PHENOTYPIC CHANGES OF BONE MARROW–DERIVED MAST CELLS AFTER INTRAPERITONEAL TRANSFER INTO W/W^v MICE THAT ARE GENETICALLY DEFICIENT IN MAST CELLS

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Mouse mast cells can be assigned to two subclasses, defined as connective tissue–type and mucosal-type mast cells. Mucosal-type mast cells can be distinguished from connective tissue–type mast cells by their anatomical location, histochemical staining properties (1), and T cell dependence for proliferation during a helminthic infection (2). Mouse serosal mast cells (SMC)[1], which are connective tissue–type mast cells, express on their surface the neutral glycosphingolipid globopentaosylceramide (which contains the Forssman epitope) (3), and contain in their secretory granules protease-resistant heparin proteoglycans of ~750,000 M_r (4, 5). Although definitive biochemical data are not available, as assessed by their histochemical staining properties (1), mouse intestinal mucosal mast cells contain little or no heparin proteoglycans.

In 1981, several laboratories reported (6–10) that cells that possessed the phenotypic characteristics of mast cells could be grown from mouse hematopoietic progenitors in the presence of conditioned medium containing T cell–derived soluble factors. These cultured mast cells exhibited a number of similarities to mouse mucosal mast cells, including alcian blue–positive/safranin-negative histochemical staining properties and dependence on T cell factors for their growth. The mouse bone marrow–derived mast cells (BMMC), when cultured in

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[1] *Abbreviations used in this paper:* BMMC, bone marrow-derived mast cells; HBA, HBSS containing BSA and sodium azide; SMC, serosal mast cells; TG, Tyrode's buffer with gelatin; TSG buffer, Tris-HCl, sodium sulfate, and guanidine-HCl.

Con A/splenocyte-conditioned medium, WEHI-3 conditioned medium, or purified IL-3, preferentially synthesize and store in their secretory granules proteoglycans of ~200,000 M_r that contain chondroitin sulfate E glycosaminoglycans (4). Only small amounts of proteoglycans that bear heparin glycosaminoglycans are detectable in these cells (11). BMMC do not have detectable amounts of globopentaosylceramide, but express on their surface the probable precursor of this glycosphingolipid, globotetraosylceramide (globoside) (12). When cocultured on mouse skin-derived 3T3 fibroblasts, the in vitro-derived BMMC become more similar to connective tissue mast cells than to mucosal mast cells in that they become alcian blue-positive/safranin-positive, increase their histamine content ~15-fold, synthesize considerably more proteoglycans bearing heparin glycosaminoglycans (13), and increase their expression of the Forssman epitope (our unpublished observations).

A possible lineal relationship between mucosal-type mast cells and connective tissue-type SMC has been indicated by in vivo studies (14, 15) in which the subclasses of mast cells detected in mast cell-deficient mice after adoptive transfer of BMMC were defined by their tissue location and staining properties. When alcian blue-positive/safranin-negative/berberine sulfate-negative BMMC from $WBB6F_1$-$^{+/+}$ mice were injected into the peritoneal cavities of mast cell deficient $WBB6F_1$-W/W^v mice, the resultant SMC that were recovered after 10 wk contained ~20 times more histamine than did the BMMC and contained granules that were alcian blue-positive/safranin-positive/berberine sulfate-positive (14). BMMC that contained abnormally large granules derived from C57BL/6-bg^J/bg^J (beige) mutant mice were used to confirm that the SMC appearing in mast cell-deficient recipients were of donor origin. That the mast cells in the glandular stomach mucosa of the reconstituted animals stained with alcian blue but not with berberine sulfate or safranin indicated that the tissue microenvironment regulated the mast cell phenotype. Moreover, in vivo studies have also revealed that partially purified (14) or single (15) SMC can give rise to cells with phenotypic characteristics of both subclasses of mast cells after injection into $WBB6F_1$-W/W^v mice.

In the present study, we demonstrate that T cell factor-dependent cultured mouse BMMC injected into the peritoneal cavities of $WBB6F_1$-W/W^v mice undergo phenotypic biochemical changes that are characterized by a marked increase in the biosynthesis of heparin proteoglycans and in the surface expression of the Forssman epitope of globopentaosylceramide.

