abbreviations used in this paper: CTMC, connective tissue mast cells; KL, *c-kit* ligand; mBMMC, mouse bone marrow-derived mast cells; mMC-CPA, mouse mast cell carboxypeptidase A; mMCP, mouse mast cell protease; PTB, PBS/Tween-20/BSA; r, recombinant; SMC, serosal mast cells; WCM, WEHI-3 cell conditioned medium.

mMCP-4 transcripts is inhibited when these cells are exposed concomitantly to either rIL-3 or rIL-4 (7–11).

The WCBF₁/J-SI/SI^d (SI/SI^d) and WBB6F₁/J-W/W^v (W/W^v) mouse strains possess abnormalities in the membrane-spanning domain of KL (6, 12) and the tyrosine kinase domain of *c-kit* (the membrane receptor for KL) (13), respectively. Both strains normally have very few mast cells in their tissues (14–16), and the levels of all mMCP transcripts in their ears and skin are below detection (17) even though large numbers of mBMMC can be generated by culturing the hematopoietic progenitor cells of these mice in the presence of rIL-3 or IL-3-enriched conditioned medium (18). Connective tissue-type mast cells (CTMC) can be elicited in the skin of the W/W^v mouse but not in the SI/SI^d mouse at sites of idiopathic chronic dermatitis (19) or by repeated application of phorbol ester (20). Because W/W^v mice reconstituted with mBMMC derived from the WBB6F₁/J-+/+ (WB-+/+) mouse can produce cells at different anatomical sites that histochemically resemble either CTMC, serosal mast cells (SMC), or mucosal mast cells (21), it was concluded that in the BALB/cJ mouse mMCP-5 (3, 22), mMCP-6 (4), and mMC-CPA (5) are early-expressed proteases and that mMCP-1 (23, 24), mMCP-2 (25), and mMCP-4 (26) are late-expressed proteases. In addition, because rKL-developed mBMMC histochemically resemble SMC and CTMC, preferentially synthesize heparin proteoglycans, and contain high steady-state levels of the mMCP-4 transcript (7, 27), it was concluded that KL and its receptor preferentially induce mast cell-committed progenitor cells to differentiate in a SMC and/or CTMC lineage.

In the present study, mBMMC were developed with rIL-3 or with IL-3-enriched WEHI-3 cell conditioned medium (WCM) in the absence of KL from mast cell-deficient strains, their +/+ littermates, BALB/cJ mice, and C57BL/6J mice. We now report that KL and its receptor are not essential for inducing expression of those granule proteases that are specifically found in SMC and CTMC.

Materials and Methods

Cell Culture. mBMMC were obtained by separately culturing bone marrow cells from the femurs and tibias of BALB/cJ, WB-+/+, WCB6F₁/J-+/+ (WC-+/+), W/W^v, SI/SI^d, and C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) for 2–5 wk in enriched medium (RPMI 1640 containing 0.1 mM nonessential amino acids, 2 mM L-glutamine, 10% FCS [GIBCO BRL, Gaithersburg, MD], 100 U/ml penicillin, 100 µg/ml streptomycin, 10 µg/ml gentamicin, and 50 µM 2-mercaptoethanol) supplemented with either 100 U/ml mouse rIL-3 (Genzyme Corp., Boston, MA) or 50% WEHI-3 cell (line TIB-68; American Type Culture Collection, Rockville, MD) CM (1, 2). As assessed by PCR analysis, WEHI-3 cells do not express KL (Cheng, H. J., and J. G. Flanagan, unpublished observation). Every 7 d, the nonadherent cells in the cultures were transferred into new flasks containing fresh culture medium. In some experiments, 3-wk-old mBMMC from BALB/cJ, WB-+/+, or WC-+/+ mice were separately cultured for 72 h in 50% WCM/50% enriched medium supplemented with 100 U/ml mouse rIL-10 alone or with both 50 U/ml mouse rIL-4 (Genzyme Corp.) and mouse rIL-10 (8–11). The concentration selected

for each cytokine was identical to or higher than that which was previously shown to elicit fully a particular biologic response in mouse mast cells.

