

DISTRIBUTION OF MAST-CELL PRECURSORS IN HEMATOPOIETIC AND LYMPHOPOIETIC TISSUES OF MICE*

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The origin of tissue mast cells has been a subject of controversy for nearly a century since the first description of the cells by Ehrlich (1, 2). Many authors believed that mast cells developed locally from certain types of connective tissue cells (1-4). Ginsburg and coworkers (5-7) and Ishizaka et al. (8, 9) claimed the importance of lymphoid cells as a source of mast cell precursors, based on the *in vitro* experiments that demonstrated the development of mast cells from thymus and lymph node cells. Burnet presented a hypothesis that mast cells, or some subpopulations of mast cells, were postmitotic derivatives of thymus-derived (T) lymphocytes (10). Recently, we have shown that mice of two mutant genotypes are useful for the investigations on the origin of mast cells. Giant granules of beige (C57BL/6-*bg^J/bg^J*, Chediak-Higashi syndrome) mice can be used as a marker for mast cells of the donor origin (11, 12). WBB6F₁-*W/W^v* mice are also useful because they lack tissue mast cells owing to a defect in mast cell precursors (13, 14). By using these mutant mice, we demonstrated the presence of mast cell precursors in the bone marrow (11-14) and peripheral blood of adult mice (15) and in the liver of mouse embryos (16). To clarify the above mentioned apparent discrepancy, we studied the distribution of the precursor cells, which differentiate into mast cells *in vivo*, in lymphoid tissues of mice by using our experimental systems. We have shown that the concentration of mast cell precursors in the thymus, lymph node, and Peyer's patch is <0.1% of the concentration in the bone marrow and that the thymus and T lymphocytes are not essential for the differentiation of the precursor cells into mature mast cells.

Materials and Methods

Mice. WBB6F₁ (WB-*W/+* X C57BL/6-*W^v/+*)-*W/W^v* and their normal littermates (WBB6F₁-*+/+*), WCB6F₁ (WC-*S1/+* X C57BL/6-*S1^d/+*)-*S1/S1^d* mice, and C57BL/6-*bg^J/bg^J* mice and their normal littermates (C57BL/6-*bg^J/+* or *+/+*) were raised in our laboratory using parental stocks originally obtained from The Jackson Laboratory, Bar Harbor, Maine, or were purchased from The Jackson Laboratory. Nude, athymic (BALB/*c-nu/nu*) mice were raised and kindly given by Dr. T. Tanabe (Research Institute for Microbiol Diseases, Osaka University), and were kept within a filter-air laminar flow enclosure.

Thymectomy. The operation was carried out under Nembutal (Abbott Laboratories, North Chicago, Ill.) anesthesia 4 wk before irradiation and cell injection. The success of the thymectomy was confirmed at the time of the sacrifice and the animals with thymus remnant were discarded.

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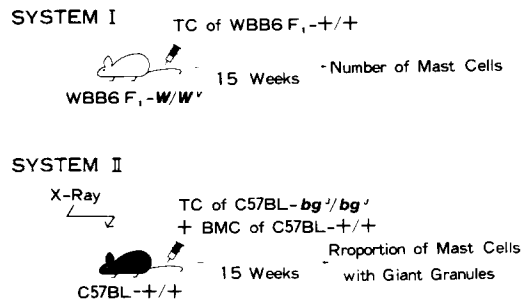


FIG. 1. Experimental design for detection of mast cell precursors in the thymus. Same for the lymph node and Peyer's patch as well.

Cell Preparation and Treatment with Anti-Thy-1.2 Serum. Cells were suspended in Eagle's medium by the method described previously (17). In one experiment, bone marrow cells (BMC)¹ were treated with anti-Thy-1.2 alloantiserum and complement according to the method described by Yutoku et al. (18). The antiserum, prepared by immunizing AKR mice with thymus cells of C3H mice (19), was kindly given by Dr. M. Yotoku (Institute for Cancer Research, Osaka University). 1 ml of 1:4 dilution of anti-Thy-1.2 serum and, as a source of complement, 1 ml by 1:4 dilution of rabbit serum which had been previously absorbed with mouse spleen cells, were added to 5×10^7 BMC in 1 ml Eagle's medium. The cells were incubated at 37°C for 45 min with shaking and then washed with cold Eagle's medium. When cell viability was determined by the trypan-blue dye exclusion technique, viability of BMC after the treatment with the antiserum and complement was $\cong 90\%$, whereas the viability of the thymus cells (TC) after the same treatment was $< 5\%$.