Materials and Methods

Materials. Penicillin, streptomycin, PWM, RPMI 1640, and DME (Grand Island Biological Co., Grand Island, NY); horse serum (Sterile Systems, Inc., Logan, UT); FCS (Irvine Scientific, Santa Ana, CA); α medium (Flow Laboratories, Inc., Irvine, Scotland); Sephadex G-25/PD-10 gel filtration columns and Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ); ^{3}H-labeled heparin (0.08 mCi/mg) and [^{35}S]sulfate (~4,000 Ci/mmol) (New England Nuclear, Boston, MA); Partisil-10 PAC amino-cyano HPLC columns (Whatman Inc., Clifton, NJ); Hydrofluor (National Diagnostics, Somerville, NY); 1,2-dimethoxyethane and butyl nitrite (Eastman Kodak Co., Rochester, NY); heparin (Sigma Chemical Co., St. Louis, MO); Zwittergent 3-12 (Calbiochem-Behring Corp., La Jolla, CA); chondroitin sulfate A, chondroitin sulfate C, *Flavobacterium heparinum* heparinase, *Proteus vulgaris* chondroitinase ABC, *Proteus vulgaris* chondro-6-sulfatase, *Proteus*

vulgaris chondro-4-sulfatase, ΔDi-OS, ΔDi-4S, and ΔDi-6S (Miles Laboratories Inc., Elkhart, IN); and fluorescein-conjugated $F(ab')_2$ fragments of affinity-purified goat anti-rat IgM (CooperBiomedical, Inc., Malvern, PA) were obtained as noted. Rat chondrosarcoma ^{35}S-labeled proteoglycan ($M_r = \sim 2 \times 10^6$) was prepared as previously described (16). The unsaturated ^{35}S-labeled disaccharide, $\Delta Di\text{-}diS_E$, was obtained by chondroitinase ABC treatment of ^{35}S-labeled mouse BMMC proteoglycan (4).

Mice. Mast cell–deficient $WBB6F_1\text{-}W/W^v$ mice (having no detectable SMC and <1% of the normal number of skin connective tissue mast cells [17]) and $WBB6F_1\text{-}^{+/+}$ mice were raised to 2–6 mo old at the Osaka University Medical School and the Shizuoka Laboratory Animal Center, Japan. The original stocks of parental strains (i.e., WB-*W*/+ and C57BL/6-W^v/+) were obtained from The Jackson Laboratory (Bar Harbor, ME). The WB-*W*/+ strain was maintained by brother-sister matings, but the W^v mutant gene was maintained in C57BL/6 mice of the Osaka University inbred colony for more than 30 backcrosses.

Culture of T Cell Factor–dependent Mouse BMMC. Mouse bone marrow cells were suspended at a concentration of 10^6/ml in α medium supplemented with 10^{-4} M 2-ME, 20% horse serum, and 10% PWM-stimulated spleen cell–conditioned medium as described previously (14). The conditioned medium was prepared by incubating spleen cells for 5 d at 2×10^6 cells/ml in α medium containing a 1:300 dilution of pokeweed mitogen, 10% FCS, and 10^{-4} M 2-ME. After centrifugation, the conditioned medium was filtered through 0.45 μm filters and stored at −80°C. Cells were maintained in a humidified atmosphere of 5% CO_2 for 4–7 wk, and the non-adherent cells were placed into fresh culture media every 7 d. At a time when the mast cell purity exceeded 95% as assessed histochemically, the BMMC were washed and 10^6 BMMC in 1.0 ml of α medium were injected intraperitoneally per mouse.

Purification of Serosal Mast Cells. For each experiment, the mast cells were isolated from the peritoneal cavities of 40–50 $WBB6F_1\text{-}^{+/+}$ mice and $WBB6F_1\text{-}W/W^v$ mice injected 15 wk earlier with $WBB6F_1\text{-}^{+/+}$–derived BMMC. Tyrode's buffer with gelatin (TG; 2 ml) was injected into the peritoneal cavity of each mouse. After gentle massage of the abdomen for ~30 s, the peritoneal cavity was opened, and the fluid containing the SMC was aspirated. The cells from groups of 10 mice were pooled, centrifuged at 150 *g* for 10 min at room temperature, resuspended in 1 ml of TG, and layered over 2 ml of 22.5% metrizamide. After centrifugation at 350 *g* for 15 min at room temperature, the mast cells were harvested at the bottom of the tube. The mast cell purity after a single metrizamide gradient sedimentation was 70–80%, and these cells were used routinely for biochemical analyses. The purity of the mast cells was >95% after a second metrizamide gradient sedimentation, and this latter population of cells was used for cytofluorographic analysis. Serosal and cultured mast cells were quantified using a hemocytometer after staining with neutral red (0.02% in 0.9% NaCl).

