PROLIFERATION OF PERITONEAL MAST CELLS IN THE SKIN OF W/W^v MICE THAT GENETICALLY LACK MAST CELLS*

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Peritoneal mast cells and macrophages are commonly used to study functions of these two types of cells (1–3). Although many investigators have studied the production of peritoneal macrophages both in vivo (4–6) and in vitro (7, 8), only a few investigators have taken an interest in production of peritoneal mast cells (9–11). Both mast cells and macrophages are the progeny of the multipotential hematopoietic stem cell (CFU-S)[1] (12, 13). Although most of the progeny of CFU-S (such as erythrocytes, neutrophils, and platelets) differentiate within hematopoietic tissues, undifferentiated precursors of mast cells leave the hematopoietic tissues, migrate in the peripheral blood (14–16), and enter connective tissues, where they proliferate, and differentiate into mast cells (17–19). Such a mode of differentiation bears some resemblance to that of macrophages, because monocytes, the precursors of macrophages, often undergo final differentiation in nonhematopoietic tissues (20). Since Lin and his co-workers (7, 8) have demonstrated the presence of macrophage precursors in the peritoneal cavity of mice and have characterized such precursors, we examined the presence of mast cell precursors in the peritoneal cavity. For this purpose, we used mice of W/W^v genotype as recipients of cells, because these mutant mice genetically lack tissue mast cells (21, 22). When a cell suspension, containing mast cell precursor(s), was injected directly into the skin of W/W^v mice, a cluster of mast cells appeared at the injection site (16). By determining the proportion of injection sites at which mast cells appeared, the concentration of mast cell precursors can be calculated with the limiting dilution analysis. Unexpectedly, the concentration of mast cell precursors in the peritoneal cavity was about five times as great as the value observed in the bone marrow. Physical characterization revealed that mast cell precursors in the peritoneal cavity differed from mast cell precursors in the bone marrow. When a single peritoneal mast cell was picked by the micromanipulator and injected into the skin of W/W^v mice, a mast cell cluster containing ~2,000

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CFU-S, spleen colony-forming unit.

cells developed at 6% injection sites. This indicated that some peritoneal mast cells kept extensive proliferative potentiality even after morphological differentiation.

Materials and Methods

Mice. Mice of (WB × C57BL/6)F$_1$ (hereafter WBB6F$_1$)-(*W/W^v*, *W/+*, *W^v/+*, *+/+*) and C57BL/6-(*bg^J/bg^J*, *+/+*) were raised in our laboratory. Giant granules of C57BL/6-*bg^J/bg^J* mice were used as a marker to identify the origin of mast cells (23, 24). Original stocks of mutant mice were derived from The Jackson Laboratory, Bar Harbor, ME, but *W^v* and *bg^J* mutant genes have been maintained in C57BL/6 mice of our own inbred colony (more than 28 and 15 backcrosses, respectively at the time of the present experiment) (25, 26). Mice were used at 2–6 months of age.

Cell Suspensions. Mice were killed by decapitation. 2 ml of Eagle's medium containing 10 IU/ml heparin and 0.1% bovine serum albumin (BSA) was injected into the peritoneal cavity, and the abdomen was massaged gently for 30 s. The peritoneal cavity was then carefully opened, and the fluid containing peritoneal cells was aspirated with a pasteur pipette. Cells from bone marrow were suspended in Eagle's medium according to the method described previously (27).

Cell Counts. Number of cells was determined with a standard hemocytometer. Mast cells were identified either by staining with toluidine blue solution containing 1% acetic acid or by using the phase contrast microscope. Both methods gave satisfactory agreement. However, when the proportion of mast cells in the examined cell suspension was low, mast cells were accurately counted only after staining with toluidine blue.

Removal of Phagocytes. The major components of mouse peritoneal cells are macrophages and small lymphocytes. Macrophages were removed according to the method described by Kubota et al. (28). The peritoneal cell suspension was adjusted to 5 × 10^6 cells/ml. Carbonyl iron (GAF Co., New York) was added to this suspension at a final concentration of 4 mg/ml and the mixture was incubated in a water bath at 37°C. Then the mixture was agitated frequently for 30 min and the iron and iron-laden cells were attracted to the bottom of a tube by using a magnet (9,000 gauss). The cells remaining in the supernatant fluid were used for nonphagocytic cells.

