Administration of 8-Methoxypsoralen and Ultraviolet A Irradiation (PUVA) Induces Turnover of Mast Cells in the Skin of C57BL/6 Mice

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Administration of 8-methoxypsoralen followed by ultraviolet A irradiation (PUVA treatment) has been used as a therapy for urticaria pigmentosa. The effect of PUVA treatment on cutaneous mast cells in mice was investigated by using giant granules of mast cells from C57BL/6-+/+ (Chediak-Higashi syndrome) mice as a marker. C57BL/6-+/+ mice were lethally irradiated and rescued by bone marrow transplantation from C57BL/6-bgl/bgl mice. In the radiation chimeras, mast cells in the skin were of +/+ type and mast-cell precursors migrating in the bloodstream were bgl/bgl. When PUVA treatment was applied to the skin of the radiation chimeras, the total number of mast cells continued to decrease until the third week after the treatment and then recovered to pre-treatment levels. The initial reduction was attributed to the decrease of +/+ type mast cells, and the subsequent recovery to be as a result of the increase of bgl/bgl-type mast cells. This observation may explain the fact that the therapeutic effect of PUVA treatment is transient. Symptoms of urticaria pigmentosa become manifest after the cessation of PUVA treatment probably because new mast cells differentiate from bone marrow-derived precursors. J Invest Dermatol 95:353–358, 1990

M ast cells are normal inhabitants of the skin, but precursors of mast cells are derived from the bone marrow [1]. Although most of the progeny of hematopoietic stem cells leave the bone marrow only after maturation, undifferentiated precursors of mast cells leave the bone marrow, migrate via the bloodstream, and invade the skin. After the invasion, they proliferate and differentiate into mast cells [5,6]. Even after their morphologic differentiation, an appreciable proportion of mast cells retain a considerable proliferative potential [7]. When production of mast cells is required in the skin, differentiated mast cells localized in the skin proliferate first, and then bone marrow–derived precursors invade and differentiate into mast cells [8].

Abnormal increase of the mast cells characterizes several dermatoses, such as urticaria pigmentosa and systemic mastocytosis. Mast cells release various chemical mediators, including vasoactive amines, eosinophil chemotactic factors, and proteolytic enzymes [9], and activated mast cells produce some biologically active metabolites of arachidonic acids [10] and cytokines, including IL-3, IL-4, IL-5, IL-6, and GM-CSF [11–13]. These substances may directly or indirectly cause itching and wealing, disabling symptoms of these dermatoses [14]. Beside treatments with antihistamines [15,16] or adrenocortical steroids [17,18], the administration of 8-methoxypsoralen (8-MOP) followed by ultraviolet A (UVA) irradiation (PUVA treatment) has been reported to be effective in these dermatoses [19–22]. Moreover, irradiation of ultraviolet B (UVB) is also reported to suppress mast cell–mediated wealing [23].

Either PUVA treatment or UVB irradiation inhibits degranulation of mast cells elicited by various chemicals such as compound 48/80 and Concanavalin A [24–26]. On the other hand, Briffa et al [20], Kolde et al [27], and Danno et al [26] reported that the number of mast cells did not change after PUVA treatment and UVB irradiation. Even if the total number of mast cells does not change, there is a possibility that the turnover of mast cells may be induced by the above-mentioned treatments. The mast cells that were located in the skin tissue before the treatments may be destroyed, and the decrease may be compensated by invasion and differentiation of bone marrow–derived precursors [28,29]. To distinguish mast cells localized in the skin from those newly derived from the bone marrow precursors, we used giant granules of mast cells from beige C57BL/6-bgl/bgl, Chediak-Higashi syndrome mice as a marker [8,30,31]. The C57BL/6-+/+ mice, which had received lethal irradiation and injection of bone marrow cells from C57BL/6-bgl/bgl donors, were used as recipients of PUVA treatment and UVB irradiation. Although the decrease in the total number of mast cells was moderate after these treatments, bgl/bgl-type mast cells increased remarkably.