[^{35}S]Sulfate Labeling of Mast Cells and Purification of ^{35}S-labeled Proteoglycans. Purified mouse SMC and BMMC ($2–10 \times 10^6$ cells) were suspended either in sulfate-free RPMI 1640 or in a 1:3 mixture of normal and sulfate-free RPMI 1640 containing 10% FCS at a concentration of 10^6 cells/ml. [^{35}S]Sulfate (100 μCi/ml) was added, and the cell suspensions were incubated for 3–20 h at 37°C in 5% CO_2. The ^{35}S-labeled mast cells were pelleted by centrifugation at 150 *g*, washed three times with RPMI 1640 medium, and then disrupted by the addition of 500 μl of 4 M guanidine-HCl containing 0.1% Zwittergent 3-12 and sufficient solid CsCl to achieve a final density of 1.4 g/ml (4). Porcine heparin glycosaminoglycan (0.1 mg) was routinely added as a carrier to prevent nonspecific loss of ^{35}S-labeled proteoglycans. Samples were centrifuged at 17°C for 48 h at 100,000 *g* using a SW40-Ti rotor (Beckman Instruments, Inc., Fullerton, CA) (18). The bottom 50% of each gradient was dialyzed against 0.1 M NH_4HCO_3, lyophilized, and then redissolved in distilled water.

Gel Filtration Chromatography of ^{35}S-labeled Mast Cell Proteoglycans before and after Treatment with Either Pronase or NaOH. The approximate M_r of the density gradient-purified ^{35}S-labeled mast cell proteoglycans before and after either Pronase (19) or alkaline treatment (4) was determined by Sepharose CL-4B chromatography. For Pronase diges-

tion, 20 μl of 10 mg/ml Pronase and 30 μl of 0.25 M Tris-HCl, pH 8.0, were added to 100-μl samples of density gradient–purified mast cell ^{35}S-labeled proteoglycans containing 2–5 × 10^3 cpm. After a 1-h incubation at 37°C, 250 μl of distilled water and 400 μl of 8 M guanidine-HCl/0.2 M sodium sulfate/0.2 M Tris-HCl, pH 7.0, were added to each Pronase digest. Replicate 100-μl samples were incubated at 22°C for 20 h in 0.2 M NaOH to remove the glycosaminoglycan side chains from the ^{35}S-labeled proteoglycans via β elimination. After neutralization with acetic acid, the samples were mixed with 300 μl of distilled water and 400 μl of 8 M guanidine-HCl/0.2 M sodium sulfate/0.2 M Tris-HCl, pH 7.0. Native, Pronase- and alkali-treated ^{35}S-labeled proteoglycan samples were applied separately to 0.8 × 90-cm columns of Sepharose CL-4B that had been previously equilibrated with 0.1 M Tris-HCl, 0.1 M Na_2SO_4, and 4 M guanidine-HCl, pH 7.0 (TSG buffer). The Sepharose CL-4B columns were eluted at a flow rate of 5 ml/h, and 0.9 ml fractions were collected. Each fraction was mixed with 0.9 ml of ethanol and 10 ml of Hydrofluor, and the radioactivities were quantified using a Tracor Analytic Mark III liquid scintillation counter.

Characterization of the ^{35}S-labeled Glycosaminoglycans Bound to Mast Cell Proteoglycans. ^{35}S-Labeled proteoglycans isolated from the different populations of mast cells were resuspended in 500 μl of water, and 100-μl samples were incubated with chondroitinase ABC (20, 21), heparinase (22), or nitrous acid (23). The resulting samples and untreated material were chromatographed on Sephadex G-25/PD-10 columns to determine the nature of the ^{35}S-labeled glycosaminoglycans bound to the different proteoglycan samples by following the shift in radioactivity from the excluded to the included volumes. For the control samples, 100 μl of each preparation was suspended in 400 μl of TSG buffer containing 200 μg of heparin glycosaminoglycan carrier. To determine the susceptibility to degradation by chondroitinase ABC, preparations were suspended in 250 μl (final volume) of Tris-enriched buffer (20) containing 10 mM NaF and 200 μg of chondroitin sulfate A, and were incubated at 37°C for 30 min with 0.5 U of chondroitinase ABC. To determine the susceptibility to degradation by heparinase, preparations were suspended in 250 μl (final volume) of 0.1 M sodium acetate buffer, pH 7.0, containing 1 mM calcium acetate, 0.1% toluene, 100 μg of heparin, and ~30 mU of heparinase, and then were incubated at 37°C for 16 h. To determine the susceptibility to degradation by nitrous acid, 100-μl samples were incubated with 12.5 μl of butyl nitrite, 125 μl of 1,2-dimethoxyethane, and 200 μg of heparin for 17 h at −20°C. At the end of the chondroitinase ABC, heparinase, and nitrous acid treatments, 250 μl of 8 M guanidine-HCl/0.2 M sodium sulfate/0.2 M Tris-HCl, pH 7.0, was added to each mixture. All samples were then applied to separate Sephadex G-25/PD-10 columns that had been equilibrated with TSG buffer. The columns were eluted with TSG buffer, and 0.5-ml fractions were collected, mixed with 0.5 ml of ethanol and 10 ml of Hydrofluor, and the radioactivity was determined.

