

Local Development of Mast Cells From Bone Marrow-Derived Precursors in the Skin of Mice

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A mechanism to control development of mast cells was investigated in mice. Although mast cells in the skin of normal C57BL/6 mice were still of host type 290 days after irradiation and injection of bone marrow cells from beige (Chediak-Higashi syndrome, C57BL/6 *bg^l/bg^l*) mice, donor-type mast cells with giant granules appeared after painting of methylcholanthrene on the dorsal skin. Since donor-type mast cells appeared only at the painted portion of the skin, with an increase

in the labeling index of such donor-type mast cells with ³H-thymidine, proliferation and differentiation of bone marrow-derived precursors of mast cells seem to be controlled locally. Although the morphologic feature of marrow-derived precursors was not identified, the finding that all fibroblasts cultured from the methylcholanthrene-treated skin were of host type may exclude the possibility that fibroblasts are the precursors of mast cells.

THE ORIGIN of mast cells has been a subject of controversy for nearly a century since the first description of the cells by Paul Ehrlich.^{1,2} Many authors speculated that mast cells developed locally from certain types of connective tissue cells.¹⁻⁴ On the other hand, we recently showed that mast cells can be derived from transplanted bone marrow cells in irradiated mice using giant granules of beige mice as markers of donor-type mast cells.⁵ However, the appearance rate of donor-type mast cells was different for each tissue.⁵ Mast cells of skin remained of host type despite the fact that most of mast cells in the intestinal canal were of donor type 10 wk after irradiation and bone marrow cell transplantation.⁵ Two possibilities were postulated to explain the different proportions observed in the skin and the intestinal canal. First, the origin of mast cells in skin may be different from that of mast cells in intestinal canal. Second, differentiation of mast cells from their precursors may be locally controlled.

The main purpose of the present study was to investigate these possibilities and to elucidate the relationship between the precursor cells derived from bone marrow and the precursor cells supposed to be fixed to connective tissues. Since Cramer and Simpson⁶ and Takeoka et al.⁴ reported that methylcholanthrene painting increased the number of mast cells in the skin before development of cancer, we used this well-known carcinogenic substance as an agent for perturbing the mast cell population in the skin.

MATERIALS AND METHODS

Mice. Beige (C57BL/6 *bg^l/bg^l*, Chediak-Higashi syndrome) mice and their normal littermates (C57BL/6 *+/+* or *bg^l/+*) were used. Mice were raised in our laboratory using parental stocks

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Submitted May 10, 1978; accepted September 14, 1978.

Supported in part by Grants-in-Aid from the Japanese Ministry of Education, Science and Culture.

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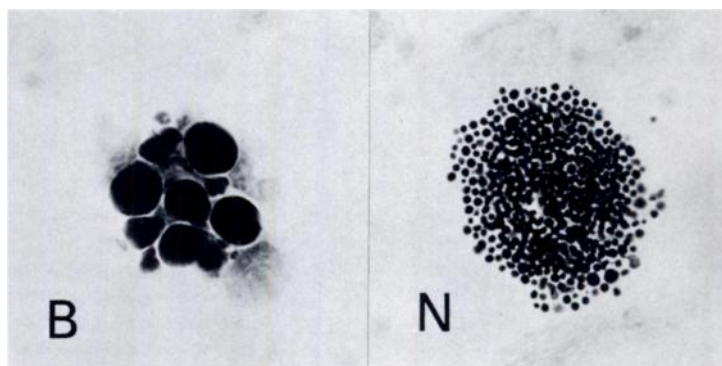


Fig. 1. Mast cells in stretch preparation of mesentery. Stretched mesentery was used to demonstrate most of granules contained in a mast cell. B, mast cell of beige C57BL/6 mouse; N, mast cell of normal C57BL/6 mouse.

originally obtained from Jackson Laboratory, Bar Harbor, Me. Animals were 3–4 mo old at time of irradiation. As shown by Chi and Lagunoff using histochemistry and electron microscopy,⁷ the size of specific granules of mast cells is abnormally enlarged and the number of them is significantly decreased (Fig. 1). Although the electron microscope shows the abnormality of mast cells of beige mice conspicuously,^{7,8} the mast cells of beige mouse origin can be easily identified by the light microscope from early stage of differentiation.

Bone marrow transplantation. The method of cell preparation⁹ and the condition of x-irradiation¹⁰ have been described. Normal mice were injected intravenously with bone marrow cells (5×10^6) of beige mice within 3 hr after whole-body irradiation (800 rad).