MATERIALS AND METHODS

Radiation Chimeras Mice of C57BL/6-(bgl/bgl) and +/+ were raised in our laboratory and used at 8 weeks of age. C57BL/6-+/+ mice were irradiated (8.5 Gy) using a Toshiba x-ray machine...
operated at 200 kV and 20 mA (target distance, 50 cm; 0.7 mm copper + 0.5 mm aluminum filter; 0.48 Gy/min). Bone marrow cells (10⁶) obtained from C57BL/6-bgJ/bgJ donors were injected into the retro-orbital sinus of irradiated C57BL/6-+/+ mice. The recipient mice were kept within a laminar-flow enclosure. Blood samples were obtained from the retro-orbital sinus of survivors 8 weeks after the bone marrow transplantation. Blood smears were stained with Sudan Black B and counterstained with hematoxylin and eosin for histologic observation, the other with acidified toluidine blue (pH 3.0) for counting mast cells. The number of hg+/hg+-type and hg+-type mast cells were counted under a microscope by using a scale inserted into the eye piece. The number of mast cells was expressed as number per square millimeter.

**Clonal Cultures of Mast Cells** Mice were killed by overinhalation of ether. Pieces of dorsal and ventral skin were removed, washed in Hank’s balanced salt solution buffered with HEPES (pH 7.3) diced into fragments (≤1 mm³), and incubated in Hank’s solution containing collagenase (Type 1, 1.5 mg/ml, Sigma Chemical Co., St. Louis MO), hyaluronidase (Type 1-s, 1.5 mg/ml, Sigma), and 20% fetal bovine serum (HyClone, Logan UT) at 37°C for 2.5 h with gentle agitation. After the incubation, the cell suspensions were passed through a nylon mesh and washed with α-medium (GIBCO, Grand Island, NY) 3 times. The number of cells was counted with a standard hemocytometer. Dispersed skin cells (10⁶) were cultured in the methylcellulose medium; the composition of the medium and culture conditions have been described by Nakamura et al [33]. As a source of growth factors, pokeweed mitogen-stimulated spleen cell-conditioned medium (PWM-SCM) was used. PWM-SCM was prepared according to the method described by Nakahata and Ogawa [34], and the content of IL-3 and IL-4 in PWM-SCM has been shown by Hamaguchi et al [35]. The numbers of mast cell colonies containing ≥50 cells were counted 16 d after the initiation of the culture.

**Staining of Cultured Cells** Individual colonies were lifted from the methylcellulose medium by using a 5-μl Eppendorf pipette under direct microscopic visualization and collected in Eppendorf microcentrifuge tubes containing 0.4 ml Eagle’s medium. After being washed twice with the medium, the samples were immediately spun in a cytocentrifuge (CytoSpin 2, Shandon Southern, Elliott, IL) at 600 rpm for 5 min. The slides were stained with May-Grunwald-Giemsa solution.

### Table I. Numbers of Mast Cells of Each Type in the Dorsal Skin of Radiation Chimeras 3 Weeks after Various Dosages of UVB Irradiation

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Irradiation Dose (mJ/cm²)</th>
<th>Number of Mice</th>
<th>Number of Mast Cells of Each Type/mm² (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+/+ Type</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>5</td>
<td>39 ± 5</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>5</td>
<td>28 ± 12</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5</td>
<td>25 ± 9</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>5</td>
<td>11 ± 4*</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>5</td>
<td>37 ± 4</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5</td>
<td>24 ± 3*</td>
</tr>
</tbody>
</table>

*p < 0.01, when compared to the value of nontreated control mice by Student t test.

### Table II. Numbers of Mast Cells of Each Type in the Dorsal Skin of Radiation Chimeras 3 Weeks after 8-MOP Administration and Various Dosages of UVA Irradiation

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment (J/cm²)</th>
<th>Number of Mice</th>
<th>Number of Mast Cells of Each Type/mm² (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+/+ Type</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>5</td>
<td>37 ± 5</td>
</tr>
<tr>
<td></td>
<td>UVA 2.5 J only</td>
<td>5</td>
<td>48 ± 9</td>
</tr>
<tr>
<td></td>
<td>8-MOP only</td>
<td>5</td>
<td>46 ± 5</td>
</tr>
<tr>
<td></td>
<td>8-MOP+1.5 J</td>
<td>6</td>
<td>10 ± 3*</td>
</tr>
<tr>
<td></td>
<td>8-MOP+2.5 J</td>
<td>5</td>
<td>7 ± 3*</td>
</tr>
<tr>
<td></td>
<td>8-MOP+3.5 J</td>
<td>5</td>
<td>9 ± 4*</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>5</td>
<td>38 ± 5</td>
</tr>
<tr>
<td></td>
<td>UVA 2.5 J only</td>
<td>5</td>
<td>46 ± 7</td>
</tr>
<tr>
<td></td>
<td>8-MOP only</td>
<td>5</td>
<td>45 ± 3</td>
</tr>
<tr>
<td></td>
<td>8-MOP+2.5 J</td>
<td>5</td>
<td>9 ± 1*</td>
</tr>
</tbody>
</table>