The nature of the ^{35}S-labeled unsaturated disaccharides liberated by chondroitinase ABC treatment of the ^{35}S-labeled mast cell proteoglycans was determined by HPLC as described by Seldin et al. (24). Replicate samples of each ^{35}S-labeled proteoglycan preparation were incubated as described above with chondroitinase ABC alone; chondroitinase ABC and 0.5 U chondro-6-sulfatase; or chondroitinase ABC, 0.5 U chondro-6-sulfatase, and 0.5 U chondro-4-sulfatase. At the end of each digestion, 1.2 ml of ethanol was added, and the samples were placed on ice for 1 h and centrifuged at 8,000 *g* for 10 min at 4°C. The supernatants were dried under a stream of nitrogen, suspended in 100 μl of 70% acetonitrile/methanol (3:1, vol/vol) and 30% 0.5 M ammonium acetate/acetic acid, pH 5.3, and chromatographed on a 4.6 × 250 mm Partisil-10 column at a flow rate of 1 ml/min. The radioactivity in each 0.5-ml fraction was determined by liquid scintillation counting. The HPLC column was standardized with the unsaturated chondroitin sulfate–derived disaccharides ΔDi-OS, ΔDi-4S, ΔDi-6S, and ΔDi-diS_E.

Analysis of Cell Surface Expression of the Forssman Epitope. The rat IgM mAb B1.1 (3) was used to evaluate the expression of the Forssman epitope on the cell surface of the different populations of mast cells. A rat monoclonal IgM anti-TNP antibody (RATNP 17.3) (obtained from Dr. P. A. LeBlanc, University of Alabama, Tuscaloosa, AL) was

used as a control reagent. Replicate samples of 5×10^5 BMMC, SMC harvested from WBB6F$_1$-$^{+/+}$ mice, and SMC recovered from WBB6F$_1$-W/W^v mice that had been reconstituted with BMMC were each placed in 1.5-ml polypropylene conical-bottomed centrifuge tubes with calcium- and magnesium-free HBSS containing 0.1% BSA and 0.02% sodium azide (HBA). The cells were centrifuged at 1,000 *g* for 1 min, and replicate pellets of each cell preparation were incubated in an ice bath for 30 min with 50 μl of either the B1.1 or the RATNP 17.3 mAb (350 μg IgM/ml). An excess of HBA was added, and the cells were washed twice by centrifugation. 50 μl of fluorescein-conjugated F(ab')$_2$ fragments of affinity-purified goat anti-rat IgM diluted 1:50 in HBA was added to each cell pellet. The cells were resuspended, incubated in the dark for 30 min in an ice bath, washed by centrifugation, and resuspended in HBA for flow cytometric analysis on a FACS IV (Becton Dickinson & Co., Oxnard, CA). Cells positive for the binding of the B1.1 mAb were defined as those exhibiting channel numbers of fluorescence greater than those of 97% of the replicate cells interacted with the RATNP 17.3 mAb. The net mean channel number of fluorescence (a measure of fluorescence intensity) was defined as the mean channel number of fluorescence of cells stained with B1.1 antibody minus that of cells incubated with the RATNP 17.3 antibody.

Results

Characterization of ^{35}S-labeled Mast Cell Proteoglycans. The three populations of mast cells that were analyzed for their cell-associated proteoglycans were the starting population of BMMC from normal +/+ mice, SMC from +/+ mice, and SMC that were recovered from the peritoneal cavities of W/W^v mice 15 wk after the injection of BMMC from +/+ mice. After density-gradient centrifugation of the detergent/guanidine-HCl lysates of the three populations of ^{35}S-labeled mast cells, in each instance >85% of the ^{35}S-labeled macromolecules were recovered in the bottom half of the gradients, consistent with the preferential incorporation of this radioisotope into proteoglycans. Samples of these partially purified ^{35}S-labeled proteoglycans were chromatographed on a Sepharose CL-4B column under dissociative conditions to determine their hydrodynamic sizes. Based on the obtained K_{av} values of 0.34 ± 0.035 (mean ± SD, $n = 8$), 0.26 ± 0.035 (mean ± SD, $n = 4$), and 0.25 ± 0.05 (mean ± SD, $n = 4$), it was concluded that the M_r of the ^{35}S-labeled proteoglycans in the starting BMMC (Fig. 1*A*), the SMC from the +/+ animals (Fig. 1*B*), and the SMC recovered from the reconstituted W/W^v animals (Fig. 1*C*) were ~350,000; ~600,000; and ~650,000 M_r; respectively. The ^{35}S-labeled proteoglycans in all studied populations of mast cells were resistant to degradation by Pronase, since their hydrodynamic size on Sepharose CL-4B did not change appreciably after enzymatic treatment (Fig. 1, *A* and *C*).