Blood smear. Blood samples were obtained from lateral tail veins. Smears were fixed in formaldehyde vapor, stained with Sudan black B, and counterstained with Giemsa solution.^{11,12} More than 100 neutrophils with distinctly segmented nuclei were scored for the presence of giant granules.

Histology. Mice were killed on various days after bone marrow transplantation. A piece of dorsal skin and one of ventral skin were removed, gently smoothed onto a piece of thick filter paper, and fixed in 10% buffered formalin (pH 7.2). Skin was embedded in paraffin; sections (5 μ m thick) were stained with acidified toluidine blue (pH 3.0).⁷ About 1000 mast cells were scored in dorsal and ventral skin of each mouse for giant metachromatic granules.⁵

Methylcholanthrene painting. 20-Methylcholanthrene (0.5% solution in benzene) was painted with a brush on the dorsal skin of the normal mice that had been irradiated and injected with the bone marrow cells of beige mice, and on the dorsal skin of untreated normal mice. The painting was carried out on every third day for 27 days (total ten times), based on the report by Takeoka et al.⁴ that an augmentation of mast cell number with an increase in the labeling index occurred after such painting of methylcholanthrene.⁴ When the irradiated and reconstituted mice were used, the painting was started on the 90th day after bone marrow cell transplantation because our previous result showed that mast cells of the donor origin were predominant in the intestinal canal of the recipient 90 days after transplantation but donor-type mast cells were rare in the skin of the same recipient⁵ (Fig. 2).

Autoradiography. Mice were injected intraperitoneally with ³H-thymidine (³H-TdR) before or during or after painting of methylcholanthrene according to the procedure described by Takeoka et al.⁴ (Fig. 2). Each mouse received 60 μ Ci ³H-TdR five times at 6-hr intervals (total dose, 300 μ Ci in 24 hr). When ³H-TdR injection and painting of methylcholanthrene were started on the same day (i.e., 90th day after bone marrow transplantation), methylcholanthrene was painted between the first and the second injections of ³H-TdR. Mice that received ³H-TdR before or during methylcholanthrene painting were killed on the 120th day after bone marrow transplantation; mice that received ³H-TdR after methylcholanthrene painting were killed on the 135th day (Fig. 2).

Paraffin sections of dorsal and ventral skin were made as described above. Sections were deparaffinized, dipped into Sakura NR-M2 emulsion, dried, and exposed for 20 days in refrigerator (4°C). After development, the sections were stained with hematoxylin and toluidine blue. About 1000 mast cells were scored in dorsal and ventral skin of each mouse for the presence of giant granules and

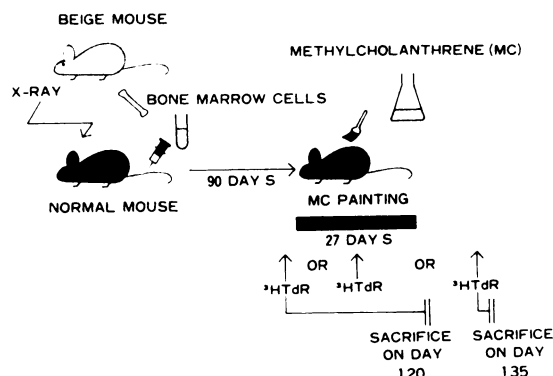


Fig. 2. Experimental design for autoradiography. $^3\text{H-TdR}$ was injected before, during, or after painting of methylcholanthrene on the dorsal skin of mice.

silver grains. The presence of more than three grains over the nucleus was considered as an indication of positive labeling.

Culture of fibroblasts. After killing of the mouse, a piece of dorsal skin (about 10×10 mm) was removed, washed in phosphate-buffered saline (PBS) thoroughly, and diced into fragments (about 1 mm^3) using a sterile razor. The fragments were put on a glass slide kept on a stainless steel grid and incubated in a plastic Petri dish containing Eagle's minimum essential medium supplemented with 10% fetal calf serum. The tissue fragments were removed on the third or fourth day after the start of incubation at 37°C in an atmosphere of 5% CO_2 in air. The remaining glass slide was transferred into a new Petri dish, submerged in fresh medium, and incubated again. Medium was changed every third day. After 10–14 days in culture, lysosomes of fibroblasts were demonstrated by removing medium and incubating cells with acridine orange ($1:10^6$) in PBS for 3–10 min at 37°C .¹³ The distribution of fluorescence on stained cells was examined by epiillumination with a Zeiss fluorescence microscope. More than 100 cells per cover slip (five cover slips for each mouse) were scored for giant lysosomes.¹³

