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REPORTS

Systemic Suppression of Contact Hypersensitivity in Mice by Psoralen Plus UVA Radiation (PUVA)

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Treatment of mice with 8-methoxypsoralen plus long-wave UV radiation (UVA, 320-400 nm) decreased their response to contact sensitizers applied subsequently to unirradiated skin. This decreased reactivity exhibited a delayed time course, it affected the afferent but not the efferent phase of the reaction, and it was associated with the development of splenic suppressor cells. These suppressor cells were antigen-specific T lymphocytes, and they prevented the induction, but not the elicitation, of contact hypersensitivity in recipient mice. In all of these characteristics, the decreased reactivity induced by treatment with psoralen plus UVA radiation (PUVA) resembled that produced by UV radiation of shorter wavelengths (< 320 nm). These studies suggest that PUVA treatment may initiate the same sequence of cellular events as does exposure to sunlamp (UVB, 280-320 nm) radiation, leading to preferential activation of the suppressor cell pathway.

Exposure of mice to UV radiation from FS40 sunlamps, which emit wavelengths predominantly in the UVB (280-320 nm) range, depresses their response to contact allergens applied subsequently to unirradiated skin [1]. This decreased reactivity is accompanied by the appearance of antigen-specific suppressor T lymphocytes (T_s cells) in the spleens of mice exposed first to UV radiation and then to the sensitizing antigen [2]. The induction of T_s cells in UV-irradiated mice has been attributed to an alteration in the presentation of certain antigens by cells of macrophage lineage [1,3-6].

Recent studies addressing the question of how UV radiation induces suppression of contact hypersensitivity (CHS) implicated DNA as a possible target for the initial photobiologic event. Analysis of the wavelength dependence of this UV radiation-induced suppression demonstrated that the most effective wavelengths lie in the range of 260-270 nm [7]. Since DNA is one of several molecular species in skin that strongly absorb radiation of these wavelengths, damage to the DNA of particular target cells in the skin might be the initiating event in the subsequent suppression of CHS. Our first approach to testing this hypothesis was to select another agent that also produces DNA damage in the skin and to determine whether treatment with this agent would produce similar immunologic alterations. The chemical photosensitizer 8-methoxypsoralen (8-MOP), in combination with long-wave UV radiation (UVA, 320-400 nm), which is designated by the acronym PUVA, induces DNA damage and elicits many of the same biologic responses in the skin of humans and laboratory animals as sunlamp irradiation. These include sunburn, melanization, damage to Langerhans cells, and probably even the induction of cancer [8-10]. Thus it was not unreasonable to suppose that PUVA treatment also might alter immunologic functions in a manner similar to that described for sunlamp irradiation.

The purpose of this study was to determine whether treatment of mice with PUVA produces systemic suppression of CHS, and, if so, whether the cellular mechanisms are similar to those associated with suppression of CHS by UVB radiation. This issue is of additional interest because of the current widespread use of PUVA for the treatment of several common skin diseases in humans, notably psoriasis and vitiligo. For this reason also, it is important to identify any potential immunologic alterations that may accompany PUVA treatment.

MATERIALS AND METHODS

Mice

Specific-pathogen-free female mice of the inbred strains C3H/HeN-(MTV^-), BALB/cAnN, and (C57BL/6N × C3H/HeN) F1 hybrids (B6C3F1) were supplied by the NCI-Frederick Cancer Research Facility's Animal Production Area. The animals were 8-12 weeks old at the start of an experiment, and within each experiment, the age of the animals did not vary by more than 1 week. The mice had free access to Purina mouse chow and chlorinated water (10-12 ppm) and were housed in rooms where ambient light was automatically regulated on a 12-h light-dark cycle.

PUVA Treatment

8-MOP was administered by i.p. injection of 0.4 mg (Hoffmann-La Roche, Inc., Nutley, New Jersey) in 0.5 ml of a 2% gelatin solution. For treatment, the dorsal fur was removed from the mice with electric clippers; they were then given 8-MOP, and 30-60 min later they were exposed to UVA radiation. The UVA radiation (> 320 nm) was delivered from a bank of 6 PUVA fluorescent bulbs (Sylvania, Danvers, Massachusetts) filtered through a 0.05-mm sheet of Mylar to eliminate wavelengths in the UVB region (< 320 nm) [11]. The output of the filtered light source was measured with an IL700 UV spectroradiometer system (International Light, Inc., Newburyport, Massachusetts), using a WBS350 filter and a SEE400 detector, which provides a measure of the irradiance for the integrated waveband between 320-400 nm. The irradiance at the level of the animals' backs averaged 5 W/m^2. During

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Abbreviations:
- C′: complement
- CHS: contact hypersensitivity
- DNFB: dinitrofluorobenzene
- LNC: lymph node cells
- 8-MOP: 8-methoxypsoralen
- OXA: oxazolone = 4-ethoxymethylene-2-phenyl-oxazol-5-one
- PUVA: 8-MOP plus UVA radiation
- T_s cells: suppressor T lymphocytes
- TNCB: trinitrochlorobenzene

the irradiation, the mice were placed in individual compartments on a shelf 20 cm below the radiation source to prevent shielding by cage-mates. Black electrical tape was used to shield the ears during the irradiation period.

UVB Treatment

The conditions of treatment of the animals with UVB radiation were identical to those described above for exposure to UVA radiation. UVB radiation was provided by a bank of 6 FS40 sunlamps (Westinghouse, Bloomfield, New Jersey). The energy output was measured with the IL700 radiometer using a #PT171C UVB detector with a WB320 filter and an A127 quartz diffuser. Approximately 80% of the radiation emitted by this source is within the 280- to 340-nm wavelength range. The irradiance at the level of the animals' backs averaged 4.5 W/m^2.

