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Effect of Ultraviolet Irradiation on Mast Cell-Deficient W/W^v Mice*

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The effect of UV irradiation on the skin was investigated in (WB-W/+)×(C57BL/6J-W^v/+)F1-W/W^v mice, which are genetically deficient in tissue mast cells. Their congenic littermates (+/+) and normal albino mice (ICR or BALB/c) were used as controls. Mice were irradiated with 500 mJ/cm^2 of UVB and the increment of ear thickness was measured before and 6, 12, and 24 h after irradiation. Ear swelling in W/W^v mice at 12 and 24 h after irradiation was significantly smaller than that in +/+ and ICR mice. In contrast, the number of sunburn cells formed 24 h after UVB irradiation (200 or 500 mJ/cm^2) was similar in W/W^v, +/+ and ICR mice. On the other hand, when mice were treated with 8-methoxypsoralen (0.5%) plus UVA irradiation (4 J/cm^2) (topical PUVA), ears of W/W^v and BALB/c mice, which were both white in color, were thickened similarly 72 h after treatment, but less swelling was observed in +/+ mice, which were black in skin color. The amount of prostaglandin D$_2$ (PGD$_2$) in ears, determined by radioimmunoassay specific for PGD$_2$, was elevated 3-fold in +/+ and ICR mice at 3 h after irradiation with 500 mJ/cm^2 of UVB in comparison with basal level without irradiation. However, such elevation was not observed in W/W^v mice. These results suggest that mast cells play an important role in UVB-induced inflammation, and PGs from mast cells are responsible at least in part for the development of this reaction. However, neither mast cells nor PGs contribute to the sunburn cell formation and ear swelling response by PUVA treatment.

Considerable information is available about the acute changes of the skin after UV irradiation occurring at cellular, molecular, and pharmacologic levels. However, the precise mechanism, nature, site, and role of the substances that evoke the reaction are not yet fully understood [1]. Mast cells play a central role in the genesis and regulation of inflammatory events [2], and the release of vasoactive substances from mast cells after UV irradiation has been postulated to produce the vasodilation that characterizes sunburn [3], though definitive studies are lacking. The present study was undertaken to examine whether mast cells are responsible for the development of UV-induced inflammation. To this end, we used a mutant mouse, (WB-W/+)×(C57BL/6J-W^v)F1-W/W^v, which is deficient in tissue mast cells [4].

MATERIALS AND METHODS

Materials

[5,6,8,9,12,14,15-^{3}H]prostaglandin D$_2$ (PGD$_2$) (100 Ci/mmol) was from New England Nuclear (Boston, Massachusetts). Synthetic PGD$_2$ was a gift from Ono Pharmaceutical Co. (Osaka, Japan). Sep-Pak C18 cartridges were from Waters Associates. All other chemicals used were of analytical grade.

Animals

Mast cell-deficient mice (W/W^v) and their normal littermates (+/+) were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. We verified that W/W^v mice used in the present study were actually deficient in tissue mast cells [5]. We used normal congenic mice (+/+) as controls. However, W/W^v mice are white and +/+ mice are black in coat color [4]. The response to UV irradiation is considered to be affected by different skin color in mice of both genotypes. Therefore, we have also used normal white mice (BALB/c or ICR) as controls. Male mice, 8–16 weeks old, were used in all experiments and mice were rested for at least 2 weeks before use.

UVB Irradiation

Five tubes of fluorescent sunlamp (Toshiba FL 20SE, Toshiba Electric Co., Tokyo, Japan) were used as a radiation source of middle-wave UV radiation (UVB) as described previously [6]. This lamp tube emits wavelengths mainly between 280–320 nm peaking at 310–315 nm. Mice were placed in a cage and the cage positioned under the UVB source. The cage, 30 cm (width) × 25 cm (length) × 2 cm (height) in size, was covered by a thin wire netting (Nakagawa Medical Product Supplies, Kyoto, Japan) so that during exposure, mice could move freely without lying one over another and received an even distribution of UVB with a total energy dose of 500 mJ/cm^2 at a distance of 30 cm, as measured with the aid of a UV radiometer (Toshiba Medical Supplies, Tokyo, Japan) with spectral sensitivity in the range of 280–320 nm. Irradiation time was approximately 30 min.