Characterization of ^{35}S-labeled Glycosaminoglycans. The ^{35}S-labeled glycosaminoglycan side chains from the starting BMMC (Fig. 2*A*), the SMC from normal +/+ mice (Fig. 2*B*), and the SMC from the reconstituted animals (W/W^v) (Fig. 2*C*) filtered on Sepharose CL-4B with K_{av} values of 0.60 ± 0.013 (mean ± SD, $n = 5$), 0.49 ($n = 1$), and 0.51 ± 0.031 (mean ± SD, $n = 3$), indicative of M_r of ~55,000; ~115,000; and ~105,000; respectively.

Replicate samples of partially purified mast cell proteoglycans were subjected to either chondroitinase ABC, heparinase, or nitrous acid treatments, and the resulting digests were chromatographed on Sephadex G25/PD-10 columns under dissociative conditions to determine the nature of the ^{35}S-labeled glycosaminoglycans. In control experiments, both untreated ^{35}S-labeled chondrosarcoma chondroitin sulfate proteoglycans and untreated ^{3}H-labeled heparin glycosami-

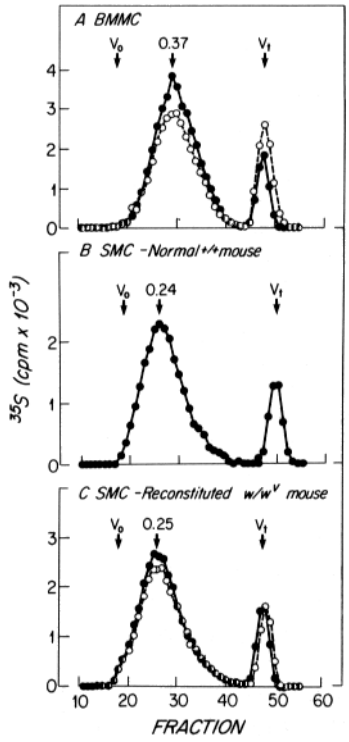

FIGURE 1. Sepharose CL-4B chromatography of the cell-associated ^{35}S-labeled proteoglycans from mouse BMMC (*A*), SMC from +/+ mice (*B*), and SMC from reconstituted *W/W*^{v} mice (*C*) before (●) and after (○) incubation with Pronase. V_o and V_t mark the columns' void volumes and total volumes, respectively. Data are from a representative experiment.

noglycans eluted in the excluded volumes of the columns. After treatment of the ^{35}S-labeled chondrosarcoma proteoglycans with chondroitinase ABC, 95 ± 1% (mean ± SD, n = 3) of the radioactivity filtered in the included volume of the column, whereas after treatment with nitrous acid and heparinase, <4% of the ^{35}S-labeled proteoglycans filtered in the included volume of the column. After incubation of the ^{3}H-labeled heparin glycosaminoglycan with heparinase, nitrous acid, and chondroitinase ABC, 79 ± 9%, 89 ± 4%, and 2 ± 1% (mean ± SD, n = 3) of the radioactivity, respectively, filtered in the included volume of the columns.

As assessed by Sephadex G25/PD-10 chromatography, 86 ± 11%, 9 ± 11%, and 10 ± 11% (mean ± SD, n = 8) of the ^{35}S-labeled glycosaminoglycans of the mouse BMMC that had been differentiated in vitro in the presence of PWM-conditioned media were degraded by chondroitinase ABC, nitrous acid, and heparinase, respectively (Fig. 3*A*). Three out of eight of these cultures differed from the BMMC cultures presented in Fig. 3*A* in that 15–32% of the ^{35}S-labeled glycosaminoglycans were degraded as heparin. When analyzed by HPLC, 58 ± 5% and 42 ± 5% (mean ± SD, n = 5) of the ^{35}S-labeled unsaturated disaccharides generated by chondroitinase ABC treatment of the BMMC samples eluted at the retention times corresponding to the ΔDi-4S and ΔDi-diS_E standards, respectively