Density Gradient Centrifugation. Peritoneal and bone marrow cells were fractionated on continuous density gradients according to the method described by Németh and Röhlich (29) and Enerbäck and Svensson (30) with a slight modification. Stock solutions (1.12 g/ml) of polyvinylpyrolidone-coated silica particles (Percoll, Pharmacia, Uppsala) were prepared by dissolving 9 parts of Percoll with 1 part of 10-fold concentrated calcium- and magnesium-free Hanks' solution containing 1% BSA and 200 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (Hepes). Continuous density gradients of Percoll were generated by centrifugation of 7 ml of Percoll stock solution with 23,000 g for 60 min at 4°C in a Hitachi RP-65 fixed angle roter. Gradients ranging from a density of 1.03 to 1.20 were obtained by this procedure. 1 ml of suspensions containing 2 × 10^7 cells was carefully layered on top of Percoll continuous gradients, and centrifugation was performed with 500 g for 30 min at 4°C in a swing-out rotor. Percoll gradient solution (0.4 ml each) was collected from the bottom of the tube by the peristaltic pump. Cells were washed and resuspended in Eagle's medium. Mast cell suspension (30–50% pure) were obtained by a single centrifugation of the phagocyte-free peritoneal cells.

To obtain mast cell suspensions of >90% purity, the above-mentioned procedure of density gradient centrifugation was repeated by using 30–50% pure mast cell suspensions. In this case, 1 ml of suspension containing 2 × 10^6 cells was layered on top of the Percoll continuous gradients.

Separation of a Single Mast Cell by Micromanipulator. Peritoneal cell suspensions containing 30–50% mast cells were prepared by the removal of phagocytes and the density gradient centrifugation. Cells other than mast cells were mostly small lymphocytes. The concentration was adjusted to 5 × 10^4 cells per ml. One drop of the suspension was poured on a slide glass. The large cell with many remarkable granules was identified,

separated, and individually aspirated with a fine microtube attached to a Leitz microma-nipulator. An aspirated single mast cell was individually transferred to a plastic tube (#2003 Falcon Plastics, Becton Dickinson Overseas Inc., Tokyo) containing 0.05 ml Eagle's medium. In another experiment, 10 individually aspirated mast cells were trans-ferred to a plastic tube.

Direct Injection of Cells into the Skin. Hairs of dorsal skin of WBB6F₁-*W/W*ᵛ mice were removed with hair clippers. Cells in 0.05 ml Eagle's medium were injected directly into the skin with a tuberculine syringe. Each mouse received six injections, the sites of which were marked by mixing India ink with the suspension medium. Mice were killed 5 wk after the cell injection. The dorsal skin was peeled off; each injection site that could be identified as a blotted black spot was cut to 1.5 × 1 cm, attached to a piece of thick filter paper to keep it flat, and fixed in 10% buffered formalin (pH 7.2). Skins were embedded in paraffin; serial sections (25 μm thick, cut parallel to the skin surface), were cut from the subcutaneous tissue to the epithelium, and stained with acidified toluidine blue (pH 3.0) (31). All serial sections were examined using a microscope. A group of more than 100 mast cells at the largest cross sections was defined as a mast cell cluster. The maximum number of clusters that could be identified at each injection site was three.

Limiting Dilution Analysis. The principle of the method has been described in detail (16). When at least one mast cell cluster appeared, the injected cell suspension was assumed to contain mast cell precursor(s). Concentration of mast cell precursors was calculated from the proportion of injection sites at which no clusters appeared (defined as "proportion of nonappearance") for various cell doses by limiting dilution analysis according to Finney (32), Porter and Berry (33), and Breivik (34).

Electron Microscopy. Peritoneal cells of WBB6F₁-+/+ mice were mixed with India ink and directly injected into the skin of WBB6F₁-*W/W*ᵛ mice. The WBB6F₁-*W/W*ᵛ mice were killed 5 wk after the injection. Each injection site, which was identified as a blotted black spot, was diced into fragments (~1 mm³) using scissors. The fragments were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 45 min, and were washed with 0.1 M cacodylate buffer containing 4.5% sucrose. After postfixation with 1% osmium tetroxide in Veronal acetate buffer (pH 7.4) for 1 h at 4°C, the tissue fragments were dehydrated in a graded series of ethanol followed by acetone, and embedded in Epon 812. Ultrathin sections were cut with the LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-12 electron microscope.