Histochemical, Immunohistochemical, Histamine, and mRNA Analyses of mBMMC. Cyto centrifuge preparations of mBMMC exposed to various combinations of cytokines were stained with toluidine blue or with alcian blue followed by safranin (28). More than 100 cells were assessed histochemically in each experiment. For immunohistochemical analysis, mBMMC in Carnoy's fixative were washed with PBS containing 0.2% Tween-20 and 0.1% BSA (PTB buffer), incubated for 30 min at 37°C in PBS containing 0.05% Tween-20 and 4% goat serum, and then incubated for 2 h at 21°C with affinity-purified anti-mMCP-2₍₅₆₋₇₁₎ IgG (11) or anti-mMCP-5₍₁₄₆₋₁₆₂₎ IgG (29) diluted in goat serum. The cells were washed in PTB buffer, incubated for 1 h at room temperature in PTB buffer supplemented with biotinylated goat anti-rabbit IgG, washed again, incubated in the alkaline phosphatase Vectastain ABC reagent (Vector Laboratories Inc., Burlingame, CA) for 30 min at room temperature, and then washed again in PTB buffer. In each protocol, control cell preparations were processed without primary antibodies.

For histamine analyses, cells were suspended in HBSS at a density of ~5 × 10⁶ cells/ml and then were sonicated at 4°C with a Branson Sonifier (15 pulses/50% pulse cycle; Branson Ultrasonics Corp., Danbury, CT). The resulting cell lysates were diluted 1/1,000 in HBSS, and their histamine contents were determined with a radioimmunoassay kit (Amac Inc., Westbrook, ME) (30).

For quantitation of steady-state protease mRNA levels in mBMMC, approximately equal amounts of total cellular RNA (isolated by the method of Chomczynski and Sacchi [31]) were applied to individual lanes of 1.3% agarose-formaldehyde gels, and the gels were subjected to electrophoresis for 16–22 h. The separated RNA was transferred to nylon membranes (MagnaGraph; Micron Separations Inc., Westboro, MA) (32). The resulting blots were analyzed with [³²P]dCTP (~3,000 Ci/mmol; DuPont/New England Nuclear, Boston, MA) random prime-labeled (Stratagene, La Jolla, CA) mMCP-1 (9, 24), mMCP-2 (25), mMCP-4 (9, 26), mMCP-5 (3), mMCP-6 (4), mMC-CPA (5), serglycin proteoglycan peptide core (33), and β-actin (34) cDNA probes. Hybridizations were performed at 68°C for 1–4 h with QuikHyb (Stratagene). The RNA blots were washed at 55–60°C in 30 mM NaCl, 3 mM sodium citrate, 0.1% SDS, and autoradiography was performed with Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY). Usually, the same blot was assessed for the presence of other transcripts after the previous radiolabeled probe was removed by incubation of the blot for 1 h at 75°C in 0.25 mM EDTA, 0.1× Denhardt's solution, 5 mM Tris-HCl, and 0.05% pyrophosphate. In some cases, relative levels of individual transcripts were quantitated by directly measuring the amount of blot-bound radioactivity with a ³²P imaging system (Betascop 603; IntelliGenetics, Inc./Betagen, Mountain View, CA). The data were then β-actin corrected for variable total RNA loading in the lanes of the gel.

The mMCP-4 probe used in the above RNA blot analyses cross-reacts weakly (9) with a gene that encodes a mast cell-like serine protease that has been designated mMCP-L (26). Thus, a RNase protection analysis was performed to confirm the presence of the mMCP-2 and mMCP-4 transcripts in WB-+/+ mBMMC. Antisense cRNA probes corresponding to residues 804–956 of a mMCP-2 cDNA and residues 497–633 of a mMCP-4 cDNA were generated from their plasmids with T3 or T7 primers and a kit from Ambion Inc. (Austin, TX). Each cRNA probe was labeled with [³²P]dUTP (~800 Ci/mmol; DuPont/New England Nuclear) and was then separately incubated overnight at 43°C with

~5 μ g of total RNA isolated from BALB/cj mBMMC and WB-+/+ mBMMC. The sizes of the protected fragments were analyzed on 6% polyacrylamide/8 M urea gels.