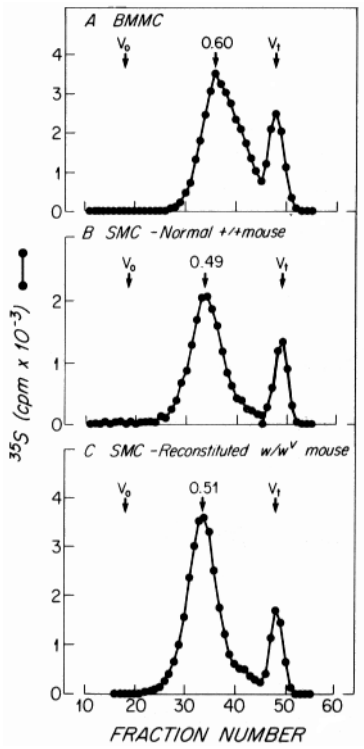

FIGURE 2. Sepharose CL-4B chromatography of the ^{35}S-labeled glycosaminoglycans β-eliminated from the proteoglycans of mouse BMMC (*A*), SMC from +/+ mice (*B*), and SMC from reconstituted *W/W*^{v} mice (*C*). V_o and V_t mark the columns' void volumes and total volumes, respectively. Data are from a representative experiment.

(data not shown). When these ^{35}S-labeled proteoglycans from BMMC were incubated with both chondro-6-sulfatase and chondroitinase ABC, almost all of the radioactivity associated with the ΔDi-diS_E peak disappeared, whereas the radioactivity associated with the ΔDi-4S and free sulfate peaks increased. Incubation of the samples with chondro-6-sulfatase, chondro-4-sulfatase, and chondroitinase ABC resulted in the elution of almost all of the radioactivity into the free [^{35}S]sulfate peak. Thus, mouse BMMC differentiated in PWM-conditioned media synthesize proteoglycans that contain primarily chondroitin sulfate E glycosaminoglycans, although variable amounts of heparin glycosaminoglycans are also present.

When ^{35}S-labeled proteoglycans produced by normal SMC from +/+ mice were incubated with chondroitinase ABC, nitrous acid, and heparinase, 5 ± 1%, 82 ± 7%, and 73 ± 6% (mean ± SD, n = 4) of the total macromolecular radioactivity was degraded (Fig. 3*B*). The mast cells recovered from the peritoneal cavities of the reconstituted *W/W*^{v} mice synthesized ^{35}S-labeled proteoglycans that were degraded 5 ± 3%, 84 ± 3%, and 72 ± 7% (mean ± SD, n = 4) by chondroitinase ABC, nitrous acid, and heparinase treatments, respectively (Fig. 3*C*). Because of the low chondroitin sulfate content of the proteoglycans present

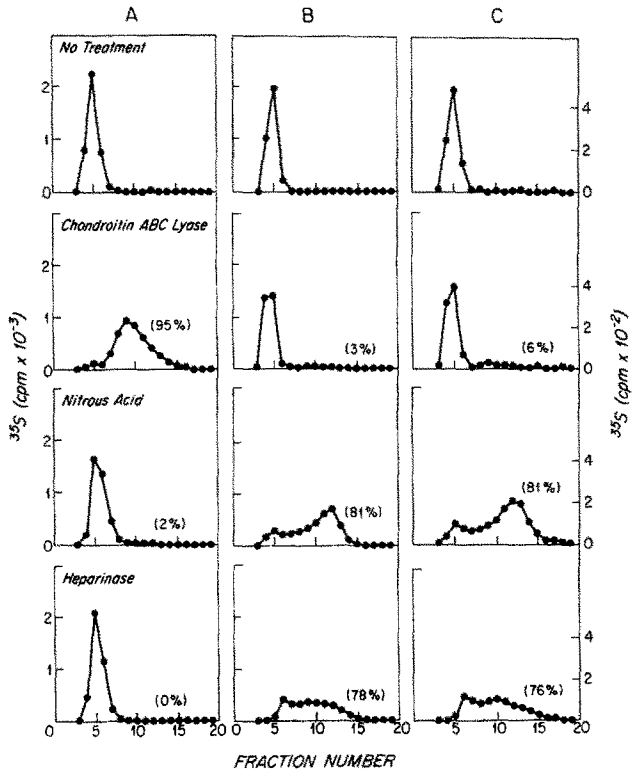

FIGURE 3. Sephadex G25/PD-10 chromatography of the ^{35}S-labeled proteoglycans from mouse BMMC (*A*), SMC from +/+ mice (*B*), and SMC from reconstituted W/W^v mice (*C*) before and after treatment with chondroitinase ABC, nitrous acid, or heparinase. The numbers in parentheses indicate the percentage of the total ^{35}S-labeled macromolecules that were degraded by each treatment. Data are from a representative experiment.

in SMC, it was not possible to analyze their unsaturated chondroitin sulfate disaccharides by HPLC.

