Mechanisms of Contact Photosensitivity in Mice. VII. Diminished Elicitation by Reserpine and Defective Expression in Mast Cell-Deficient Mice

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The involvement of mast cells in the elicitation of contact photosensitivity (CPS) was examined in mice treated with pharmacologic agents and in genetically mast cell-deficient W/W^v mice. Contact photosensitivity responses were diminished by pretreatment with reserpine, which may have been due to depletion of vasoactive amines in mast cells. This inhibition was almost reversed by the monoamine oxidase inhibitor, pargyline-HCl, which prevented reserpine-induced depletion of vasoactive amines such as serotonin. Defective CPS was also found in W/W^v mice, but not in their congenic littermates (+/+). Abnormal CPS in mast cell-deficient mice was due to a defect in the elicitation of CPS rather than a defect in the induction of effector T cells, since the ability to elicit CPS could be transferred to normal +/+ mice by photosensitized cells from mast cell-deficient mice. These findings favor the view that mast cells are involved in the elicitation of CPS. *J Invest Dermatol 87:39–41, 1986*

Cutaneous mast cells have been suggested to be involved in the elicitation of delayed-type hypersensitivity (DTH) responses in mice since agents such as reserpine, which deplete mast cells of vasoactive amines, inhibit DTH [1–4]. According to this view [5], T cells in actively sensitized animals produce an antigen-specific factor that sensitizes tissue mast cells. When they encounter an antigen, as occurs at a DTH challenge site, these mast cells release serotonin, which opens gaps between adjacent endothelial cells, and a second T-cell population enters the extravascular space to interact with local antigen. Thus, DTH is mediated by the sequential activity of 2 functionally different T cells [6,7].

The ability of mast cell-deficient mice to elicit DTH has been reported by several workers; however, the results are controversial. Askenase et al [8] demonstrated the impaired elicitation of DTH reaction in these mice, while other groups found no deficiency in tissue swelling or leukocyte infiltration associated with DTH [9–11]. More recently, Mekori and Galli [12] found that W/W^v mice have no detectable abnormalities in the induction of immunologic tolerance.

The purpose of the present study is to determine whether mast cells are involved in contact photosensitivity (CPS), one of the DTH reactions, in mice. We have been investigating the mechanisms of CPS using our mouse model [13] especially on its immunologic aspects as well as on the differences from ordinary contact sensitivity (CS) [14–18]. We have examined the effect of reserpine that affects the storage of serotonin and pargyline-HCl that affects the catabolism of monoamines such as serotonin, and mast cell-deficient W/W^v mice.

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Abbreviations:
BL: black light, i.e., long-wave UV (320–400 nm)
CPS: contact photosensitivity
CS: contact sensitivity
DTH: delayed-type hypersensitivity
HBSS: Hanks' balanced salt solution
TCSA: 3,3′,4′,5-tetrachlorosalicylanilide

MATERIALS AND METHODS

Animals Mast cell-deficient mice (WB-W/+) × (C57BL/6J-W^v/+) F_1-W/W^v (W/W^v) [19], their normal littermates (+/+), and BALB/c mice were obtained from Shizuoka Experimental Animal Co., Hamamatsu, Japan. The W/W^v mice used in the present study were verified to actually be deficient in tissue mast cells [20]. Male mice, 8–12 weeks old were used in all experiments and mice were maintained for at least 2 weeks before use.

Chemicals 3,3′,4′,5-Tetrachlorosalicylanilide (TCSA) was purchased from Eastman Kodak Co., Rochester, New York. Reserpine (Apoplon for parenteral injection) was obtained from Dai-ichi Pharmaceutical Co., Tokyo, Japan. Pargyline-HCl was purchased from Sigma Chemical Co., St. Louis, Missouri.

Light Twenty-watt Toshiba FL-20BLB tubes (black light, BL) (Toshiba Electric Co., Tokyo, Japan) were used for irradiation. The BL emitted UVA ranging mainly between 320–400 nm with a peak at 360 nm. The energy output of 3 tubes arranged in parallel was 2.7 mW/cm^2 at 360 nm and 0.17 mW/cm^2 at 305 nm at a distance of 15 cm.

