Presence of Mast Cell Precursors in Peripheral Blood of Mice Demonstrated by Parabiosis

By Y. Kitamura, K. Hatanaka, M. Murakami, and H. Shibata

The presence of mast cell precursors in peripheral blood was examined. The beige C57BL/6 (bg'/bg', Chediak-Higashi syndrome) mouse was parabiosed with the normal C57BL/6 mouse. Mast cells containing giant granules and originating in the beige partner appeared in the normal parabiont. A comparable proportion of normal-mouse-type mast cells developed in the beige parabiont as well. In spite of the low radiosensitivity of mature mast cells, irradiation of the normal parabiont reduced the proportion of normal-mouse-type mast cells appearing in the beige partner. It was concluded, therefore, that the precursors of mast cells can migrate through the bloodstream.

Although both tissue mast cells and blood basophils have granules that contain histamine and heparin, the former are considered to be fixed to the tissues, and the latter wander in peripheral blood. However, we have recently shown that tissue mast cells are derived from grafted bone marrow cells in irradiated genetically normal mice and in nonirradiated W/W' mice that are genetically deprived of tissue mast cells, and therefore there is a possibility that precursors of mast cells migrate in the bloodstream. We examined this possibility in the present study using parabiosis, a more physiologic system than bone marrow transplantation. Pairs of normal and beige (Chediak-Higashi syndrome) C57BL/6 mice, differing with respect to the size of mast cell granules, were parabiosed, and 9 wk later significant numbers of mast cells of the opposite partners’ type were observed in various tissues.

Materials and Methods

Beige (C57BL/6-bg'/bg') mice and their normal littermates (C57BL/6- +/- or -bg'/+) were raised in our laboratory using parental stocks that had been obtained from the Jackson Laboratory, Bar Harbor, Maine. At the age of 2 mo, pairs of beige and normal mice were parabiosed with skin-to-skin anastomosis according to Bunster and Meyer, but the celiac cavity was not opened. Parabiotic pairs consisting of two normal mice were used as controls. After the operation, blood samples were obtained from a lateral tail vein of each parabiont once a week. Two smears were made from each blood sample. A smear was fixed in formaldehyde vapor, stained with Sudan black B, and counterstained with Giemsa solution. The other smear was fixed in methanol and stained with Giemsa solution alone. One hundred neutrophils with distinctly segmented nuclei were scored for the presence or absence of giant granules in the smear stained with Sudan black B, and counterstained with Giemsa solution. The other smear was fixed in methanol and stained with Giemsa solution alone. One hundred neutrophils with distinctly segmented nuclei were scored for the presence or absence of giant granules in the smear stained with Sudan black B, and 100 nucleated cells were examined for the appearance of cells containing basophilic granules in the smear stained with Giemsa solution alone. Parabiotic pairs showing chimerism of neutrophils were killed 4 or 9 wk after the operation.

In some cases the normal parabiont was x-irradiated (850 rads) 4 wk after the operation while the beige parabiont was shielded by lead. Conditions of irradiation have been described in a previous publication. These parabiotic pairs were killed 5 or 15 wk after the irradiation (i.e., 9 or 19 wk after the
Fig. 1. Migration of neutrophils between normal and beige parabionts at various weeks after parabiosis. Neutrophils of beige-mouse type increased in normal partners after irradiation of the latter, whereas neutrophils of normal-mouse type decreased in the shielded beige partners. Each point is the mean of 5-7 mice.

RESULTS

As shown in Fig. 1, about 20% of neutrophils in peripheral blood of normal partners had giant granules 1 wk after parabiosis and were considered to come from the beige partners; similarly, about 20% of neutrophils in the beige parabionts were of the normal-mouse type. Neutrophils of the opposite partners' type rose to about 35% 3 wk after the operation, and this level of chimerism was maintained thereafter. When the normal partners were irradiated 4 wk after parabiosis, over 90% of neutrophils became of beige-mouse type in the normal mice (Fig. 1). In contrast, neutrophils of normal-mouse type dropped to less than 10% in the beige partners (Fig. 1).

Only 6 cells containing basophilic granules were found in 78,000 nucleated cells of blood smears obtained from parabiosed animals on various days after the operation. All of these 6 cells were identified as mast cells by morphologic criteria, and none of them was of the opposite partners' type.