Irradiation. A Toshiba X-ray machine was operated at 180 kV and 20 mA with a 1-mm aluminum filter. Mice were kept in a polycarbonate box during irradiation. Target distance was 72 cm when mice received <850 rad (61 rad/min), and 45 cm when mice received >1,200 rad (155 rad/min). When peritoneal cells of irradiated mice were used, the cells were harvested within 1 h after irradiation for transplantation.

Assay of Spleen Colony-forming Unit (CFU-S). The method of Till and McCulloch (35) was used to determine the number of CFU-S (35). Peritoneal cells (2 × 10⁶) from WBB6F₁-+/+ mice were injected intravenously into WBB6F₁-(+/+, *W*/+, or *W*ᵛ/+) mice exposed to 850 rad. The mice were killed on the eighth day after the injection; spleens were removed and fixed in Bouin's solution. Colonies were counted with a dissection microscope (× 7). More than 10 mice were used to determine each value.

Origin of Mast Cell Precursors. Origin of mast cell precursors were determined by using giant granules of *bg*ᴶ/*bg*ᴶ mice as a marker (22, 24). In one experiment, bone marrow cells of C57BL/6-*bg*ᴶ/*bg*ᴶ mice were injected intravenously to nonirradiated WBB6F₁-*W/W*ᵛ mice. Two months after the transplantation, peritoneal cells of WBB6F₁-*W/W*ᵛ recipients were harvested, and injected into the skin of the other WBB6F₁-*W/W*ᵛ mice. 5 wk after the injection, types of mast cells that appeared at the injection sites were examined (Fig. 1*A*).

In another experiment, types of mast cell precursors in the peritoneal cavity, peripheral blood, and bone marrow of C57BL/6-+/+ recipients were determined 2 months after irradiation (800 rad) and bone marrow transplantation from C57BL/6-*bg*ᴶ/*bg*ᴶ donors. Cell suspensions were prepared from the above-mentioned tissues and injected into the
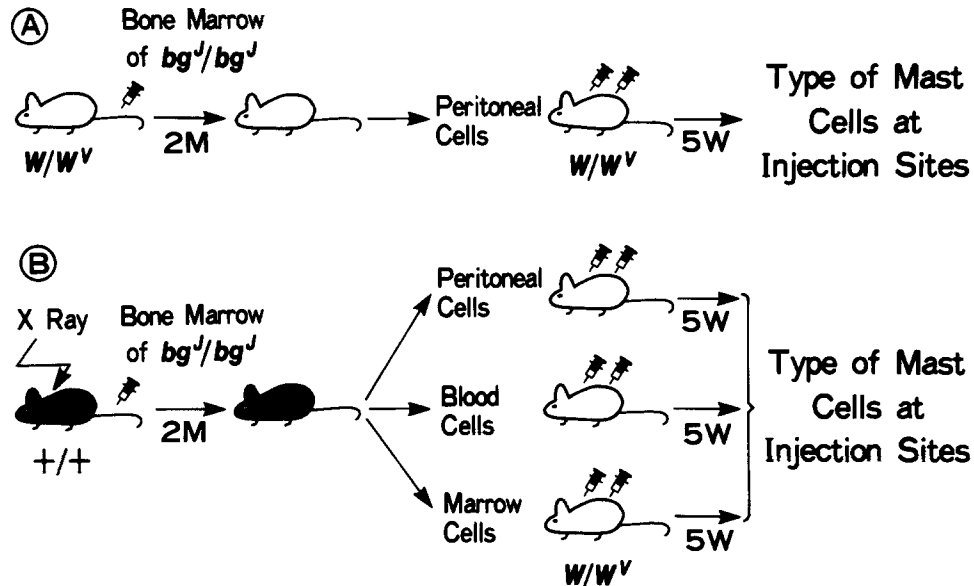
FIGURE 1. Experimental design for the origin of mast cell precursors. Giant granules of mast cells of bg^J/bg^J mice were used as a marker.
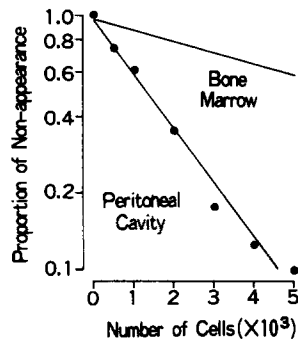

FIGURE 2. Proportion of injection sites at which mast cells did not appear 5 wk after direct injection of different numbers of peritoneal cells from WBB6F$_1$-+/+ mice. Each value was obtained by examining 42–48 injection sites. The logarithmic regression line is described by the equation $\ln p = -0.48 \times 10^{-3} X - 0.08$ (p = proportion of nonappearance; X = number of cells). The regression line for bone marrow cells (16) is also shown for comparison.

skin of WBB6F$_1$-W/W^v mice. 5 wk after the injection, types of mast cells that appeared at the injection sites were determined (Fig. 1 B).