RESULTS

Appearance of donor-type mast cells in skin. Mast cells of skin remained of host type even 290 days after irradiation and injection of bone marrow cells from beige mice if no additional treatment had been carried out. When methylcholanthrene was painted on the dorsal skin, mast cells with giant granules appeared at the painted site despite the fact that mast cells in the ventral skin of the same mice remained of the host type (Table 1, Fig. 3). On the other hand, no mast cells with such giant granules appeared when methylcholanthrene was painted on the dorsal skin of normal mice in the absence of the bone marrow cell transplantation. No

Table 1. Effect of Methylcholanthrene Painting on Appearance of Donor-type Mast Cells

Methylcholanthrene Painting	Days After Bone Marrow Transplantation	No. of Mice	Percentage of Mast Cells with Giant Granules (Mean \pm SE)	
			Dorsal Skin	Ventral Skin
No	90	7	0.1 ± 0.1	NE
No	120	5	2.6 ± 2.3	0.8 ± 0.3
No	190	6	2.2 ± 0.9	NE
No	290	3	1.7 ± 1.4	NE
Yes	120	12	$22.7 \pm 3.7^*$	$0.4 \pm 0.2^\dagger$

NE, not examined.

* $p < 0.01$ when compared by Student's t test to the value for mice not painted with methylcholanthrene.

† $p < 0.01$ when compared to the value for dorsal skin, but $p > 0.3$ when compared to the value for mice not painted with methylcholanthrene.

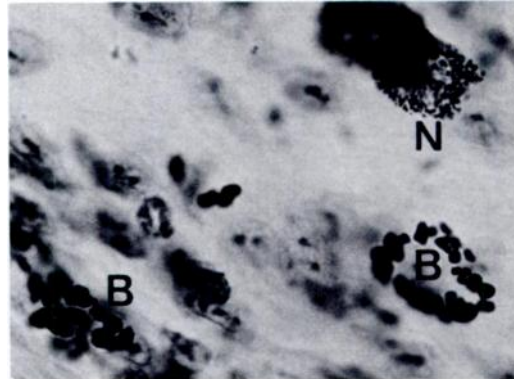


Fig. 3. Appearance of beige mouse-type mast cells in the skin of normal C57BL/6 mouse after methylcholanthrene painting. Normal C57BL/6 mouse had been irradiated and injected with the bone marrow cells of the beige C57BL/6 mice. B, mast cells of the beige mouse type; N, mast cell of normal mouse type.

mitoses of mast cells were detectable in the skin of irradiated and reconstituted mice or in that of nonirradiated mice after methylcholanthrene painting.

In the next experiment, mice were injected with $^3\text{H-TdR}$ before, during, or after painting of methylcholanthrene. Both donor-type and host-type mast cells were labeled (Fig. 4). The labeling index of donor-type mast cells in the dorsal skin was not significantly different from that of host-type mast cells when $^3\text{H-TdR}$ was injected before painting of methylcholanthrene. In contrast, the labeling index of donor-type mast cells was significantly higher than that of host-type mast cells when $^3\text{H-TdR}$ was injected during or after painting of methylcholanthrene ($p < 0.001$, χ^2 test) (Table 2). Since donor-type mast cells were scarcely detected in the ventral skin, the labeling index of donor-type mast cells could not be calculated.

Origin of fibroblasts. Since connective tissue cells without metachromatic granules as well as mast cells were labeled after injection of $^3\text{H-TdR}$, the origin of fibroblasts was examined as a representative sample of connective tissue cells other than mast cells. Mice irradiated and reconstituted with bone marrow cells of beige mice were used after confirming that most peripheral granulocytes were of beige mouse origin. After painting of methylcholanthrene, a piece of dorsal skin was removed and cut into two parts. One part was fixed in formalin to examine mast cells. Fibroblasts were cultured from the other part. Although the examination of

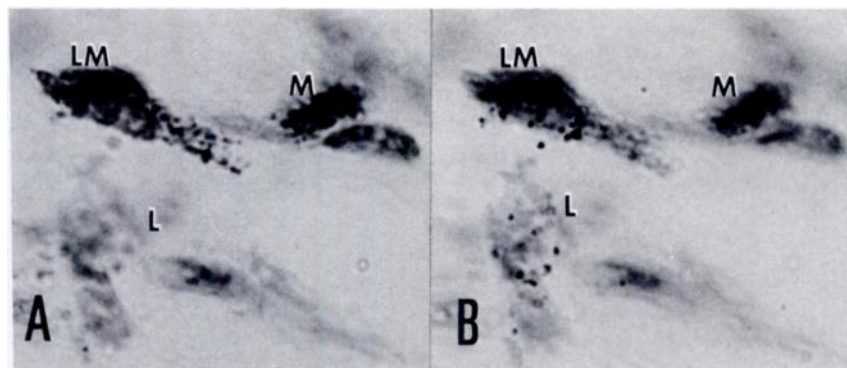


Fig. 4. Labeled mast cell of normal mouse type. A, focus on metachromatic granules; B, focus on silver grains. LM, labeled mast cell; M, unlabeled mast cell; L, labeled cell without metachromatic granules.