Cell Transfer. Two experimental systems were used. Cells were transplanted between histocompatible donors and hosts in both systems to avoid complicate allogenic effects.

In the system I, WBB6F₁-W/W^v mice were injected with cells of congenic +/+ mice intravenously at 6 wk of age. Repeated biopsies of the skin of the back were carried out under ether anesthesia to find the variation of mast-cell number in one animal. The W/W^v recipient mice were killed 15 wk after the cell injection, a piece of dorsal skin, the stomach, cecum, and mesentery were removed (Fig. 1).

In the system II, cells were injected intravenously into C57BL/6-+/+ mice after whole body x-irradiation (800 rads). The condition of irradiation has been described (20). To examine the presence of mast cell precursors in the thymus and lymph node, TC or lymph node cells (LNC) of C57BL/6-*bg*^d/*bg*^d mice were mixed with BMC of C57BL/6-+/+ mice, and then injected into irradiated C57BL/6-+/+ mice (Fig. 1). The cells with giant granules were assumed to be derived from TC or LNC of C57BL/6-*bg*^d/*bg*^d mice. The BMC of C57BL/6-+/+ mice were added because the injection of TC or LNC alone does not rescue 800-rad irradiated mice.

Blood Examination. Blood samples were obtained from lateral tail veins at various times after cell transfer. When cells of WBB6F₁-+/+ mice were injected into WBB6F₁-W/W^v mice, number of erythrocytes was counted with hemocytometer. When cells from beige C57BL/6 mice were injected into normal C57BL/6 mice, blood smears were fixed in formaldehyde vapor, stained with Sudan Black B, and counterstained with Giemsa solution (21, 22). 100 neutrophils with distinctly segmented nuclei were scored for the presence or absence of giant granules.

Determination of Mast Cell Development. A piece of dorsal skin, the stomach, and cecum were gently smoothed onto a piece of thick filter paper to keep them flat and fixed in 10% buffered formalin (pH 7.2). The stretch preparation of the mesentery was also fixed in 10% formalin. Tissues were embedded in paraffin; 5- μ m thick sections, and stretched mesentery were stained with acidified toluidine blue (pH 3.0).

In the system I (Fig. 1), the number of mast cells was evaluated according to the previously

¹ Abbreviations used in this paper: BMC, bone marrow cell(s); LNC, lymph node cell(s); PPC, Peyer's patch cell(s); SC, spleen cell(s); TC, thymus cell(s).

TABLE I
Criteria for Increase of Erythrocytes and Mast Cells in WBB6F₁-W/W^v Mice by Injection of Cells from Congenic +/+ Mice

| Mice | Mean number of cells ± SE (range)* | | | | | |
|--|------------------------------------|--------------------------|------------------------|------------------------|---------------------------|--------------------------|
| | Erythrocytes | Mast cells‡ | | | | |
| | | Skin | Forestomach | Glandular stomach | Cecum | Mesentery |
| | × 10 ⁶ /mm ³ | | | | | |
| WBB6F ₁ -+/+ | 9.3 ± 0.2 (8.4 - 10.8) | 393 ± 34 (204 - 674) | 124 ± 16 (44 - 242) | 103 ± 19 (42 - 324) | 5.7 ± 1.2 (0.4 - 14.6) | 659 ± 73 (156 - 1263) |
| WBB6F ₁ -W/W ^v (non-treated) | 5.2 ± 0.3 (3.1 - 7.1) | 0.8 ± 0.1 (0.2 - 2.2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Criteria for increase | >8.0 | >20 | >20 | >20 | >5 | >50 |

* Values obtained from 15 WBB6F₁-+/+ mice and 15 WBB6F₁-W/W^v mice, 18-24 wk of age at the time of study.

‡ Number of mast cells per centimeter in the skin, forestomach, glandular stomach, and cecum; per centimeter squared of the membranous part of the mesentery.

described method under the microscope (13). The number of mast cells in the skin, stomach, and cecum was expressed as mast cells per centimeter. The number of mast cells in the mesentery was expressed as mast cells per centimeter squared.