Results

Presence of Mast Cell Precursors in the Peritoneal Cavity. Various doses of peritoneal cells of WBB6F$_1$-+/+ mice were directly injected into the skin of WBB6F$_1$-W/W^v mice, and the development of mast cells at the injection sites was examined. The concentration of mast cell precursors was calculated by the limiting dilution analysis (Fig. 2). The concentration in the peritoneal cavity was ~5 times as much as the value observed in the bone marrow (Table I). In

Table I

Concentration of CFU-S and Mast Cell Precursors in the Peritoneal
Cavity and Bone Marrow of WBB6F$_1$-+/+

	Mean no. per 10^5 cells*	
Origin of cells	CFU-S	Mast cell precursor
Peritoneal cavity	0.21 (0.16–0.26)	48 (42–55)
Bone marrow[‡]	20 (18–22)	10 (8–12)

* The 95% confidence intervals are shown in parentheses.
[‡] Data that have been reported by us (16) are shown for comparison.

contrast, the concentration of CFU-S in the peritoneal cavity was much lower than the concentration in the bone marrow (Table I). This suggested that peritoneal precursors measured by the present method were committed to the mast cell differentiation.

When the concentration of mast cell precursors was measured by the limiting dilution analysis, only the appearance of >100 mast cells was questioned. However, when mast cells appeared at injection sites, they usually appeared in groups (Fig. 3A). Although the largest cluster contained ~8,000 mast cells, usual clusters contained ~2,000 mast cells. We examined the clonal nature of these mast cell clusters. Peritoneal cells from C57BL/6-bg^J/bg^J mice (5 × 10^3) and those from C57BL/6-+/+ mice (5 × 10^3) were mixed and injected into the skin of WBB6F$_1$-W/W^v mice; a total of 179 clusters developed in 144 injection sites. As shown in Table II, most clusters contained either bg^J/bg^J-type mast cells alone (Fig. 3B) or +/+-type mast cells alone (Fig. 3C). This indicated that the development of mast cell clusters resulted from proliferation and differentiation of a single precursor rather than from aggregation of differentiated mast cells.

Electron microscopy was used to confirm that the cells, appearing in the skin after the direct injection of peritoneal cells, were in fact mast cells. As shown in Fig. 3D, the nucleus was not segmented; cytoplasmic granules were numerous, small and electron dense. These characteristics are consistent with the ultrastructural features of the mature mast cell, which have been described by Zucker-Franklin (36), Galli et al. (37), and Dvorak et al. (38).