*p < 0.01, when compared to the value of nontreated control mice by Student t test.
The backs of radiation chimeras were irradiated with various doses of UVB. Macroscopically, UVB irradiation with 250 or 500 mJ/cm² resulted in erythema of the skin, and irradiation with 750 mJ/cm² induced ulcers. The mice were killed 3 weeks after irradiation, and the skin tissues were examined microscopically.

Acanthosis of epidermis, infiltration of neutrophils and lymphocytes, and fibrosis were observed in the skin of mice irradiated with 750 mJ/cm² UVB. The total number of mast cells showed a rather moderate change. However, when mast cells were classified into +/+ and bg/j bgl types, the former decreased and the latter increased significantly (Table I). Two separate experiments were done; although the decrease of +/+ mast cells in 500 mJ/cm²-irradiated mice was significant only in experiment 2, the results of both experiments were consistent (Table I). Since 750 mJ/cm² UVB irradiation appeared to cause a secondary effect through the development of ulcers, we hereafter used 500 mJ/cm² UVB irradiation.

Administration of 8-MOP was followed by various doses of UVA irradiation. As macroscopic and microscopic alterations of the skin observed after 8-MOP administration and 3.5 J/cm² UVB were comparable to those observed after 750 mJ/cm² UVB irradiation, we selected 2.5 J/cm² UVA irradiation as a PUVA treatment. Although neither administration of 8-MOP alone nor irradiation of UVA alone induced significant change in the number of mast cells, a combination of 8-MOP and UVA resulted in a significant decrease in the total number of mast cells (Table II). When the types of mast cells were classified, +/+ mast cells decreased and bg/j bgl mast cells increased remarkably (Fig 1, Table II). Two separate experiments were done, and the results of both experiments were consistent.

Another experiment was done to follow the sequential change in numbers of mast cells. Mice were killed at various times after either 500 mJ/cm² UVB irradiation or PUVA treatment (UVA 2.5 J/cm²). As all mice received one of these treatments to the dorsal skin, the numbers of mast cells in the ventral skin were used as a control. For this purpose, samples were harvested from both the dorsal and ventral skin. In the dorsal skin that received either UVB irradiation or PUVA treatment, the total number of mast cells continued to decrease until the third week after irradiation and then recovered to pre-irradiation levels. The initial reduction was attributed to the decrease of +/+ mast cells, and the subsequent recovery was considered to be a result of the increase of bg/j bgl mast cells (Figs 2 and 3). Although a statistically significant increase of bg/j bgl mast cells was observed in the ventral skin 4 and 5 weeks after PUVA treatment (Fig 3), the biologic significance was not clear.

Types of mast cell colony-forming cells were investigated 4 weeks after UVB irradiation or PUVA treatment. Skin-cell suspensions were prepared from the dorsal skin and plated in methylcellulose. Each mast-cell colony consisted of either +/+ mast cells alone or bg/j bgl mast cells alone (Fig 4). Mast cells of +/+ type were predominant in the skin cell suspensions, but mast cell colonies of bg/j bgl type were predominant in methylcellulose cultures (Table III).

**DISCUSSION**

The results of the present study showed that the number of mast cells in the skin of mice decreased after UVB irradiation and PUVA treatment. As compared to UVB, PUVA seemed to be more potent in the ability to decrease mast cells without eliciting inflammation. Granerus et al [21] reported that amelioration of symptoms such as itching and weal in patients with urticaria pigmentosa was observed within 2 weeks after starting PUVA, and the patients were symptomless after 3–6 weeks. Such amelioration of the clinical symptoms after the phototherapy is considered to be related to the decrease in number of skin mast cells. On the other hand, sequential examinations of the skin of radiation chimeras revealed that the number of mast cells returned to the pre-treatment level within 4 weeks. This observation is consistent with the fact that the clinical
Figure 3. The sequential change in the numbers of skin mast cells after PUVA treatment. A, dorsal skin; B, ventral skin; open square, total mast cells; open circle, bgJ/bgJ-mast cell; closed circle, bgJ/bgJ-mast cell. A result of single experiment; each point represents mean ± SE of five to nine mice. In total, 35 mice were used. * p < 0.05; ** p < 0.01, when compared to the value of non-treated control mice by Student t test.

effects of phototherapy are transient and symptoms become manifest after the cessation of the therapy [20].