In the system II (Fig. 1), the proportion of mast cells with giant granules was scored. More than 200 mast cells were counted in the skin, stomach, and mesentery; >50 mast cells were counted in the cecum.

Criteria for Increase of Mast Cells and Erythrocytes. The number of mast cells in each tissue of individual WBB6F₁-W/W^v recipient mice was considered to be increased by the injection of cells from WBB6F₁-+/+ mice if the number exceeded the arbitrarily determined values shown in Table I. Criterion for the increase of peripheral erythrocyte number is also shown in Table I.

Skin Graft. A piece of skin ≈ 20 × 25 mm in its full thickness, including panniculus carnosus, was removed. A small piece (≈ 20 × 3 mm) was cut from the skin to determine the value of mast cells before grafting. The remaining part of the skin was grafted on the back of the recipient mouse under Nembutal anesthesia and was kept in place by wound clips. Each mouse with a graft was kept in an individual cage. Recipient mice were killed 6 wk after grafting; grafts were removed for counting the number of mast cells. A piece of the dorsal skin of the recipient mice was also removed for comparison.

Results

Distribution of Precursor Cell. WBB6F₁-W/W^v mice were injected with a fixed dose (2 × 10⁷) of BMC, spleen cells (SC), TC, LNC, and Peyer's patch cells (PPC) of congenic +/+ mice at 6 wk of age (Fig. 1). Biopsies of the dorsal skin were done 5 and 10 wk after the cell injection. The number of mast cells in the skin increased significantly 10 wk after the injection of BMC and SC (Fig. 2). In contrast, such increase of mast cells was not observed after the injection of TC, LNC, and PPC (Fig. 2).

WBB6F₁-W/W^v recipient mice were killed 15 wk after the cell injection, and number of mast cells in the skin, stomach, cecum, and mesentery was evaluated. Although injection of BMC and SC increased the number of mast cells in all tissues examined, the injection of TC, LNC, and PPC increased the number only in the stomach and cecum of <30% of WBB6F₁-W/W^v recipients (Table II).

In the next experiment, the dose of TC was increased to 10⁸, and that of BMC was decreased to 10⁴ for detailed comparison of the concentration of mast cell precursors between the thymus and bone marrow. As shown in Fig. 3 and Table III, the

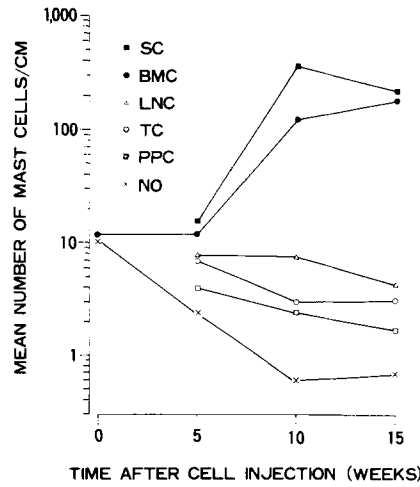


FIG. 2. Number of mast cells in a unit length of the skin of WBB6F₁-W/W^v mice at various weeks after injection of 2 × 10⁷ hematopoietic or lymphopoietic cells from congenic +/+ mice. x—x: Values for control WBB6F₁-W/W^v mice receiving no cells. Each point, mean of 6–18 mice.

TABLE II
Increase of Erythrocytes and Mast Cells in WBB6F₁-W/W^v Mice 15 Wk after Injection of a Fixed Dose (2 × 10⁷) of Various Cells from Congenic +/+ Mice

| Cells injected | Proportion of cases in which increase was detected | | | | | |
|----------------|--|------------|-------|-------------|-------------------|-------|
| | Erythrocytes | Mast cells | | | | |
| | | Blood | Skin | Forestomach | Glandular stomach | Cecum |
| BMC | 11/11 | 11/11 | 11/11 | 11/11 | 11/11 | 10/11 |
| SC | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| TC | 0/10 | 0/10 | 2/10 | 3/10 | 0/9 | 0/10 |
| LNC | 0/12 | 0/12 | 2/12 | 3/12 | 3/12 | 0/12 |
| PPC | 0/12 | 0/12 | 0/12 | 1/12 | 3/12 | 0/12 |

development of mast cells after the injection of 10⁸ TC was significantly inferior to that after the injection of 10⁵ BMC, and was comparable to the value obtained by the infection of 10⁴ BMC.