Sensitization and Elicitation of CPS to TCSA The procedure was as described previously [13]. In brief, 50 μl of 1% TCSA in acetone was applied to the shaved abdominal wall skin of mice and, within 30 min, the site was irradiated with 3 tubes of BL at a distance of 15 cm for 2 h on days 0 and 1. Light passed through a pane of window glass (3 mm thick). On day 5, all mice were challenged on both sides of the ear lobe with an application of 20 μl of 0.1% TCSA in ethanol and subsequent irradiation with 3 tubes of BL at a distance of 15 cm for 2 h. Before elicitation, the basal line thickness of both ears was measured with a dial thickness gauge (Peacock, Tokyo, Japan). Ear thickness was measured 24 h after the BL irradiation and expressed as the mean increase in thickness above the basal line control values.

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Treatment with Drugs That Affect Serotonin Reserpine, an amine-depleting drug, and pargyline-HCl, a monoamine oxidase inhibitor, were used as described by Gershon et al [1]. Pargyline-HCl was dissolved in saline and administered (100 mg/kg) i.p. After 30 min, reserpine was injected i.p. at a dose of 5 mg/kg 2 h before contact photoelicitation.

Adoptive Transfer of CPS Axillary, brachial, and inguinal lymph nodes were collected on day 5 from either W/W^v mice or +/+ mice. Single cell suspensions were prepared by teasing the lymph nodes in Hanks' balanced salt solution (HBSS, Nissui Seiyaku, Japan) containing penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were washed twice with HBSS by centrifugation at 1000 rpm for 7 min at 4°C. Cells (10^8) in 0.4 ml of HBSS were injected i.v. into normal W/W^v or +/+ recipients. The control mice were not injected with cells. Within 1 h after cell transfer, the recipient and control mice were challenged on the ears with TCSA plus irradiation, and ear swelling response was measured after 24 h.

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

RESULTS

Effects of Monoamine Depletion on CPS to TCSA As shown in Table I, pretreatment of mice with reserpine 2 h before challenge diminished the CPS response occurring 24 h after photoelicitation with TCSA. This suppression was not observed when reserpine was injected 2 h after the challenge. Pargyline-HCl partially reversed the reserpine-induced suppression when it was administered 30 min before the injection of reserpine. Pargyline-HCl pretreatment alone had no significant effect. Reserpine injected prior to contact photosensitization had no inhibitory effect on sensitization of CPS to TCSA (data not shown).

Contact Photosensitivity to TCSA in Mast Cell-Deficient W/W^v Mice (Table II) A time course study revealed that CPS reached a peak 24 h after the challenge, when the response in mast cell-deficient W/W^v mice was significantly weakened as compared with +/+ control mice. Since W/W^v mice are white and +/+ mice are black, photoelicitation may be affected by the different skin colors. Since we have already demonstrated that CPS is inducible in various mouse strains irrespective of H-2 haplotypes and that albino mice seem to be more readily contact photosensitized [13], we used BALB/c normal white mice as controls. The CPS response in BALB/c mice was comparable to that in +/+ mice. In contrast to the previous reports on contact sensitivity [6,7], we could not detect the early 2-h component of the CPS reaction in any strain examined. Since the photochallenge procedure requires a 2-h BL irradiation, we measured the changes in ear thickness 1, 2, and 4 h after the BL irradiation, i.e., during the photochallenge procedure, immediately after, and 2 h after the photochallenge. No significant ear swelling was observed at any point examined.

Table I. Effect of Monoamine Depletion on CPS to TCSA

Drug Treatment[a]	Ear Swelling[b] (24 h) ($\times 10^{-3}$ cm ± SD)	N[c]	*p* Value[d]
Saline	4.7 ± 1.4	5	Control
Reserpine (−2 h)	1.5 ± 0.5	5	$p < 0.01$
Reserpine (+2 h)	3.5 ± 0.4	5	$p < 0.2$
Pargyline (−2.5 h) + reserpine (−2 h)	3.3 ± 0.4	5	$p < 0.2$
Pargyline (−2.5 h)	5.3 ± 1.1	5	N.S.

[a]Reserpine (5 mg/kg) was i.p. injected 2 h before (−2 h) or 2 h after (+2 h) challenge. Pargyline-HCl was dissolved in saline and administered i.p. 2.5 h before (−2.5 h) challenge.

[b]Mice were immunized for TCSA-CPS and ear-challenged 5 days later. Increases in ear thickness were measured at 24 h. In each experiment, nonimmunized mice were challenged similarly and their background ear swelling responses were subtracted from those of appropriately immunized mice.

[c]Number of mice examined.

[d]Compared with control by Student's *t*-test. N.S. = not significant.

