Decrease of Mast Cells in $W/W^v$ Mice and Their Increase by Bone Marrow Transplantation

By Y. Kitamura, S. Go, and K. Hatanaka

Production of tissue mast cells was evaluated in genetically anemic mice of $W/W^v$ genotype and was found to be abnormal. In the skin of adult $W/W^v$ mice the number of mast cells/cm was less than 1% of the number observed in the congeneric +/+ mice. No mast cells were detectable in other tissues of the $W/W^v$ mice. After transplantation of bone marrow cells from +/+ mice the number of mast cells in the skin, stomach, caecum, and mesentery of the $W/W^v$ mice increased to levels similar to those of the +/+ mice. These results show that the $W/W^v$ mouse is a useful tool for the investigations concerning the physiologic roles and the origin of mast cells.

Mice of the $W/W^v$ genotype have a severe macrocytic anemia, and a decrease of granulocytes and megakaryocytes in the bone marrow of $W/W^v$ mice has been reported as well. Since the anemia of $W/W^v$ mice can be cured by bone marrow transplantation from congeneric +/+ mice and since bone marrow cells of $W/W^v$ mice are deprived of the ability to make macroscopic colonies in the spleen of irradiated congeneric mice, the cause of the hematologic changes in $W/W^v$ mice are thought to be due to a qualitative defect of hematopoietic stem cells (CFU-S).

Recently, Kitamura et al. showed that mast cells could be derived from transplanted bone marrow cells in irradiated mice. This result suggests a possibility that mast cells may be descendants of the CFU-S contained in the transplanted bone marrow. If mast cells originate from the CFU-S, it is plausible that some defect of mast-cell production could arise in $W/W^v$ mice owing to the defect of direct precursors, whose small number results from defective CFU-S in these mice. In order to examine the possibility, we compared the number of mast cells in $W/W^v$ mice to that in +/+ mice. We found that the number of mast cells was conspicuously less in $W/W^v$ mice and that the number of mast cells in $W/W^v$ mice can be increased to a level similar to that observed in the congeneric +/+ mice by bone marrow transplantation from the +/+ mice.

**MATERIALS AND METHODS**

**Mice.** WBB6F1 (WB/Re-W/+ × C57BL/6J-W'/+)(W/W', W/+ , W'/+, +/+) mice were used. WBB6F1 mice were raised in our laboratory using the parental stocks originally obtained from Jackson Laboratory, Bar Harbor, Me. The $W$ genotype of all animals was inferred from the pattern of white spots and dilution of the coat color.

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Bone marrow transplantation. The method of cell preparation was described previously. Cells were injected within 2 hr after the death of donors. Donors and recipients of the cells were of the same sex.

Blood cell count. Blood was collected from the lateral tail veins. Erythrocyte counts were carried out with the conventional technique.

Determination of mast cell number. To find the variation of mast cell number in one animal, repeated biopsies of the skin of the back were carried out under ether anesthesia. The biopsy measured roughly (surface area) 20 × 2 mm. Each new set of biopsies was remote from the previous scar. At autopsy a piece of dorsal skin, the stomach, and the caecum were removed, 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 mesentry was also fixed in 10% formalin. Tissues were embedded in paraffin; sections (5 μm thick) and the stretched mesentry were stained with acidified toluidine blue (pH 3.0). In the section of the skin, mast cells between epithelium and paninculus carnosus were counted under the microscope. In the section of the stomach cut so as to include both the forestomach and the glandular stomach and in the longitudinal section of the caecum, mast cells in whole layers (i.e., mucosa, submucosa, and muscle) were counted. The number of mast cells thus obtained in the skin, stomach, and caecum was divided by the length of each section and expressed as mast cells/cm. The number of mast cells in the unit area of the stretched mesentry was counted using a square-ruled ocular micrometer and expressed as mast cells/cm². In some W/Wmice sections of bone marrow, spleen, thymus, heart, lung, kidney, liver, and brain were also examined microscopically after staining with toluidine blue.

RESULTS

Decrease of mast cells in W/W mice. The number of mast cells was counted in the dorsal skin of adult mice of different genotypes. The white spots seen in the ventral skin of W/+ and W/+ mice were also examined. The number of mast cells in the skin of the W/Wmice was less than 1% of the number of mast cells in the skin of the mice of other genotypes (Table 1). The number of mast cells slightly but significantly less in the skin of W/+ mice (p < 0.01 compared to the value of +/+ mice). Although the rank order of mast-cell number among the mice with different genotypes was same as that of erythrocyte number (i.e., W/+ > +/+ > W/+ > W/W), in W/Wmice the reduction of the mast-cell number was much more conspicuous than that of the erythrocyte number (Table 1). The number of mast cells in the white spots of W/+ and W/+ mice was comparable to the number in the dorsal skin of the mice with corresponding genotype (Table 1).