Experiment using the system II also showed the low concentration of mast cell precursors in lymphoid tissues. Although a considerable number of mast cells with giant granules appeared in the stomach and cecum of C57BL/6-+/+ mice after irradiation and injection of 5 × 10⁵ BMC of C57BL/6-bg^J/bg^J mice, beige-type mast cells scarcely developed after injection of either 10⁸ TC or 5 × 10⁷ LNC from C57BL/6-bg^J/bg^J mice (Table IV).

Insigificance of T Lymphocytes and Thymus. T lymphocytes are known to exist in the bone marrow (23, 24). Although the concentration of mast cell precursors in the thymus is negligible when compared to that in the bone marrow, it does not necessarily exclude the possibility that T lymphocytes in the bone marrow may help the differentiation of precursor cells into mast cells. To examine this possibility, BMC of WBB6F₁-+/+ mice were injected into WBB6F₁-W/W^v mice after removal of T lymphocytes by treatment with anti-Thy-1.2 serum and complement. As shown in

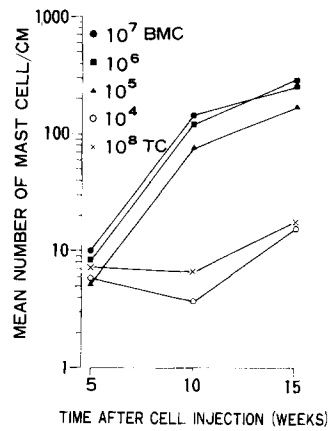


FIG. 3. Number of mast cells in a unit length of the skin of WBB6F₁-W/W^v mice at various weeks after injection of TC (10⁸) or BMC (10⁴-10⁷) from congenic +/+ mice. Each point, mean of 7-15 mice.

TABLE III
Ability of BMC and TC of WBB6F₁-+/+ Mice to Increase Erythrocytes and Mast Cells of WBB6F₁-W/W^v Mice

| Cells injected | Cell dose | Proportion of cases in which increase was detected | | | | | |
|----------------|-----------------|--|------------|-------------|-------------------|-------|-----------|
| | | Erythrocytes | Mast cells | | | | |
| | | Blood | Skin | Forestomach | Glandular stomach | Cecum | Mesentery |
| TC | 10 ⁸ | 3/15 | 3/15 | 8/15 | 8/15 | 8/15 | 5/15 |
| BMC | 10 ⁴ | 1/9 | 1/9 | 5/9 | 6/9 | 4/9 | 0/9 |
| | 10 ⁵ | 8/9 | 8/9 | 9/9 | 9/9 | 9/9 | 7/8 |
| | 10 ⁶ | 7/8 | 8/8 | 7/7 | 8/8 | 8/8 | 8/8 |
| | 10 ⁷ | 7/7 | 7/7 | 7/7 | 7/7 | 7/7 | 7/7 |

TABLE IV
Appearance of Beige-type Cells in Irradiated Normal C57BL/6 Mice 15 Wk after Injection of Various Cells

| Cell injected | | | | No of mice | Mean proportion of beige-type cells | | | | | |
|---------------------|---------------------|-----------------|---------------------|------------|-------------------------------------|------|-------------|-------------------|-------|-----------|
| BMC beige | BMC normal | TC beige | LNC beige | | Neutrophils | | Mast cells | | | |
| | | | | | Blood | Skin | Forestomach | Glandular stomach | Cecum | Mesentery |
| 5 × 10 ⁵ | 0 | 0 | 0 | 7 | 79 | 0 | 17 | 57 | 39 | 0 |
| 5 × 10 ⁶ | 0 | 0 | 0 | 7 | 99 | 0 | 15 | 67 | 79 | 3 |
| 0 | 5 × 10 ⁶ | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 5 × 10 ⁶ | 10 ⁸ | 0 | 6 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| 0 | 5 × 10 ⁶ | 0 | 5 × 10 ⁷ | 11 | 0 | 0 | 0 | 0.2 | 0 | 0 |

Fig. 4, T-lymphocyte-depleted BMC did not cure the anemia of WBB6F₁-W/W^v mice as reported by Wiktor-Jedrzejczak et al. (24). However, the number of mast cells was increased not only in the skin (Fig. 4) but also in the stomach, cecum, and mesentery (data not shown) by the injection of such T-lymphocyte-depleted BMC as well as by the injection of nontreated BMC.