Table II. Contact Photosensitivity to TCSA in Mast Cell-Deficient W/W^v Mice

Strain	Ear Swelling Response ($\times 10^{-3}$ cm ± SD)[a] 2 h	24 h	48 h	N[b]
W/W^v	0.0 ± 0.7	1.4 ± 0.6	0.4 ± 0.6	5
+/+	0.0 ± 0.6[c]	4.8 ± 0.7[d]	2.2 ± 1.1[e]	5
BALB/c	0.6 ± 0.7[c]	5.0 ± 1.0[d]	2.5 ± 0.9[e]	5

[a]Mice were immunized for TCSA-CPS and ear-challenged 5 days later. Increases in ear thickness were measured at 2, 24, and 48 h. In each experiment, nonimmunized mice were challenged similarly and their background ear swelling responses were subtracted from those of appropriately immunized mice.

[b]Number of mice examined.

[c]Increment of ear thickness is not significant compared with W/W^v mice.

[d]Significantly different from the values in W/W^v mice, $p < 0.005$.

[e]Compared with W/W^v mice, $p < 0.1$.

Cell Transfer of CPS to TCSA in Mast Cell-Deficient W/W^v Mice Table III demonstrates that actively sensitized W/W^v mice have defective CPS to TCSA, and also that sensitized lymph node cells of immunized W/W^v mice can transfer the ability to elicit CPS to +/+ recipients, but not to W/W^v recipients. Similarly +/+ immune lymph node cells could transfer the ability to elicit CPS to +/+ recipients, but not to W/W^v recipients.

DISCUSSION

Pretreatment of mice with reserpine diminished the elicitation of CPS reaction and pargyline-HCl substantially reversed this suppression. As reserpine had no effect on elicitation when given 2 h after challenge, the agent may block CPS by affecting the initial steps of the elicitation reaction. Defective CPS was found in mast cell-deficient W/W^v mice. Adoptive transfer experiments suggested that abnormal CPS in mast cell-deficient mice is due to a defect in elicitation of CPS rather than a defect in inducing sensitization of T cells. This is because the ability to elicit CPS could be transferred to normal +/+ mice by sensitized cells from mast cell-deficient mice, but sensitized cells from +/+ mice could not transfer CPS responsiveness to mast cell-deficient mice. These findings suggest that mast cells are involved in the elicitation of CPS reaction.

Based on studies using pharmacologic agents as well as mast cell-deficient mice, Askenase et al [1–5] hypothesized that cutaneous mast cells are essential for the elicitation of DTH in mice. On the other hand, other investigators [9–12] have shown that W/W^v mice have normal DTH responses, indicating that the role of mast cells in DTH is questionable. We do not have any data to draw a conclusion on the role played by mast cells in *ordinary*

Table III. Cell Transfer of CPS to TCSA in Mast Cell-Deficient W/W^v Mice

Active Sensitization Strain	Adoptive Transfer: Donor Strain	Recipient Strain	Ear Swelling (24 h)[a] ($\times 10^{-3}$ cm ± SD)	N[b]
W/W^v			1.8 ± 0.6	4
+/+			5.0 ± 0.4	4
	W/W^v	W/W^v	0.4 ± 0.4	4
	W/W^v	+/+	2.9 ± 0.4	4
	+/+	W/W^v	0.1 ± 0.2	4
	+/+	+/+	3.2 ± 1.0	4

[a]Mice were immunized for TCSA-CPS, and 5 days later were ear-challenged or lymph node cells were harvested and transferred i.v. to recipient mice. Increases in ear thickness were measured at 24 h. In each experiment, nonimmunized mast cell-deficient and +/+ controls were challenged similarly and their background ear swelling responses were subtracted from those of appropriately immunized mice.

[b]Number of mice examined.

CS. However, our findings seem to favor the view that cutaneous mast cells are important in eliciting 24-h challenge reaction of *CPS*. It can be argued that reserpine exerts some activity apart from its effect on vasoactive amines, thus impairing the elicitation of DTH response [9]. Alternatively, it is possible that the mast cell is not the site of the amines involved in CPS and that the defective CPS reaction in W/W^v mice may be ascribable partly to an unknown effect of BL irradiation on mast cell-deficient mice.

We could not detect the early component of DTH, which preceded the classical 24- to 48-h DTH skin reaction, even though mice were serially assayed. Thus, it is still unclear whether CPS lacks the early skin swelling reaction that reaches a peak 2 h after challenge, or the early component of CPS is masked by photochallenge process, which requires 2-h BL irradiation

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