Results

Protease mRNA Levels in mBMMC Generated from Different Strains. 4-wk-old IL-3-dependent mBMMC from BALB/cj mice contained high steady-state levels of the mMCP-5, mMCP-6, and mMC-CPA transcripts but undetectable levels of the mMCP-1, mMCP-2, and mMCP-4 transcripts (Fig. 1). mBMMC generated from *W/W^v* mice, *Sl/Sl^d* mice, and their +/+ littermates contained not only high steady-state levels of the mMCP-5, mMCP-6, and mMC-CPA transcripts but also high steady-state levels of the mMCP-2 and mMCP-4 transcripts. The presence of the mMCP-2 and mMCP-4 transcripts in WB-+/+ mBMMC was confirmed by RNase protection assays. Total RNA from these mBMMC protected a radiolabeled 136-bp fragment of the mMCP-4 probe and a 152-bp fragment of the mMCP-2 probe from degradation (data not shown). C57BL/6J mBMMC contained high steady-state levels of the mMCP-5, mMCP-6, and mMC-CPA transcripts but not the mMCP-1, mMCP-2, or mMCP-4 transcripts (data not shown).

mBMMC from WB-+/+ mice and BALB/cj mice were assessed for their histochemistry and time course of appearance of the steady-state levels of the mMCP-2, mMCP-4, mMC-CPA, and serglycin transcripts. No cells in the starting bone marrow from either strain stained with toluidine blue; only a few stained with toluidine blue at day 3. However at day 10, 40–60% of the cells in the cultures from both strains contained metachromatic granules when stained with toluidine blue. The serglycin transcript was detected in the starting bone marrow cells from both strains (Fig. 2), but the steady-state level of this transcript was notably higher at day 3 and the level of this proteoglycan peptide core transcript remained very high throughout the subsequent 28 d of culture. A substantial increase in the steady-state level of the mMC-CPA transcript also occurred at day 3. No mMCP-2 or mMCP-4 transcripts were detected in the BALB/cj mBMMC cultures at days 0, 3, 7, 10, 14, 21, and 28. In contrast, WB-+/+ mBMMC contained detectable steady-state levels of the

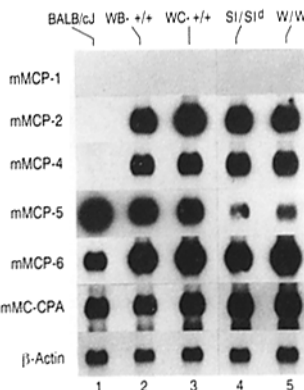


Figure 1. RNA blot analyses of the steady-state levels of the mMCP-1, mMCP-2, mMCP-4, mMCP-5, mMCP-6, mMC-CPA, and β -actin transcripts in mBMMC derived by culturing bone marrow cells from BALB/cj (lane 1), WB-+/+ (lane 2), WC-+/+ (lane 3), *Sl/Sl^d* (lane 4), and *W/W^v* (lane 5) mice for 4 wk in enriched medium supplemented with 100 U/ml rIL-3. Similar data were obtained in a repeat experiment, and in two additional experiments with mBMMC derived with WCM rather than rIL-3.