Immunofluorescence Analysis of the Surface Expression of the Forssman Epitope. As assessed by indirect immunofluorescence staining and flow cytometric analysis in two experiments, BMMC were 1.2% (Fig. 4*A*) and 7.3% positive for the binding of the B1.1 rat monoclonal anti-Forssman epitope antibody, with net mean channel numbers of fluorescence of 2.0 and 5.5, respectively. SMC purified to ~98% from $WBB6F_1$-$^{+/+}$ mice were 69.7% (Fig. 4*B*) and 93.5% positive for B1.1, with net mean channel numbers of fluorescence of 34.3 and 44.4, respectively. SMC purified to ~98% from the peritoneal cavities of $WBB6F_1$-W/W^v mice 15 wk after the injection of BMMC were 75.0% (Fig. 4*C*) and 68.4% positive for the binding of the B1.1 antibody with net mean channel numbers of 31.7 and 51.1, respectively.

Discussion

In this report, we demonstrate that when T cell factor–dependent cultured $WBB6F_1$-$^{+/+}$ BMMC, which preferentially synthesize ~350,000 M_r chondroitin sulfate E proteoglycans and express little of the Forssman epitope, are injected

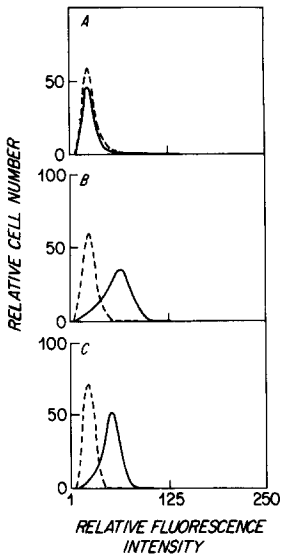

FIGURE 4. Flow cytometric analysis of the surface binding of B1.1 mAb to BMMC (*A*), SMC from +/+ mice (*B*), and SMC from reconstituted W/W^v mice (*C*). Cells were incubated in either negative control antibody (- - -) or B1.1 antibody (——), washed, incubated with fluorescein-conjugated $F(ab')_2$ goat anti-rat IgM, and analyzed on a FACS IV cytofluorograph.

into the peritoneal cavities of genetically mast cell-deficient $WBB6F_1$-W/W^v mice, they give rise to SMC that, like SMC obtained directly from $WBB6F_1$-$^{+/+}$ mice, preferentially synthesize and store ~600,000 M_r heparin proteoglycans and express the Forssman epitope on their cell surface. These findings confirm our previous morphological and histochemical studies showing that T cell factor–dependent mouse BMMC can give rise to mast cells with phenotypic characteristics of SMC (connective tissue-type mast cells) following injection into mast cell-deficient $WBB6F_1$-W/W^v mice. Whereas no cells in the initial populations of BMMC had granules that stained detectably with either berberine sulfate or safranin, berberine sulfate–positive/safranin-positive mast cells developed in those anatomical sites that in normal mice contain connective tissue mast cells (e.g., skin, peritoneal cavity, and gastric muscularis propria) (14). In contrast, berberine sulfate–negative/safranin-negative, mucosal-like mast cells developed in the gastric mucosa of the reconstituted animals. Although the BMMC used in these original reconstitution studies appeared to be homogeneous morphologically and histochemically, they were not cloned. More recently, a T cell factor–dependent population of mast cells was cloned in methylcellulose from cells derived from the peritoneal cavity of $WBB6F_1$-$^{+/+}$ mice. These cloned mast cells, which are berberine sulfate–negative/safranin-negative gave rise to berberine sulfate–positive/safranin-positive SMC 10 wk after they were injected into $WBB6F_1$-W/W^v mice (25). Further, both subclasses of mast cells were detected when a single SMC from a $WBB6F_1$$^{+/+}$ mouse was injected into these mast cell–deficient mice (15). That some populations of BMMC in the present study contained detectable amounts of heparin proteoglycans but did not stain with

either safranin or berberine sulfate may reflect a lesser sensitivity of the staining methods compared to the biochemical techniques.