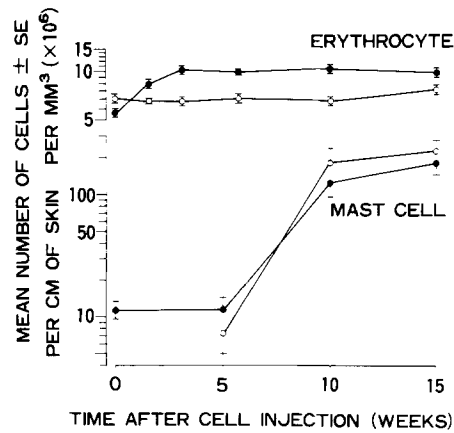


FIG. 4. Number of peripheral erythrocytes and mast cells in the skin of WBB6F₁-W/W^v mice injected with either nontreated BMC or BMC which had been treated with anti-Thy-1.2 serum and complement. Each point, mean of 7-16 mice. ●, nontreated, ○, anti-Thy 1.2 + C'.

TABLE V

*Effect of T-lymphocyte Removal from Injected BMC and Thymectomy of the Hosts on Appearance of Donor-type Cells in Irradiated Mice **

| Treatment with anti-Thy-1.2 serum | Thymectomy of host | No. of mice | Mean proportion of beige-type cells (%) | | | | | |
|-----------------------------------|--------------------|-------------|---|------|-------------|-------------------|-------|-----------|
| | | | Neutrophils | | | Mast cells | | |
| | | | Blood | Skin | Forestomach | Glandular stomach | Cecum | Mesentery |
| No | No | 10 | 100 | 1.3 | 11 | 59 | 72 | 1.2 |
| Yes | No | 7 | 100 | 0.1 | 5 | 64 | 83 | 1.2 |
| Yes | Yes | 8 | 100 | 2.0 | 5 | 60 | 80 | 1.7 |

* Normal C57BL/6 mice were injected with 5×10^6 BMC of beige C57BL/6 mice after irradiation, and killed 15 wk later.

TABLE VI

Development of Mast Cells in the Skin of WBB6F₁-W/W^v Mice Grafted on the Back of Nude Athymic Mice

| Skin | Number of mast cells/cm ± SE* | |
|-------------------------------|-------------------------------|---------------------|
| | Before grafting | 6 wk after grafting |
| Graft from W/W ^v | 0.8 ± 0.2 (10) | 487 ± 45 (9) |
| Graft from Sl/Sl ^d | 0.3 ± 0.2 (8) | 1.3 ± 0.6 (8) |
| Skin of nude host | NE‡ | 416 ± 43 (9) |

* Number of mice is shown in parentheses.

‡ NE, not examined.

Insignificance of T lymphocytes for mast cell differentiation was also demonstrated by using the system II. Neither removal of T lymphocytes from the donor BMC nor thymectomy of the recipient mice affected the development of donor-type mast cells in the irradiated recipients (Table V).

In the last experiment, a piece of the skin of WBB6F₁-W/W^v and WCB6F₁-Sl/Sl^d mice was grafted on the back of nude athymic mice to confirm the insignificance of the thymus for mast-cell differentiation. The number of mast cells increased to the normal level in the skin grafted from WBB6F₁-W/W^v mice 6 wk after grafting (Table VI), but not a significant number of mast cells appeared in the skin graft from

WCB6F₁-*Sl/Sl^d* mice, in which mast cells are depleted because of a defect in microenvironment for differentiation of mast cells (14).

Discussion

Concentration of mast cell precursors in the thymus is found to be <0.1% of that in the bone marrow. As the concentration in the lymph node and Peyer's patch is comparable to that in the thymus, and as the spleen and fetal liver (16) contained a considerable number of mast cell precursors, the main source of the precursor cells in the body seems to be hematopoietic tissues rather than lymphopoietic tissues. This result is not necessarily contradictory to the *in vitro* development of mast cells from lymphoid cells reported by Ginsburg et al. (5-7) and Ishizaka et al. (8, 9) because mast cells developed in one-half of WBB6F₁-*W/W^v* mice injected with a large dose of TC (10⁸) and because the *in vitro* technique (5-9) may be more sensitive than *in vivo* method used in the present study. Because improvement of *in vitro* culture is possible by using fibroblasts of mast cell-depleted WBB6F₁-*W/W^v* mice as a feeder layer and giant granules of beige mice as a marker for the origin of mast cells, development of mast cells from the bone marrow should be tested by such *in vitro* system.