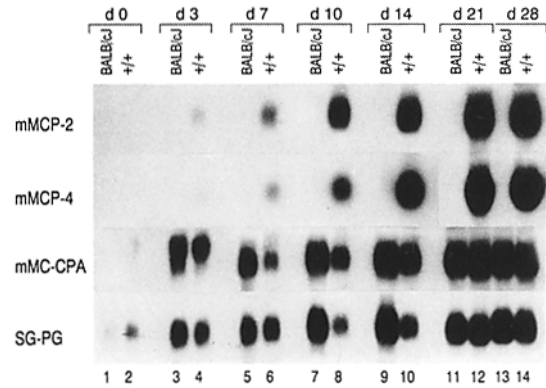


Figure 2. RNA blot analyses of the steady-state levels of the mMCP-2, mMCP-4, mMC-CPA, and serglycin (SG-PG) transcripts in starting bone marrow cells from BALB/cj mice (lane 1) and WB-+/+ mice (lane 2); bone marrow cells from BALB/cj mice cultured for 3 d (lane 3), 7 d (lane 5), 10 d (lane 7), 14 d (lane 9), 21 d (lane 11), and 28 d (lane 13) in the presence of WCM; and bone marrow cells from WB-+/+ mice cultured for 3 d (lane 4), 7 d (lane 6), 10 d (lane 8), 14 d (lane 10), 21 d (lane 12), and 28 d (lane 14) in the presence of WCM. Similar data were obtained in two repeat experiments of 10- and 14-d duration.

mMCP-2 transcript at day 3, moderate levels at day 7, and maximal levels at days 10–14. The mMCP-4 transcript was detected in cultures of WB-+/+ mBMMC at day 7, but maximal levels of this protease transcript were not detected until day 14.

Histochemistry, Immunohistochemistry, and Histamine Content of mBMMC from Different Strains. Whether grown in the presence of rIL-3 or WCM, mBMMC from BALB/cj, WB-+/+, WC-+/+, *Sl/Sl^d*, *W/W^v*, and C57BL/6J mice were alcian blue⁺/safranin⁻ when stained, suggesting that the mBMMC from these strains exhibit no substantial difference in the amount of heparin they synthesize. Whether derived with rIL-3 or WCM, mBMMC from BALB/cj, WB-+/+, WC-+/+, *Sl/Sl^d*, or *W/W^v* mice all contained <300 ng histamine/10⁶ cells. The mean percentages of mBMMC (>325 cells examined in each strain) that reacted with anti-mMCP-5₍₁₄₆₋₁₆₂₎ IgG were 63% for WC-+/+ (Fig. 3), 23% for WB-+/+ (data not shown), 65% for *W/W^v* (Fig. 3), and 54% for BALB/cj (Fig. 3) mice. Whereas 53, 48, and 72% of the mBMMC from the WC-+/+, WB-+/+, and *W/W^v* strains, respectively, reacted with anti-mMCP-2₍₅₆₋₇₁₎ IgG, only 1% of the mBMMC from the BALB/cj and C57BL/6J mouse strains reacted with mMCP-2. Approximately 1% of the mBMMC in all preparations were reactive when incubated without primary anti-mMCP-2₍₅₆₋₇₁₎ IgG, or without primary anti-mMCP-5₍₁₄₆₋₁₆₂₎ IgG.

Effects of Stimulatory and Inhibitory Cytokines on the Steady-State mMCP mRNA Levels in mBMMC from WB-+/+, W/W^v, and BALB/cj Mice. Since it had been previously shown that rIL-10 induces and rIL-4 suppresses the expression of mMCP-1 and mMCP-2 in BALB/cj mBMMC, mBMMC from WB-+/+ and *W/W^v* mice were exposed to WCM and rIL-10 in the presence or absence of rIL-4 to assess the ability of these two recombinant cytokines to alter protease expression. After exposure to rIL-10 for 72 h, both