Topical PUVA Treatment

Mice were treated with PUVA by the following procedure: 10 μl of 0.5% 8-methoxypsoralen solution (Oxsoralen Lotion, Taisho Pharm. Co., Tokyo, Japan) was painted on the ears of mice 1 h prior to blacklight irradiation. Twenty-watt Toshiba FL-20 BLB (blacklight) (Toshiba Electric Co., Tokyo, Japan) was used for irradiation. The blacklight emits UVA ranging mainly from 320–400 nm with a peak emission at 360 nm through a window glass. Mice received a total energy dose of 4.0 J/cm^2 at a distance of 30 cm, as measured with the aid of a UV radiometer (Toshiba Medical Supplies, Tokyo, Japan) with spectral sensitivity in the range of 320–400 nm. Irradiation time was approximately 30 min.

Ear Swelling Response

Before UV irradiation the baseline thickness of both ears was measured with a dial thickness gauge (Peacock, Tokyo, Japan) under anesthesia with pentobarbital sodium (50 mg/kg body weight) as described previously [6]. Ear thickness was measured 6, 12, and 24 h after UVB irradiation and 24, 48, and 72 h after PUVA treatment. These time intervals were selected because the increment of ear thickness reached a maximum at 24 h after UVB irradiation in ICR mice and at 72 h after PUVA treatment in BALB/c mice. Ear swelling was expressed as the mean increment in thickness above baseline control values.

Sunburn Cell Counting

Tips of ears were removed 24 h after completion of UVB irradiation; the specimens were stained with hematoxylin-eosin and the total number of sunburn cells in the epidermis was counted and the length of the epidermis was measured as described previously [6]. Sunburn cell counts per millimeter of the epidermis were calculated after observing at least 2 different sections per ear specimen.

Radioimmunoassay of PGD$_2$

The amount of PGD$_2$ in mice ears at 3 h after irradiation with 500 mJ/cm^2 of UVB was determined by radioimmunoassay and compared

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Abbreviations:
PG: prostaglandin
PUVA: psoralen photochemotherapy

with basal level without irradiation. Both ears (32–47 mg) of each mouse were removed and directly dropped into liquid nitrogen. Frozen tissues were homogenized with a Polytron homogenizer in 10 ml of distilled ethanol precooled at −20°C. [^{3}H]PGD_2, 10,000 cpm, was added to samples as a tracer. After homogenates were centrifuged at 1000 *g* for 10 min, supernatant solutions were applied to Sep-Pak catridges, and PGD_2 was purified by the method of Powell [7]. Amounts of PGD_2 were measured in duplicates at 4 dilutions by specific radioimmunoassay for PGD_2 and values obtained were corrected for recoveries of [^{3}H]PGD_2 added. Detailed radioimmunoassay procedures and the specificities of the antiserum used were reported previously [5,8]. PGD_2 amounts measured by this radioimmunoassay were well correlated to those obtained by either bioassay or gas chromatography-mass spectrometry procedures [8].

Statistical Analysis

Student's *t*-test was employed to determine the statistical difference between means.

RESULTS

Ear Swelling Response after UVB Irradiation (Table I)

Ear thickness in +/+ and ICR mice was significantly increased after UVB irradiation as shown in Table I. On the other hand, in mast cell-deficient W/W^v mice, ear swelling was not observed at 6 and 12 h, but was significantly ($p < 0.05$) increased at 24 h after irradiation.

However, the increment of ear thickness was significantly ($p < 0.001$) smaller than that in +/+ and ICR mice at 24 h after irradiation. Ear swelling response reached a maximum at 24 h after UVB irradiation and gradually returned to the normal level within 5 days in +/+ and ICR mice.

Sunburn Cell Formation after UVB Irradiation (Table II)

Histologically, considerable edema in the dermis was observed in the ears of +/+ and ICR mice but not of W/W^v mice 24 h after irradiation. No significant difference in the number of sunburn cells formed in W/W^v, +/+, and ICR mice after UVB irradiation with 500 mJ/cm^2 was observed. Similar results were also obtained with irradiation with 200 mJ/cm^2 of UVB. Our preliminary experiment revealed that 200 mJ/cm^2 of UVB was a saturated dose for the sunburn cell formation. Therefore, under this experimental condition, the sunburn cell counts did not seem to be affected by the presence of pigment, and the two different doses of UVB (200 and 500 mJ/cm^2) produced similar numbers of sunburn cells in every strain.