Since mast cell precursors in nonhematopoietic tissues such as the skin (39) and the lymph node (31) have been shown to originate from the bone marrow, we examined whether peritoneal mast cell precursors were also derived from the bone marrow (Fig. 1A). Bone marrow cells (2 × 10^7) from C57BL/6-bg^J/bg^J mice were injected intravenously into the WBB6F$_1$-W/W^v mice. The first recipients were killed 2 months after the bone marrow transplantation; peritoneal cells were harvested and divided into two aliquots. One was used to determine the concentration of differentiated mast cells and the other to examine the presence of mast cell precursors. Mast cells of bg^J/bg^J type were observed in the peritoneal cavity of the first recipients. When the peritoneal cells of the first recipients were injected into the skin of the second WBB6F$_1$-W/W^v recipients, clusters containing only bg^J/bg^J-type mast cells appeared at the injection sites. This clearly indicated the bone marrow origin of peritoneal mast cells and peritoneal mast cell precursors.
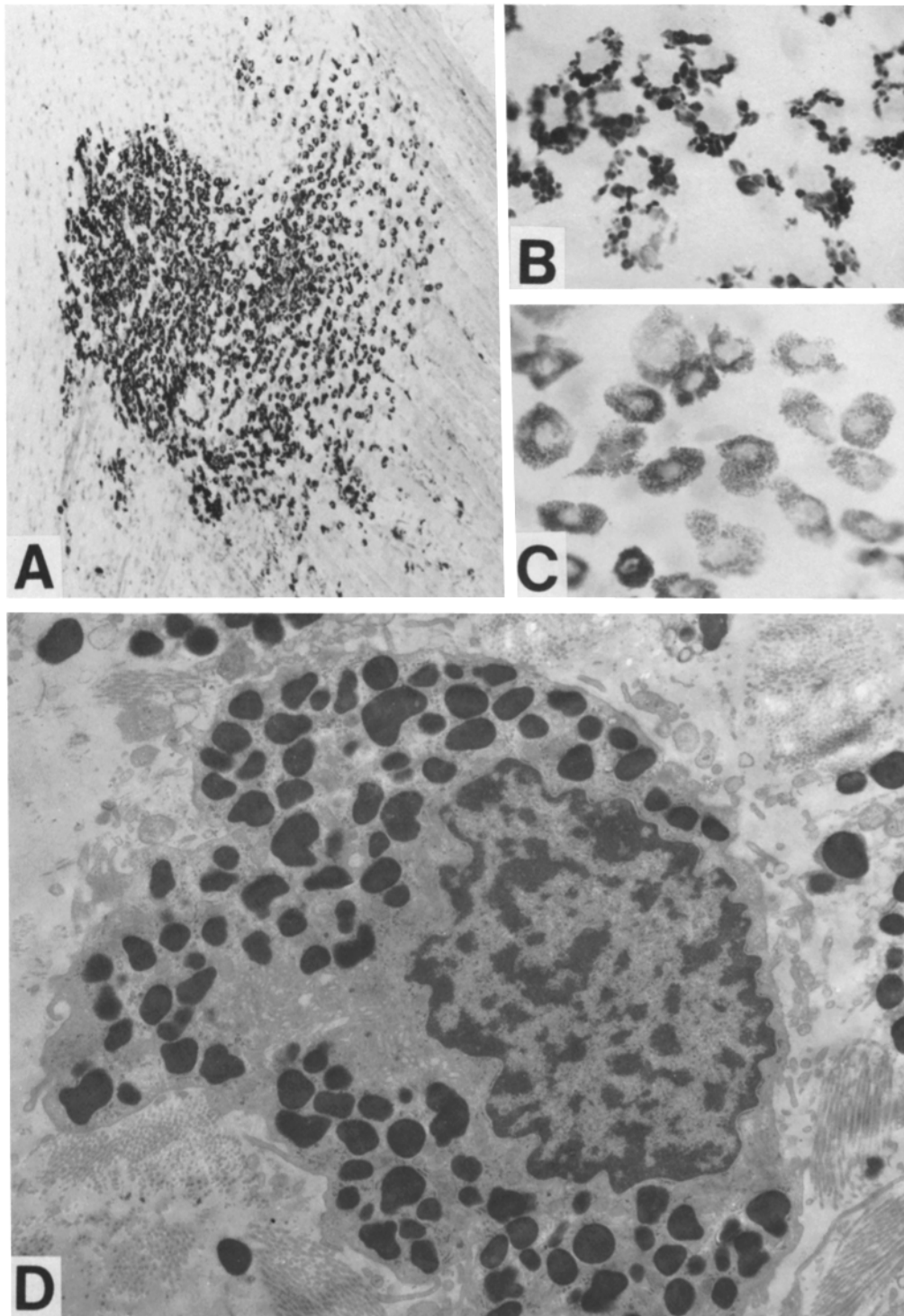
FIGURE 3. (A) A mast cell cluster that developed in the skin of WBB6F$_1$-W/W^v mice 5 wk after injection of peritoneal cells from WBB6F$_1$-+/+ mice, stained with toluidine blue, × 87. (B) A part of the cluster containing only bg^J/bg^J-type mast cells, stained with toluidine blue, × 590. (C) A part of the cluster containing only +/+-type mast cells, stained with toluidine blue, × 590. (D) The ultrastructure of a mast cell that developed in the skin of WBB6F$_1$-W/W^v mice 5 wk after injection of peritoneal cells from WBB6F$_1$-+/+ mice, × 8,800.