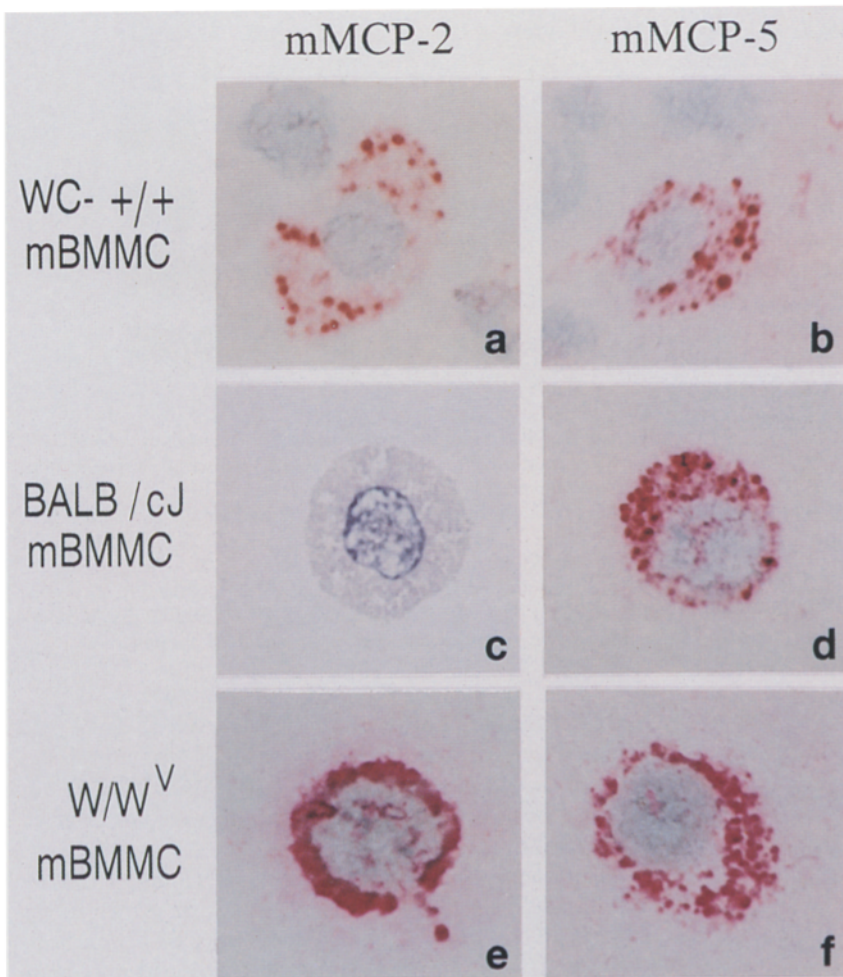


Figure 3. WC-*+/+*, BALB/*cJ*, and *W/W^v* mBMMC, obtained by culturing progenitor cells for 3 wk in WCM, were stained with anti-mMCP-2₍₅₆₋₇₁₎ IgG (panels *a*, *c*, and *e*) or anti-mMCP-5₍₁₄₆₋₁₆₂₎ IgG (panels *b*, *d*, and *f*). The red alkaline phosphatase immunoreaction product overlies mast cell granules (panels *a*, *b*, *d*, *e*, and *f*). BALB/*cJ* mBMMC do not react with anti-mMCP-2₍₅₆₋₇₁₎ (panel *c*). WB-*+/+* mBMMC (not shown) react in the same manner as WC-*+/+* and *W/W^v*-mBMMC.

BALB/*cJ* mBMMC and WB-*+/+* mBMMC contained high steady-state levels of the mMCP-1 and mMCP-2 transcripts (Fig. 4, lanes 2 and 5). In the depicted experiment, the levels of the mMCP-1, and mMCP-2 transcripts in BALB/*cJ* mBMMC exposed concomitantly to WCM, rIL-10, and rIL-4 (lane 3) were 55 and 64% less, respectively, than the levels in BALB/*cJ* mBMMC exposed to WCM and rIL-10 (lane 2). In contrast, the level of the mMCP-1 and mMCP-2 transcripts in WB-*+/+* mBMMC exposed concomitantly to WCM, rIL-10, and rIL-4 (lane 6) were only 3 and 9% less, respectively, than the levels in WB-*+/+* mBMMC exposed to WCM and rIL-10 (lane 5). In a second experiment, the level of mMCP-1 mRNA in BALB/*cJ* mBMMC was 51% less when the cells were exposed concomitantly to WCM, rIL-10, and rIL-4 than when they were exposed to WCM and rIL-10; there was no decrease in the level of this protease transcript in WB-*+/+* mBMMC exposed concomitantly to WCM, rIL-10, and rIL-4. Similar findings were obtained in a third experiment with *W/W^v* mBMMC (data not shown). Thus, as it did in BALB/*cJ* mBMMC, rIL-10 induced high steady-state levels of the mMCP-1 transcript in *W/W^v* and WB-*+/+* mBMMC. However, unlike its effect on BALB/*cJ* mBMMC, rIL-4 was not able to suppress the level of the induced mMCP-1 and constitutive mMCP-2 transcripts in mBMMC obtained from the *W/W^v* and WB-*+/+* strains.