TABLE I. *Increment in ear thickness of mice after UVB irradiation*

Strain	Ear swelling response (× 10^{-3} cm ± SD)[a]			N[b]
	6 h	12 h	24 h	
W/W^v	0.95 ± 1.10[c]	0.67 ± 0.82	1.80 ± 2.48[d]	15
+/+	2.10 ± 1.60[e]	2.50 ± 1.18[f]	5.90 ± 2.47[f]	10
ICR	2.25 ± 2.26[g]	5.83 ± 5.54[e]	13.34 ± 8.39[f]	12

[a] Mice were irradiated with 500 mJ/cm^2 of UVB and ear thickness was measured before and at 6, 12, and 24 h after irradiation. Ear swelling expressed as mean increment in thickness above baseline control values before irradiation.
[b] Number of ears examined.
[c] Increment of ear thickness is not significant compared with baseline control values before irradiation.
[d] Increment of ear thickness is significant ($p < 0.05$) compared with baseline control values before irradiation, but significantly ($p < 0.001$) smaller compared with that in +/+ and ICR mice 24 h after irradiation.
[e] Compared with baseline control values before irradiation, $p < 0.002$.
[f] Compared with baseline control values before irradiation, $p < 0.001$.
[g] Compared with baseline control values before irradiation, $p < 0.02$.

TABLE II. *Sunburn cell formation of mice after UVB irradiation*

Strain	Number of sunburn cells (counts/mm ± SD)[a]			
	200 mJ/cm^2	N[b]	500 mJ/cm^2	N[b]
W/W^v	3.80 ± 1.13[c]	13	3.72 ± 1.16[c]	14
+/+	2.70 ± 1.14[d]	8	3.31 ± 1.07[d]	10
ICR	3.26 ± 0.77	12	2.95 ± 1.39	12

[a] Tips of ears were removed 24 h after irradiation with 200 or 500 mJ/cm^2 of UVB; the specimens were stained with hematoxylin-eosin and sunburn cell counts per millimeter of the epidermis were calculated.
[b] Number of ears examined.
[c] Values are not significantly different from those in +/+ and ICR mice.
[d] Values are not significantly different from those in ICR mice.

TABLE III. *Increment in ear thickness of mice after topical PUVA treatment*

Strain	Ear swelling response (× 10^{-3} cm ± SD)[a]			N[b]
	24 h	48 h	72 h	
W/W^v	1.9 ± 1.7[c]	22.7 ± 5.5[d]	28.2 ± 2.0[d]	10
+/+	1.0 ± 1.1[c]	1.0 ± 1.9[e]	3.8 ± 2.4[f]	10
BALB/c	1.0 ± 0.5[c]	17.9 ± 8.3[d]	37.0 ± 4.4[d]	10

[a] Mice were treated with 8-methoxypsoralen (0.5%) plus UVA irradiation (4.0 J/cm^2) (topical PUVA) and ear thickness was measured before and 24, 48, and 72 h after treatment. Ear swelling was expressed as the mean increment in thickness above baseline control values before treatment.
[b] Number of ears examined.
[c] Increment of ear thickness is not significant compared with baseline control values before PUVA treatment.
[d] Significantly different from the values in +/+ mice at 48 and 72 h after treatment, $p < 0.001$.
[e] Compared with baseline control values before treatment, $p < 0.05$.
[f] Compared with baseline control values before treatment, $p < 0.001$.

TABLE IV. *Level of PGD_2 in mouse ears after UVB irradiation*

Strain	PGD_2 content (ng/g wet weight ± SD)[a]				
	No irradiation	N[b]	3 h after irradiation	N[b]	*p* value
W/W^v	6.8 ± 2.0	4	8.9 ± 3.5	4	N.S.[c]
+/+	8.2 ± 2.8	4	23.2 ± 9.8	4	$p < 0.05$
ICR	7.6 ± 2.8	3	26.0 ± 1.4	4	$p < 0.001$

[a] Both ears of each mouse were removed and directly dropped into liquid nitrogen; the amount of PGD_2 in ears was determined by radioimmunoassay as described under *Materials and Methods*.
[b] Number of samples (mice) examined; both ears of each mouse were assayed as one sample.
[c] Not significant.

Ear Swelling Response after Topical PUVA Treatment (Table III)

Twenty-four hours after PUVA treatment, no significant ear swelling was observed in W/W^v, +/+, and BALB/c mice (Table III). Forty-eight and 72 hours after treatment, a similar increment of ear thickness was observed in W/W^v and BALB/c mice, which are both white in skin color. In +/+ black mice, less but significant ear swelling was noted at 48 h ($p < 0.05$) and 72 h ($p < 0.001$) after treatment. This poor response in +/+ mice might be due to their skin color, but we cannot explain the reason at the present stage of our investigation.

The PGD_2 Content in Ears after UVB Irradiation (Table IV)

The PGD_2 content in ears of ICR mice reached a maximum at 3 h after irradiation with 500 mJ/cm^2 of UVB, and gradually decreased and returned to the basal level at 24 h after irradiation (data not shown). Therefore, we compared the value at 3 h after irradiation among W/W^v, +/+, and ICR mice. As shown in Table IV, the PGD_2 content in ears of +/+ and ICR mice was elevated 3-fold at 3 h after UVB irradiation in comparison with basal level without irradiation. However, such elevation was not observed in W/W^v mice.