In the present study, we differentiated mast cells that were localized in the skin tissue from mast cells that were newly derived from the bone marrow by using giant granules of mast cells from beige mice. Because we identified mast cells by staining with toluidine blue, the possibility that the initial decrease of mast cells is due to the loss of stainability of the granules cannot be excluded. However, bgJ/bgJ-type but not bgJ/bgJ-type mast cells increased after the initial decrease, and we consider that the decrease represents the loss of skin-localized mast cells induced by UVB irradiation or by PUVA treatment, and that the recovery is attributed to the invasion and differentiation of bone marrow-derived precursors. This is consistent with the result of Sonoda et al [8], who described a comparable change after painting, 7,12-dimethylbenz(a)anthracene (DMBA) ont the dorsal skin of radiation chimeras. Kanakura et al [36] and Waki et al [37] recently demonstrated that the presence of differentiated mast cells inhibited both the invasion of mast-cell precursors into the peritoneal cavity and the differentiation of these precursors into mast cells. The presence of bgJ/bgJ-type skin mast cells probably suppressed the invasion of bgJ/bgJ-type precursors into the skin. The loss of bgJ/bgJ mast cells that resulted from UVB irradiation, PUVA treatment, or DMBA administration may induce the invasion of bgJ/bgJ-mast-cell precursors and their differentiation into morphologically identifiable mast cells. Hyperplasia of epithelium and remarkable infiltration of inflammatory cells were observed after the administration of DMBA, whereas a minimal erythema dose of UVB irradiation or minimal phototoxic dose of PUVA treatment is enough to induce a significant decrease of skin localized mast cells. Therefore, UVB irradiation and PUVA treatment are more suitable than DMBA administration as an experimental procedure to induce the turnover of mast cells.

When skin cells of radiation chimeras that had received UVB irradiation or PUVA treatment were cultured in methylcellulose, mast cell colonies of both bgJ/bgJ and bgJ/bgJ types developed (Table III). This indicates that mast cell colony-forming cells of bgJ/bgJ origin survived after these treatments, and suggested that mast cell damage induced by these treatments may be compensated by proliferation of mast cell precursors existing in the skin in addition to cell division. However, when the proportion of morphologically identifiable bgJ/bgJ mast cells was compared to that of bgJ/bgJ mast cell colony-forming cells in the skin which received UVB irradiation or PUVA treatment, the former was significantly larger than the latter (Table III). Although the morphology of mast cell colony-forming cells in the skin has not been determined, Kanakura et al [33] suggested that some morphologically identifiable skin mast cells themselves may form mast cell colonies in methylcellulose. In fact, about 30% of morphologically identifiable mast cells in the peritoneal cavity produce mast cell colonies [34]. Some explanations are possible for the results shown in Table III. Skin mast cells with colony-forming potential may be more sensitive to UVB irradiation or PUVA treatment than skin mast cells without colony-forming potential. Thus, after UVB irradiation or PUVA treatment, bgJ/bgJ-type cells were remarkable when the skin cells were investigated morphologically, but bgJ/bgJ-type colonies were apparently predominant when the skin cells were
Table III. Proportion of Morphologically Identifiable Mast Cells and Mast Cell Colony-Forming Cells of Each Type 4 Weeks After UVB Irradiation or PUVA Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type</th>
<th>Cells</th>
<th>+/+ Type (%)</th>
<th>bg1/bg1 Type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB irradiation</td>
<td>Mast cell*</td>
<td>85</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mast cell</td>
<td>21</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>colony-forming cell</td>
<td>56</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>PUVA treatment</td>
<td>Mast cell</td>
<td>17</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>colony-forming cell</td>
<td>56</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

* Skin cell suspensions of six radiation chimeras were pooled and plated in methylcellulose; 500 mast cells and 155 mast-cell colonies were classified.

† Skin cell suspensions of six radiation chimeras were plated and methylcellulose; 400 mast cells and 207 mast-cell colonies were classified.

‡ The proportion of +/+ type (or bg1/bg1-type) mast cells were significantly different from the population of +/+ type (or bg1/bg1-type) mast cell colony-forming cells after either UVB irradiation or PUVA treatment (p < 0.01, when compared by x² test).

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REFERENCES