The results obtained by the system I (+/+ donors and *W/W^v* recipients) were consistent with the results obtained by the system II (*bg^J/bg^J* donors and irradiated +/+ recipients) with regard to the concentration of mast cell precursors. However, beige-type mast cells scarcely appeared in the skin of C57BL-+/+ mice which had been irradiated and injected with BMC from C57BL/6-*bg^J/bg^J* donors, in contrast with a significant increase of mast cells in the skin of WBB6F₁-*W/W^v* mice after the injection of BMC from the congenic +/+ donors. The presence of mature mast cells might inhibit the differentiation of mast cells from their precursors. Although we have recently shown that the development of beige-type mast cells can be induced in the skin of beige-to-normal radiation chimeras by painting methylcholanthrene (12), it remains unclear whether methylcholanthrene painting affects the differentiation of mast cells through destruction of mature mast cells.

As reported by Wiktor-Jedrzejczak et al. (24), the T-lymphocyte-depleted BMC of WBB6F₁-+/+ mice did not cure the anemia of WBB6F₁-*W/W^v* mice. However, mast cells appeared after the injection of such BMC without T lymphocytes. Accordingly, T lymphocytes do not seem to play an essential role for differentiation of mast cells. This interpretation was confirmed by the experiment in which T-lymphocyte-depleted BMC of C57BL/6-*bg^J/bg^J* mice were injected into thymectomized and irradiated C57BL/6-+/+ mice. Number of mast cells with giant granules was as many as in the nonthymectomized C57BL-+/+ mice injected with nontreated BMC of C57BL/6-*bg^J/bg^J* mouse origin.

A significant number of mast cells appeared in the skin of WBB6F₁-*W/W^v* grafted on the back of nude, athymic mice. The fact that mast cells did not appear in the skin grafted from WCB6F₁-*Sl/Sl^d* mice, which have a defect in the stroma for mast cell differentiation (14), suggests that the appearance of mast cells in the skin of *W/W^v* mice is a result of differentiation from precursor cells rather than simple infiltration from neighboring tissues. The interpretation is supported by the result of Viklický et al. who demonstrated, by radioautography, the proliferation of mast cell precursors in the skin of A/H mice grafted to nude, athymic hosts (25). Although Ishizaka et al. (8) speculated a possible role of thymic factor on mast cell differentiation, our present

results do not support the concept. Because mast cells develop not only in the skin but also in the stomach, cecum, and mesentery of WBB6F₁-*W/W^v* mice after the injection of BMC from congenic +/+ donors, the mast cells in the skin would not be different from mast cells in other tissues. Therefore, the present results suggest that the differentiation of mast cells may not depend on the thymus in any part of the body.

Summary

Two experimental systems were used to investigate the origin of precursor cells which differentiate into tissue mast cells *in vivo*. (a) Increase of mast cell number was examined in the skin, stomach, cecum, and mesentery of genetically mast cell-depleted WBB6F₁ (WB × C57BL/6)-*W/W^v* mice after the injection of various hemato-lymphoid cells of congenic +/+ mice. (b) Appearance of mast cells with giant granules was studied in irradiated C57BL/6-+/+ mice after the injection of lymphoid cells of C57BL/6-*bg^J/bg^J* (beige, Chediak-Higashi syndrome) mice. Concentrations of mast cell precursors in the thymus, lymph node and Peyer's patch were <0.1% of the concentration in the bone marrow. Neither treatment of donor bone marrow cells with anti-Thy-1.2 serum and complement nor thymectomy of the recipient mice affects the development of mast cells in the skin, stomach, cecum, and mesentery. Moreover, the number of mast cells increased to normal level when the skin of WBB6F₁-*W/W^v* mice was grafted on the back of nude athymic (BALB/c-*nu/nu*) mice. These results indicate that mast cell precursors are derived from hematopoietic tissues rather than lymphopoietic ones and that the differentiation of the precursor cells does not depend on T lymphocytes or the thymus.

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