The chemical definition of the culture-derived BMMC and the phenotypically altered mast cells recovered after adoptive transfer in mast cell–deficient mice is also fully consistent with our parallel in vitro studies (13). Although it has been reported (4) that T cell factor–dependent BMMC cultured in WEHI-3-conditioned media do not synthesize sufficient amounts of heparin glycosaminoglycans to be detected in whole cell extracts, these cells have recently been shown (11) to synthesize small amounts of heparin proteoglycans and to exocytose them preferentially in a complex of $>10^7$ M_r. The finding that the biosynthesis of proteoglycans containing heparin glycosaminoglycans is substantially increased relative to that of proteoglycans containing chondroitin sulfate E glycosaminoglycans when BMMC are cocultured with mouse 3T3 fibroblasts indicates that the phenotype of mast cells is in part regulated by connective tissue fibroblasts (13).

Recent findings suggest that the different phenotypes of the tissue mast cells could reflect differential regulation of a limited number of genes. The mRNA encoding the peptide core of the proteoglycans of mouse BMMC and rat SMC has been analyzed with a cDNA probe derived from a rat yolk sac tumor cell (26) that encodes a serine-glycine rich peptide core. These studies revealed a single, possibly identical, species of mRNA in both mast cell populations (27), consistent with an interpretation that different subclasses of mast cells polymerize different types of glycosaminoglycans onto a common peptide core. As the sequences of the initial four monosaccharides of heparin and chondroitin sulfate E glycosaminoglycans are believed to be the same (28), the regulation of glycosaminoglycan biosynthesis may occur through differential expression of the two glycosyl transferases that mediate the addition of the fifth monosaccharide to the respective chains. However, differential posttranslational modification of a common peptide core that in turn directs these proteins to different glycosaminoglycan-synthesizing compartments in the Golgi, or varied rates of catabolism of heparin-containing proteoglycans versus chondroitin sulfate–containing proteoglycans could also account for the presence of proteoglycans that preferentially bear either heparin or chondroitin sulfate E glycosaminoglycans in the secretory granules of the two types of mast cells. The biosynthesis of globopentaosylceramide is believed to occur by the addition of a single *N*-acetyl-galactosamine to globotetraosylceramide (29). Thus, the switch in the expression of globoside on the cell surface of BMMC to globopentaosylceramide on the cell surface of SMC could also occur by the regulation of a single enzymatic step.

Summary

The ability of mouse IL-3-dependent, bone marrow culture–derived mast cells (BMMC) to generate serosal mast cells (SMC) in vivo after adoptive transfer to mast cell–deficient mice has been defined by chemical and immunochemical criteria. BMMC differentiated and grown from $WBB6F_1$-$^{+/+}$ mouse progenitor cells in medium containing PWM/splenocyte–conditioned medium synthesized a ~350,000 M_r protease-resistant proteoglycan bearing ~55,000 M_r glycosaminoglycans, as defined by gel filtration of each. ~85% of the glycosaminoglycans

bound to the cell-associated BMMC proteoglycans were chondroitin sulfates based upon their susceptibility to chondroitinase ABC digestion; HPLC of the chondroitinase ABC–generated unsaturated disaccharides revealed these glycosaminoglycans to be chondroitin sulfate E. As determined by heparinase and nitrous acid degradations, ~10% of the glycosaminoglycans bound to BMMC proteoglycans were heparin. In contrast, mast cells recovered from the peritoneal cavity of congenitally mast cell–deficient WBB6F$_1$-*W*/*W*^{v} mice 15 wk after intraperitoneal injection of BMMC synthesized ~650,000 M_r protease-resistant proteoglycans that contained ~80% heparin glycosaminoglycans of ~105,000 M_r. Thus, after adoptive transfer, the SMC of the previously mast cell–deficient mice were like those recovered from the normal WBB6F$_1$-$^{+/+}$ mice that were shown to synthesize ~600,000 M_r proteoglycans that contained ~80% heparin glycosaminoglycans of ~115,000 M_r.

As assessed by indirect immunofluorescence staining and flow cytometry using the B1.1 rat mAb (an antibody that recognizes an epitope located on the neutral glycosphingolipid globopentaosylceramide), ~5% of BMMC bound the antibody detectably, whereas ~72% of the SMC that were harvested from mast cell–deficient mice 15 wk after adoptive transfer of BMMC were B1.1-positive; ~82% of SMC from WBB6F$_1$-$^{+/+}$ mice bound the antibody. These biochemical and immunochemical data are consistent with the results of previous adoptive transfer studies that characterized mast cells primarily on the basis of morphologic and histochemical criteria. Thus, IL-3-dependent BMMC developed in vitro, cells that resemble mucosal mast cells, can give rise in vivo to SMC that express phenotypic characteristics of connective tissue mast cells.

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