In the next experiment, the dorsal skin and stomach of mice of various ages

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Color of Skin</th>
<th>No. of Cells (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W/W</td>
<td>White</td>
<td>Mast Cells/cm Skin, Erythrocytes/mm³ (x 10⁶)</td>
</tr>
<tr>
<td>W/+</td>
<td>Gray</td>
<td>228 ± 17 (21)<em>, 8.9 ± 0.2 (13)</em></td>
</tr>
<tr>
<td>W/+</td>
<td>White†</td>
<td>259 ± 23 (5)*</td>
</tr>
<tr>
<td>W/+</td>
<td>Black</td>
<td>367 ± 43 (12)*</td>
</tr>
<tr>
<td>W/+</td>
<td>White†</td>
<td>375 ± 52 (1)*</td>
</tr>
<tr>
<td>W/+</td>
<td>Black</td>
<td>337 ± 29 (1)*</td>
</tr>
</tbody>
</table>

Mice were 90–240 days old at time of study. Number of mice is shown in parentheses.
*p < 0.001 when compared to the value for the W/W mice.
†White spots of ventral skin.
SKIN

Fig. 1. Number of mast cells/cm of skin of +/+ and W/W' mice on various days after birth. Each point, mean of 5-14 mice.

were examined. The number of mast cells was constant regardless of their age in skin of +/+ mice, whereas the number of mast cells in skin varied with age in W/W' mice (Fig. 1). The mast-cell number increased from the 5th day after birth until 25th day and then began to decrease. In the stomach, mast cells were rarely observed even in +/+ mice on the 5th day after birth. The number of

STOMACH

Fig. 2. Number of mast cells/cm of glandular stomach of +/+ and W/W' mice on various days after birth. Each point, mean of 4-9 mice. Similar change observed in fore-stomach also.
mast cells started to increase thereafter in +/- mice, but no mast cells were detectable in the stomachs of $W^--W^-$ mice of any age (Fig. 2).

No mast cells were found in the caecum, mesentery, bone marrow, spleen, thymus, heart, lung, kidney, liver, or brain of $W^--W^-$ mice of any age.

Increase of mast cells by bone marrow transplantation. Bone marrow cells ($2 \times 10^7$) of +/- mice were injected intravenously into 40-day-old $W^--W^-$ mice. On various days after transplantation counting of erythrocytes and biopsy of the dorsal skin were carried out. The numbers of erythrocytes rose to normal levels on the 20th day after transplantation. In contrast, the numbers of mast cells in the skin remained at about 5% of normal even 35 days after transplantation. The mast-cell number in the skin eventually increased to one-third of normal on the 70th day and were half of normal when the mice were killed on the 105th day (Fig. 3). The number of mast cells increased significantly in the stomach, caecum and mesentery also (Table 2). In the stomach, the mast-cell number was greater in $W^--W^-$ mice that had been injected with +/- marrow cells than in normal +/- mice of similar age (Table 2).

### Table 2. Number of Mast Cells in Various Tissues of $W^--W^-$ Mice 105 Days After Transplantation of Bone Marrow Cells From +/- Mice

<table>
<thead>
<tr>
<th>Genotype of Mice</th>
<th>Bone Marrow Transplantation</th>
<th>No. of Mice</th>
<th>Mean Number of Mast Cells$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W^--W^-$</td>
<td>No</td>
<td>7</td>
<td>0.9, 0, 0, 0, 0</td>
</tr>
<tr>
<td>$W^--W^+$</td>
<td>Yes</td>
<td>11</td>
<td>178, 373, 209, 2.3, 507</td>
</tr>
<tr>
<td>+/-</td>
<td>No</td>
<td>7</td>
<td>420, 93, 137, 3.9, 567</td>
</tr>
</tbody>
</table>

$^*$Number of mast cells/cm in the skin, forestomach, glandular stomach, and caecum; per cm$^2$ of the mesentery.

$^\dagger$At the time of killing the $W^--W^-$ mice were 145 days old and the +/- mice were 130–160 days old.

$^\ddagger$Mast cells were counted only in the membranous part of the mesentery. However, no mast cells were detectable in other parts of the mesentery in the untreated $W^--W^-$ mice.
DISCUSSION

The number of mast cells was significantly less in the skin of the $W/W'$ mice. Since mast cells were not detectable in other tissues of $W/W'$ mice, the adult $W/W'$ mice can be regarded as "mast cell–free" animals and may be useful for studies concerning the physiologic roles of mast cells.

Since $W/W'$ mice also lack melanocytes in their skin, the present results seem to be consistent with the claim of Okun that mast cells and melanocytes have a common precursor. However, this claim is not plausible because the white spots of $W'/+$ and $W/+ $ mice, which also lack melanocytes, had as many mast cells as their pigmented dorsal skin (Table 1).

The transplantation of bone marrow cells from the $+/$ donors increased the number of mast cells in the $W/W'$ mice. Although giant granules of the beige (Chediak-Higashi syndrome) mouse were used to show the donor origin of mast cells in our previous study, there was no marker indicating the origin of mast cells in the present study. Thus the present results can be explained in several ways: (1) The increase of mast cells might be due to the proliferation and differentiation of precursor cells of host origin. The proliferation of the precursors might be a result of events induced by bone marrow transplantation, such as the cure of anemia. (2) Wiktor-Jedrzejczak et al. recently reported a deficiency of such theta-sensitive cells in $W/W'$ mice as are required in the promotion of differentiation of CFU-S into erythrocytes. The increase of mast cells may be the result of supply of some kind of helper cells that promote the differentiation of mast cells from their precursors. In this case, the precursor cells could be of either host or donor origin. (3) The increase of mast cells may be due to the supply of precursor cells whose small number in $W/W'$ mice was the result of defective CFU-S in these mice. The bone marrow transplantation from beige ($C57BL/6J-bg'/bg'$) mice was reported to cure the anemia of WBB6F1-$W/W'$ mice, and such giant granules of $C57BL/6J-bg'/bg'$ mice were used as a marker of mast cells. The origin of mast cells that would appear in $W/W'$ mice after bone marrow transplantation from $bg'/bg'$ mice is under study. The role of theta-sensitive cells for the differentiation of mast cells will be also studied.

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