DISCUSSION

Considerable knowledge about the chemical and biologic characteristics of UV-induced inflammation has been accumulated [1]. However, the precise mechanism of this unique

response remains poorly understood despite extensive studies of the skin of humans and experimental animals. Vasoactive substances released from mast cells might play an important role in the vasodilatation which characterizes the acute sunburn reaction [3,9]. To evaluate the role of mast cells in the development of UV-induced inflammation, we used mast cell-deficient W/W^v mice. In the present study, we employed ear swelling response and sunburn cell formation of mice as indicators of UV-induced inflammatory reaction as described previously [6]. Ear swelling response induced by UVB irradiation was suppressed by betamethasone or indomethacin [10], whereas sunburn cell formation was inhibited by exogenously added superoxide dismutase, suggesting that the former reaction was mediated by PG and the latter by superoxide anion (O_2^-) [10]. Furthermore, ear swelling response caused by PUVA treatment was not inhibited by indomethacin. Therefore, ear swelling response in mouse ears after UVB irradiation is an equivalent to erythematous reaction in the skin of humans and guinea pigs [11–13].

As shown in Table I, ear swelling response induced by UVB irradiation in W/W^v mice was smaller than that of control mice. This finding suggests that mast cells play an important role at least in part in UVB-induced acute cutaneous reaction, although mast cell-deficient W/W^v mice also show macrocytic anemia, lack of melanocytes in the skin, and sterility [4,14]. However, these defects do not seem to be responsible for the lowered ear thickness of W/W^v mice after UVB irradiation. Furthermore, W/W^v mice have normal T helper and B cell functions for antibody production [15], normal delayed [16] and immediate [17] hypersensitivity responses, and normal Langerhans cells (our preliminary observation). To completely confirm the contribution of mast cells to ear swelling response, it is important to increase mast cells in the dermis of W/W^v mice by bone marrow transplantation from congenic +/+ mice, if possible. However, it seems to be difficult to enhance ear swelling response in W/W^v mice by bone marrow transplantation because the number of mast cells in the skin remains at half of normal even 105 days after transplantation [4].

There have been several reports about the effect of UV irradiation on tissue mast cells [3,9]. Valtonen et al [3] studied the effect of UV irradiation on the degranulation of mast cell in the skin of mice. They observed that mast cells decreased up to 55% of the control value, with a maximum of degranulation corresponding well with the maximum point of erythema, and suggest that UV irradiation causes a release of vasoactive substances from mast cells. More recently, Gilchrest et al [9] also observed similar findings in human skin.

The skin of W/W^v mice contains only negligible amounts of histamine (about 1% of that in congenic +/+ mice [5,18]), although no difference in PGD_2 amounts and PGD synthetase activity in the skin was found between W/W^v and +/+ mice [5]. Therefore, it could be assumed that the amount of histamine was responsible for the ear swelling response after UVB irradiation. However, histamine contributes only to a transient immediate erythema which lasts about 30–60 s in the early phase of sunburn reaction [1]. Furthermore, pretreatment of mice with an injection of either diphenhydramine (30 mg/kg) or a combination of diphenhydramine and cimetidine (10 mg/kg) could not suppress the increase of ear swelling after UVB irradiation (data not shown), suggesting that other factors than histamine may be involved in full development of ear swelling response in mice. However, further experiments will be necessary to determine the role of histamine in ear swelling response.

In mast cells, PGD_2 is the major PG [19,20] and is released upon immunologic challenge or stimulation by calcium ionophore A-23187 [21,22]. To evaluate the role of PGD_2 in ear swelling response, we determined the PGD_2 content in mouse ears by radioimmunoassay specific for PGD_2 as described previously [5,8]. As shown in Table IV, 3 h after UVB irradiation the PGD_2 content in ears of +/+ and ICR mice was elevated 3-fold in comparison with basal level without irradiation. However, such elevation was not observed in W/W^v mice. These results, together with the finding that ear swelling response was suppressed by indomethacin [10], indicated that mast cells play an important role in UVB-induced inflammation, and perhaps PGs derived from mast cells are responsible at least in part for the development of this reaction. In marked contrast, neither mast cells nor PGs contribute to the sunburn cell formation and ear swelling response by PUVA treatment because these responses were similarly observed in W/W^v and control normal mice and were not inhibited by indomethacin.

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