In contrast with the significant exchange of peripheral neutrophils (Fig. 1), mast cells of the opposite partners' type were not observed in various tissues of mice killed 4 wk after parabiosis (Table 1). Few mast cells were detectable in four longitudinal sections of the spleen, femur, and lymph nodes at that time. However, when mice were killed 9 wk after parabiosis, significant numbers of mast cells of the opposite partners' type appeared in the spleen, mesentery, stomach, and cecum of both normal and beige parabionts (Table 1). When normal partners were irradiated 4 wk after parabiosis, the development of mast cells of the opposite partners' type occurred predominantly in the normal parabionts. In fact, more than half of the mast cells were of beige-mouse origin in the spleen, bone marrow, stomach, and cecum of the irradiated parabionts 19 wk after parabiosis, whereas few mast cells of normal-mouse origin were detectable in the various tissues of the beige parabionts (Table 1). In contrast with the considerable development of mast...
MAST CELL PRECURSOR IN PERIPHERAL BLOOD

Table 1. Appearance of Mast Cells of Opposite-Partner Origin After Parabiosis

<table>
<thead>
<tr>
<th>No. of Weeks</th>
<th>Genotype of Mice</th>
<th>No. of Mice</th>
<th>Percentage of Mast Cells of Opposite-Partner Type (Mean)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>X-ray Operation</td>
</tr>
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<tr>
<td></td>
<td>Beige</td>
<td>2</td>
<td>0(0)</td>
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<td>Normal</td>
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<tr>
<td></td>
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<td>3</td>
<td>39(478)</td>
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<tr>
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<td>5</td>
<td>3(249)</td>
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<td>4(240)</td>
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<tr>
<td></td>
<td>Beige</td>
<td>2</td>
<td>5(524)</td>
</tr>
</tbody>
</table>

* Normal partners were x-irradiated (850 rads) 4 wk after parabiosis, whereas beige partners were shielded by lead.
† Average numbers of mast cells of host and partner origin counted in each tissue of an individual parabiont are shown in parentheses.

cells of opposite-partner origin in the hematopoietic tissues and the intestinal canal, mast cells of the beige-mouse type were rarely found in the skin of the normal parabionts even 19 wk after parabiosis (Table 1). Histologic examination of the joined portion of skin revealed that mast cells of one parabiont did not intermingle with those of the other across the operatively united zone.

Although thymus was examined histologically, no more than 20 mast cells were counted in most of parabiosed animals. No mast cells containing giant granules were detected in the various tissues of the normal mice that had been parabiosed with normal partners.

DISCUSSION

Our present results clearly show that mast cells of the opposite partners' type appeared after parabiotic union of mice. There are three reasons that the mast cells of opposite-partner type found in various tissues seemed to be derived from migrated precursor cells rather than from migration of mature mast cells: (1) Mast cells of opposite-partner type were not detected in any tissues 4 wk after parabiosis in spite of the considerable exchange of neutrophils. (2) Although the number of mature mast cells did not decrease in the irradiated animal (especially in the skin, where mast cells are most plentiful), irradiation of the normal partner reduced the appearance of normal-mouse-type mast cells in the beige partner. (3) Mast cells of one parabiont did not intermingle with those of the other parabiont across the operatively united zone.

The present results also show that tissue mast cells may live virtually forever in the skin and that the turnover of mast cells in other tissues is obviously very slow. Thus, if the cells developing into mast cells have a fast blood transit time, their concentration in the peripheral blood will be negligible. Since a rapid blood transit time is characteristic of all granular leukocytes, the paucity of peripheral blood basophils that was demonstrated in the present study and reported previously by others does not permit any definite conclusion whether mast cell precursors in the peripheral blood are granular or nongranular.

The proportions of mast cells of opposite-partner origin were different in various tissues (Table 1). This result is consistent with our previous report that mast cells in the skin of normal mice remain of the host type up to 290 days after irradiation and injection of bone marrow cells from beige mice. When irradiated normal mice injected with beige bone marrow are subjected to local stimulation by painting
20-methylcholanthrene on the skin, beige-mouse-type mast cells develop in the treated skin. It may be concluded, therefore, that the differences in the proportions of mast cells of opposite-partner origin are due to locally controlled differentiation of mast cells from their precursor cells. These precursor cells are derived from the hematopoietic tissues and migrate through the bloodstream to their sites of differentiation.

ACKNOWLEDGMENT

The authors wish to thank Professor L. G. Lajtha, Dr. B. I. Lord, and Dr. K. J. Mori for reviewing the manuscript and Professor K. Matsumoto and Dr. M. Seki for valuable discussions and encouragement.

REFERENCES

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