To assess further the ability of rIL-4 to regulate protease levels in WB-*+/+* mBMMC, these cells were cultured for an additional 2 wk in the presence of rIL-3 alone or in the presence of both rIL-3 and rIL-4. In two separate experiments,

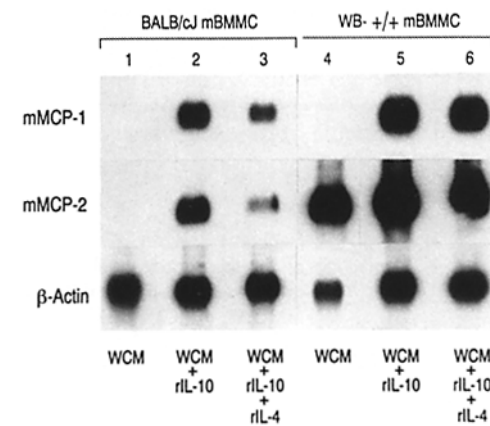


Figure 4. RNA blot analyses of the effect of rIL-4 on the rIL-10-induced expression of mMCP-1 and mMCP-2 in mBMMC. BALB/*cJ* mBMMC (lanes 1-3) and WB-*+/+* mBMMC (lanes 4-6) were cultured for an additional 72 h in WCM alone (lanes 1 and 4), WCM supplemented with rIL-10 (lanes 2 and 5), or WCM supplemented with rIL-10 and rIL-4 (lanes 3 and 6). *W/W^v* BMMC (not shown) react in the same manner as WB-*+/+* mBMMC.

rIL-4 did not reduce the constitutive high steady-state levels of the mMCP-2 or mMCP-4 transcripts in the cells of this strain (data not shown).

Discussion

BALB/cJ mouse SMC express mMCP-4 but not mMCP-1, whereas BALB/cJ mouse mucosal mast cells express mMCP-1 but not mMCP-4 (9, 22–24). We previously reported that rIL-10-treated BALB/c mBMMC express high steady-state levels of the mMCP-1 transcript but not the mMCP-4 transcript (9), whereas KL-treated mBMMC express the same panel of proteases as SMC, including mMCP-4 (7). Because *W/W^v* and *Sl/Sl^d* mice do not contain appreciable numbers of SMC (15), it was concluded that *c-kit* receptor and its ligand induce mast cell-committed progenitor cells to differentiate in a SMC lineage. We report in this study that mBMMC developed from *W/W^v* mice, *Sl/Sl^d* mice, and their *+/+* littermates differ from mBMMC developed from C57BL/6J mice and BALB/cJ mice in that they additionally have high steady-state levels of mMCP-2 and mMCP-4 transcripts (Fig. 1) and mMCP-2 protein (Fig. 3). High steady-state levels of the mMC-CPA and serglycin transcripts were obtained 3 d after bone marrow cells from WB-*+/+* and BALB/cJ mice were exposed to WCM. WB-*+/+* mBMMC contained measurable levels of the mMCP-2 and mMCP-4 transcripts at day 7 and maximal levels at day 14. However, even at day 28, the steady-state levels of the mMCP-2 and mMCP-4 transcripts were below detection in BALB/cJ mBMMC (Fig. 2). The observation that *W/W^v* and *Sl/Sl^d* mBMMC express every protease that is found in the SMC of their normal *+/+* littermates (Fig. 1) now indicates that *c-kit*-mediated signal transduction is not essential for inducing transcription of SMC-specific protease genes.