TABLE II

Constitution of Mast Cell Clusters Detected in the Skin of WBB6F₁-
W/Wᵛ Mice 5 Wk after Direct Injection of a Mixture of Peritoneal
Cells from C57BL/6-bgᴶ/bgᴶ Mice and C57BL/6-+/+ Mice*

Type of clusters	No. of clusters
Cluster containing bgᴶ/bgᴶ-type alone	98
Cluster containing +/+-type alone	73
Cluster containing both types	8
Total‡	179

* 5×10^5 peritoneal cells from C57BL/6-bgᴶ/bgᴶ mice and 5×10^5 peritoneal cells of C57BL/6-+/+ mice were mixed.
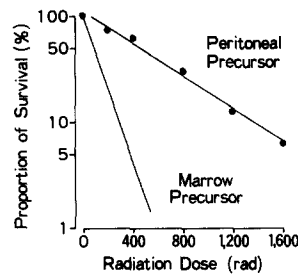‡ Total 179 clusters appeared in 144 injection sites.

FIGURE 4.   Effect of irradiation on the concentration of mast cell precursors in the peritoneal cavity of WBB6F₁-+/+ mice. The D_0 value (dose required to reduce the surviving fraction by a factor of 0.37) is 565 ± 51 rad. Each value was obtained by examining 64–92 injection sites. The result about mast cell precursors in the bone marrow is also shown for comparison; D_0 value for bone marrow precursors is 123 ± 9 rad (40).

Difference between Peritoneal and Bone Marrow Mast Cell Precursors.   Although mast cell precursors localized in the skin originate from the bone marrow, the biological characters of skin precursors are different from those of marrow precursors (18, 26, 40). We compared the characters of the peritoneal precursors to those of marrow precursors. First, we measured the radiosensitivity of peritoneal precursors. Peritoneal cells were removed from 200- to 1,600-rad-irradiated WBB6F₁-+/+ mice; concentrations of mast cell precursors in the cell suspensions were measured by the limiting dilution analysis; proportion of surviving precursors were plotted against radiation doses. As shown in Fig. 4, peritoneal precursors were less susceptible to irradiation than marrow precursors.

Low radiation sensitivity of peritoneal mast cell precursors were also demonstrated in another experiment (Fig. 1B). Radiation chimeras were produced by grafting bone marrow cells (2×10^7) of C57BL/6-bgᴶ/bgᴶ mice into 800-rad–irradiated C57BL/6-+/+ mice. Cells were harvested from the peritoneal cavity, the peripheral blood, and the bone marrow of C57BL/6-+/+ recipients 2 months after the transplantation. The cells from each tissue were injected into the skin of WBB6F₁-W/Wᵛ mice, and types of mast cells that appeared at the injection sites were examined. Mast cells of bgᴶ/bgᴶ-type appeared at the injection sites of bone marrow cells and at the injection sites of peripheral blood cells, whereas mast cells of +/+-type appeared at the injection sites of peritoneal cells (Table III). In spite of the eradication of host-type precursors in the bone marrow and

TABLE III

*Survival of Host-type Mast Cell Precursors in the Peritoneal Cavity of
C57BL/6-+/+ Mice 2 Months after 800 rad Irradiation and
Injection of 2 × 10^7 Bone Marrow Cells from C57BL/6-bg^J/bg^J
Mice**

Tissues	No. of cells injected (× 10^5)	Proportion of injection sites at which mast cells of each type appeared		
		bg^J/bg^J-type alone	+/+-type alone	Both types
Peritoneal cavity	1	0/48	47/48	1/48
Peripheral blood	10	31/32	0/32	1/32
Bone marrow	5	32/32	0/32	0/32

* Experimental procedures is shown in Fig. 1A.

TABLE IV

*Comparison of Density between Peritoneal and Bone Marrow Mast
Cell Precursors of WBB6F$_1$-+/+ Mice**

Origin of cells	Proportion of injection sites at which mast cells appeared[‡]	
	Light fraction (≦1.07 g/ml)	Heavy fraction (≧1.09 g/ml)
Peritoneal cavity	7/40	37/37
Bone marrow	19/32	3/48

* Peritoneal cells and bone marrow cells were separated by density gradient centrifugation; fractions lighter than 1.07 g/ml and fractions heavier than 1.09 g/ml were pooled, respectively. 5 × 10^5 cells were injected into the skin of WBB6F$_1$-W/W^v mice.

‡ P < 0.01, when compared between peritoneal cavity and bone marrow by χ^2 test.

peripheral blood, mast cell precursors of the host origin were maintained in the peritoneal cavity 2 months after 800 rad of whole body irradiation.