Table 2. Labeling Index of Donor-type and Host-type Mast Cells in Dorsal and Ventral Skin of Mice Painted With Methylcholanthrene (MC)

Time of Treatment*			No. of Mice	Mean Labeling Index (%)		
³ H-TdR Injection	MC Painting	Kill		Dorsal Skin		Ventral Skin Host Type
				Donor Type	Host Type	
83	90-117	120	3	2.4†	2.8	3.6
87	90-117	120	4	4.7†	1.3	2.9
90	90-117	120	3	13.4‡	1.5	0.6
105	90-117	120	2	40.2‡	6.6	1.2
113	90-117	120	2	23.7‡	2.6	0.9
117	90-117	120	4	31.1‡	4.0	3.6
132	90-117	135	2	12.3‡	2.9	4.2

* Days after bone marrow transplantation.

† $p > 0.2$ when compared by χ^2 test to value for host-type mast cells in dorsal skin.

‡ $p < 0.001$ when compared to value for host-type mast cells in dorsal skin.

skin sections showed the presence of mast cells of donor origin, all fibroblasts examined with a fluorescence microscope were of host type (Table 3).

DISCUSSION

Although mast cells of skin remained of host type even 290 days after irradiation and injection of bone marrow cells from beige mice, mast cells with giant granules appeared after painting of methylcholanthrene, clearly showing that mast cells of skin can be derived from the bone marrow. Since appearance of donor-type mast cells was restricted to the site of methylcholanthrene painting, development of mast cells seems to be controlled locally.

After injection of ³H-TdR, silver grains could be observed on nuclei of both donor-type and host-type mast cells in the dorsal skin (Fig. 4). Donor-type mast cells were scarce in the ventral skin; most of the labeled mast cells in the ventral skin were of host type. There is a possibility that mature mast cells or precursors of mast cells, which are of host origin and are fixed to connective tissue, can divide after 800-rad irradiation.

Although mitotic figures of mast cells were rarely detectable after methylcholanthrene painting as described previously by Cramer and Simpson,⁶ the labeling index of donor-type mast cells was increased by this procedure when ³H-TdR injection was carried out during or after methylcholanthrene painting. Thus methylcholanthrene painting seems to induce the division of bone marrow-derived precursors. Since donor-type mast cells appeared only at the painted site, and since ³H-TdR injection before painting of methylcholanthrene did not raise the labeling

Table 3. Origin of Cultured Fibroblasts From Dorsal Skin of Mice Painted With Methylcholanthrene

Mouse Number	Percentage of Cells With Giant Granules		
	Neutrophils*	Mast Cells*	Fibroblasts*
M801	100 (100)	13.5 (1235)	0† (566)
M802	100 (100)	10.5 (1214)	0† (593)
M803	100 (100)	10.2 (960)	0† (546)

Normal mice received irradiation and injection of bone marrow cells of beige mice 90 days before methylcholanthrene painting.

* Number of counted cells is shown in parentheses.

† $p < 0.001$ when compared by χ^2 test to value for mast cells.

index of donor-type mast cells, the division of bone marrow-derived precursors seems to occur at the painted site. However, since the present experimental system did not allow us to distinguish strictly between the proliferation of bone marrow-derived precursors and the following differentiation of the latter into mature mast cells, further investigations will be necessary to clarify these two steps in mast cell differentiation.

All fibroblasts cultured from the skin after methylcholanthrene painting were of host type in spite of the fact that mast cells of donor origin appeared in the same place. This observation seems to work against the speculation that mast cells are derived from fibroblasts.^{3,14} However, there is a possibility that fibroblasts of donor origin could not be detected because of their poor growth in culture. A method for identifying the donor-type fibroblasts in situ should be developed to examine this possibility.

ACKNOWLEDGMENT

The authors wish to thank Dr. K. J. Mori, Dr. M. Seki, Dr. O. Takeoka, and Dr. K. Matsumoto for valuable discussions.

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