It was previously shown that rKL elicits the expression of mMCP-4 in BALB/cJ mBMMC (7), whereas rIL-9 or rIL-10 in the presence of rKL elicit the expression of mMCP-1 and mMCP-2 in these cells (8). When present concomitantly, rIL-3 and rIL-4 each suppress the rIL-9-induced accumulation of the mMCP-1 and mMCP-2 transcripts and the rKL-induced accumulation of the mMCP-4 transcript in BALB/cJ mBMMC (7, 8). Although mBMMC from the WB-*+/+* and WC-*+/+* strains constitutively express mMCP-2 and all of the other granule proteases of SMC, they still require rIL-10 to induce the mucosal mast cell-specific protease (23) mMCP-1 (Fig. 4). rIL-4 was not able to suppress the rIL-10-induced expression of the mMCP-1 transcript in WC-*+/+* mBMMC and *W/W^v* mBMMC or any other protease transcript in these mast cells (Fig. 4). The resistance of the mBMMC from the *W/W^v*, *Sl/Sl^d*, and *+/+* strains to the suppressive effects of rIL-3 and rIL-4 could be explained by a difference in the mast cell-committed progenitor cells in

their bone marrows. During the first week of culture of bone marrow cells in the presence of rIL-3 or WCM, mast cells are a small proportion of the total cells in the culture (1, 2, 18). Thus, an alternative explanation for the expression of mMCP-2 and mMCP-4 in WB-*+/+* mBMMC and WC-*+/+* mBMMC is that the contaminating cell types present during the first week of culture of the cells from these strains, but not from the BALB/cJ strain, release factors that are able to counteract the suppressive effects of IL-3 and IL-4 on the expression of certain proteases. If so, it is unlikely that these autocrine factors include IL-9 or IL-10, inasmuch as mBMMC from the *+/+* strains do not express mMCP-1 (Fig. 1) unless they are exogenously stimulated (Fig. 4).

The *Sl/Sl^d* mouse and the *W/W^v* mouse both have substantially diminished numbers of mast cells in their tissues. The *Sl/Sl^d* mouse does not produce the membrane-bound form of KL (6, 12) and the *W/W^v* mouse possesses an altered *c-kit* (13). The daily administration of 30 μ g/kg of rat rKL to WC-*+/+* mice and *Sl/Sl^d* mice produces a dramatic increase in the number of dermal mast cells (35). Thus, whether acting directly or indirectly, KL is an important regulatory cytokine for this mast cell subclass in vivo. That bone marrow cells from both mast cell-deficient mouse strains give rise to large numbers of mBMMC in vitro (18) indicates that these mice do not have reduced numbers of mast cell-committed progenitor cells. Furthermore, mBMMC from both *Sl/Sl^d* mice and *W/W^v* mice are indistinguishable from mBMMC of their *+/+* littermates in terms of their expression of high steady-state levels of the mMCP-2, mMCP-4, mMCP-5, mMCP-6, and mMC-CPA transcripts (Fig. 1). Thus, mBMMC obtained from *Sl/Sl^d* and *W/W^v* mice with WCM or with rIL-3 alone express the five granule proteases found in the SMC and CTMC of their *+/+* littermates. Gordon and Galli (20) reported that substantial numbers of mast cells can be elicited in the phorbol ester-treated skin of the *W/W^v* mouse but not the skin of the *Sl/Sl^d* mouse. In addition, Ody et al. (36) reported that normal numbers of mature safranin⁺ mast cells appear in the skin of the *W/W^v* mouse when high concentrations of rIL-3 are perfused into the peritoneum continuously for 28 d. That *W/W^v* mBMMC contain high steady-state levels of five granule protease transcripts expressed by mature SMC and CTMC of their normal *+/+* littermates suggests that in this strain, IL-3 can compensate for the signal transduction abnormality in *c-kit* to promote the viability and differentiation of progenitor cells into mast cells that exhibit the granule protease phenotype of SMC and CTMC. Nevertheless, the failure to elicit mast cells in inflammatory sites in the skin of the *Sl/Sl^d* mouse suggests that *c-kit* and its ligand are essential for the homing, retention, and/or life span of mast cell-committed progenitors in the skin.

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