In the next experiment, the density of peritoneal mast cell precursors was compared to that of bone marrow precursors. Peritoneal and bone marrow cells of WBB6F$_1$-+/+ mice were fractionated by density gradient centrifugation. Cells in light fractions (≦1.07 g/ml) and those in heavy fractions (≧1.09 g/ml) were pooled, respectively; cells in middle fractions were discarded. Then, cells from the light fractions and those from the heavy fractions were injected into the skin of WBB6F$_1$-W/W^v mice. Mast cell precursors in the peritoneal cavity were more commonly observed in the heavy fractions, whereas mast cell precursors in the bone marrow were more commonly observed in the light fractions (Table IV).

Separation of Peritoneal Mast Cell Precursors. Mast cells accounted for only 0.5% of mouse peritoneal cells. Since the proportion of both morphologically identifiable mast cells and mast cell precursors increased in the heavy fraction of peritoneal cells, we investigated the relationship between differentiated mast cells and mast cell precursors. Since macrophages and small lymphocytes are major components of mouse peritoneal cells, macrophages that phagocytized carbonyl iron were firstly omitted. The proportion of both differentiated mast

TABLE V

Concentration of Morphologically Identifiable Mast Cells and Mast Cell Precursors in
Fractionated Peritoneal Cells of WBB6F$_1$-+/+ Mice

Experi-ment	Cells examined	Mean number per 10^5 cells		Mast cell/precusor ratio
		Mast cells	Precursors	
1	Nontreated peritoneal cells	520	51	10
	Peritoneal cells without phagocytes	1,610	136	12
	Mast cell–poor fraction*	60	6.7	9
	Mast cell–rich fraction*	92,000	4,980	19
2	Nontreated peritoneal cells	620	53	12
	Mast cell–rich fraction*	95,000	5,810	16

* Mast cell–poor and –rich fractions were separated by density gradient centrifugation after removal of phagocytes. To obtain the mast cell–rich fraction, density gradient centrifugation should be carried out two times.

TABLE VI

Development of Mast Cell Cluster at the Injection Site of
Morphologically Identified Mast Cell*

No. of mast cells injected	Proportion of injection site at which mast cells appeared
1	10/168
10	9/20

* The mast cell was individually picked up by the micromanipulator.

cells and mast cell precursors was tripled by the removal of macrophages (Table V).

Then, small lymphocytes and mast cells were separated by density gradient centrifugation. Each fraction was examined for the proportion of mast cells; fractions that scarcely contained mast cells and fractions composed mostly of mast cells were selected. Concentrations of mast cell precursors in "mast cell-poor" and "mast cell-rich" fractions were determined by the limiting dilution analysis. For example, mast cell clusters appeared at 21 out of 32 injection sites of 20 cells from 95% pure mast cell fraction. Most clusters contained >1,000 mast cells. The concentration of mast cell precursors roughly paralleled the concentration of morphologically identifiable mast cells. This suggested that a part of morphologically identifiable mast cells possessed remarkable proliferative potentiality.

Injection of a Single Mast Cell into the Skin. Practically, it was impossible to get 100% pure mast cell fraction by the removal of phagocytes and density gradient centrifugation. Thus, we picked up a differentiated mast cell by the micromanipulator, and injected it directly into the skin of WBB6F$_1$-W/W^v mice. Mast cell clusters developed in 10 out of 168 injection sites (Table VI). The mast cell clusters contained 401–4,408 mast cells (mean ± SE, 1,918 ± 436). When 10 mast cells were collected by the micromanipulator and injected into a single injection site, mast cell clusters appeared in 9 out of 20 injection sites (Table VI). This clearly indicated that some morphologically identifiable mast cells themselves may function as mast cell precursors.

Discussion

The present result clearly demonstrated the presence of mast cell precursors in the peritoneal cavity of mice. The electron microscopic features of the cell, which appeared at the injection site of the peritoneal precursor, were consistent with the characteristics of the mature mast cell described by Zucker-Frankline (36), Galli et al. (37), and Dvorak et al. (38). Since a mast cell cluster derived from a single peritoneal precursor contained ~2,000 cells, its proliferative potentiality was comparable to that of the mast cell precursor in the bone marrow (19). Although the peritoneal mast cell precursors were derived from the bone marrow, the concentration of mast cell precursors in the peritoneal cavity was about five times as high as the concentration in the bone marrow. Moreover, the physical character of peritoneal precursors is different from that of bone marrow precursors. The peritoneal precursors were heavier than the bone marrow precursors. The former were more resistant to irradiation than the latter. Since the presence of mast cell precursors in the peripheral blood has been shown (14–16), the bone marrow–derived precursors seemed to migrate in the circulation, enter into the peritoneal cavity, proliferate, and differentiate into peritoneal precursors.

When bone marrow cells of C57BL/6-bg^J/bg^J mice were injected into intact WBB6F$_1$-W/W^v mice, mast cell precursors of bg^J/bg^J-type appeared in the peritoneal cavity. In contrast, when bone marrow cells of C57BL/6-bg^J/bg^J mice were injected into irradiated C57BL/6-+/+ mice, mast cell precursors of bg^J/bg^J type did not appear in the peritoneal cavity. This suggests that the circulating precursor of mast cells did not enter the peritoneal cavity that contains sufficient number of mast cell precursors. A similar phenomenon was observed in the skin of mice (39). The mechanism that regulates this phenomenon remains to be clarified.

Czarnetski et al. (11) have also shown the presence of mast cell precursors in the peritoneal cavity of rats. They obtained mast cell–depleted peritoneal cells 5 d after intraperitoneal injection of distilled water. Mast cells appeared 2 wk after starting the culture of such mast cell–depleted peritoneal cells. Since most of mast cell–depleted peritoneal cells showed phagocytic activity at the start of the culture, they claimed that mononuclear phagocytes were mast cell precursors (11). In this point, their result is not consistent with the present result, because the removal of phagocytic cells increased the concentration of mast cell precursors (Table V). At present, we cannot explain the discrepancy. A single cell with phagocytic activity should be picked up, and its differentiation potentiality should be examined for further clarification of this problem.

When peritoneal mast cells were purified by the removal of phagocytes and the density gradient centrifugation, 1 out of 16 cells from 95% pure mast cell fraction could make a mast cell cluster in the skin of WBB6F$_1$-W/W^v mice. Moreover, 1 out of 17 mast cells that were morphologically identified and picked up by the micromanipulator could also make a mast cell cluster. Therefore, some morphologically identifiable mast cells in the peritoneal cavity may function as the mast cell precursors. Since peritoneal mast cells appear to float in the fluid, the proliferative potentiality of some peritoneal mast cells reminds us of the suspension culture system of mast cell–like cells that has been devised in many

laboratories (41–46). However, mitosis of peritoneal mast cells is seldom observed in intact animals (10). Moreover, peritoneal mast cells are typical connective tissue mast cells, whereas mast cell–like cells in the suspension culture are considered to resemble mucosal mast cells (47). Biochemical, functional, and immunological characterization revealed the difference between connective tissue mast cells and mucosal mast cells (47, 48). There is a possibility that the peritoneal mast cell with proliferative potentiality may have characteristics similar to mast cell–like cells that developed in vitro. A method for separating proliferative mast cells from nonproliferative ones is necessary for further clarification.

Summary

Presence of mast cell precursors in the mouse peritoneal cavity was demonstrated, and the precursors were characterized. When a cell suspension, containing mast cell precursor(s), was directly injected into the skin of genetically mast cell–deficient WBB6F$_1$ (WB × C57BL/6)-W/W^v mice, a cluster composed of ~2,000 mast cells appeared at the injection site. By determining the proportion of injection sites at which the mast cell cluster appeared, the concentration of mast cell precursors can be calculated by limiting dilution analysis. The concentration in the peritoneal cavity was about five times as great as the concentration in the bone marrow. Although peritoneal mast cell precursors were shown to originate from the bone marrow, physical characterization revealed that the peritoneal precursors differed from the marrow precursors. The peritoneal precursors were less susceptible to irradiation than the marrow precursors; the former were heavier than the latter. When a 95% pure mast cell suspension was prepared from the peritoneal cells by the removal of phagocytes and the density gradient centrifugation, 1 out of 16 cells had the potentiality to make a mast cell cluster in the skin of the W/W^v mice. Moreover, when a single mast cell was identified under the phase contrast microscope and picked up with the micromanipulator, 1 out of 17 mast cells made the cluster. This indicated that some peritoneal mast cells kept extensive proliferative potentiality even after morphological differentiation. In other words, some peritoneal mast cells themselves may function as the committed precursors.

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