Induction of Contact Hypersensitivity

The method of Asherson and Ptak [12] was used to induce CHS. Briefly, the abdominal fur was removed from the mice with electric clippers; the abdomen was then shaved with a razor blade to remove all traces of hair. This surface was painted with 50 µl of 5% trinitrochlorobenzene (TNCB) in acetone, 30 µl of 0.5% dinitrofluorobenzene (DNFB) in acetone, or 100 µl of 3% oxazolone (OXA) in ethanol. The mice were tested for CHS 5 or 6 days later by applying 5 µl of 1% TNCB in acetone, 0.2% DNFB in acetone, or 3% OXA in olive oil to both surfaces of each ear. Ear thickness was measured with a spring-loaded micrometer (model 7309, Mitutoyo, Japan) before and 24 h after application of the challenge dose. The specific ear swelling was obtained by subtracting the amount of swelling produced in mice that were challenged on the ears but not sensitized.

Preparation of Suppressor Cell Populations

Cell suspensions were prepared by teasing spleens with forceps into RPMI 1640 medium. The cells were filtered through nylon gauze, washed, resuspended, and refiltered prior to counting. For unfractionated preparations, this cell suspension was injected i.v. at a dose of 1 $\times 10^8$ viable nucleated cells per recipient. All cell suspensions injected contained > 80% viable cells, as determined by trypan blue staining.

Plastic-adherent cells were removed by resuspending the cells in RPMI 1640 medium with 10% fetal bovine serum and plating for 1 h at 37°C on 150 mm-diameter tissue culture dishes (2 spleens per plate in 15 ml of medium). Nonadherent cells were collected by rinsing with RPMI 1640. Approximately 60% of the cells were recovered; no change in the percentage of T lymphocytes could be detected in the preparation after the adherence procedure, as determined by immunofluorescence staining.

To remove T lymphocytes, the spleen cell suspensions were incubated with monoclonal anti-Thy 1.2 serum (New England Nuclear, Boston, Massachusetts) at a dilution of 1:500 for 30 min at 4°C. The cells were then washed 3 times and incubated with rabbit complement (C'; Pel-Freeze, Rogers, Arkansas) at a dilution of 1:16 for 60 min at 37°C. The cells were washed twice and then injected i.v. Control cells were incubated without antiserum, but with C'. In these experiments, the percentage of T lymphocytes in the spleen cell suspensions was reduced from approximately 40% to 50% (in the control group treated with C' only) to between 5% and 15% in the groups treated with antibody plus C' as measured by indirect immunofluorescence. The proportion of cells with surface Ig increased following antibody plus C' treatment. The methods for these techniques have been published [13].

To test for suppressor cell activity, mice were injected with various spleen cell preparations and immediately contact-sensitized; they were challenged 6 days later. The percent suppression was calculated as follows: % suppression = 100[1−(A−B/C−B)], where the letters represent ear swelling in mice: A, sensitized and given spleen cells intravenously; B, not sensitized; and C, sensitized.

RESULTS

Systemic Suppression of CHS with PUVA

A summary of several experiments with the same dose of 8-MOP given i.p., different exposures to UVA radiation, and different strains of mice is presented in Table I. Either single or repeated treatments with PUVA reduced the CHS reaction to TNCB. Neither 8-MOP alone nor UVA radiation alone reduced CHS; furthermore, the background level of ear swelling in unsensitized mice was not affected by any of the treatments. In dose-response studies, it was determined that in the pigmented strains of mice ($C3H^-$ and B6C3F1), a single 90-min exposure

TABLE I. *Suppression of CHS to TNCB by PUVA treatment*

Strain	8-MOP[a]	UVA (kJ/m^2)	UVB[b]	Ear swelling ± SEM ($cm \times 10^{-3}$)				
				−TNCB	+TNCB	Δ[c]	%S[d]	p[e]
C3H[−f]	+	22.5	−	3.2 ± 1.4	11.4 ± 2.5	8.2	1	
	+	202.5	−	2.8 ± 0.4	7.0 ± 2.8	4.2	49	<0.01
	+	−	−	3.1 ± 2.3	14.8 ± 3.3	11.7	0	
	−	202.5	−	2.9 ± 1.6	12.1 ± 2.4	9.2	0	
	−	−	−	3.5 ± 1.9	11.8 ± 2.6	8.3		
BALB/c[g]	+	9	−	4.3 ± 0.9	14.7 ± 1.1	10.4	34	<0.001
	+	18	−	5.0 ± 0.5	12.7 ± 0.8	7.7	51	<0.001
	+	−	−	4.9 ± 0.5	19.6 ± 2.3	14.7	7	
	−	−	−	5.5 ± 0.5	21.3 ± 0.7	15.8		
C3H[g]	+	9	−	4.7 ± 0.6	13.4 ± 0.7	8.7	0	
	+	18	−	5.3 ± 0.4	9.1 ± 0.9	3.8	46	<0.02
	+	36	−	4.3 ± 0.6	8.5 ± 0.8	4.2	40	<0.01
	+	−	−	3.9 ± 0.5	12.3 ± 0.6	8.4	0	
	−	36	−	6.0 ± 0.5	16.9 ± 1.4	10.9	0	
	−	−	+	4.9 ± 0.8	7.4 ± 0.4	2.5	64	<0.001
	−	−	−	5.5 ± 0.5	12.5 ± 0.9	7.0		
B6C3F1[g]	+	27	−	2.1 ± 0.3	3.5 ± 0.3	1.4	77	<0.001
	−	−	+	0.9 ± 0.2	2.1 ± 0.6	1.2	80	<0.001
	−	−	−	2.4 ± 0.2	8.4 ± 1.0	6.0		

[a] 0.4 mg in 0.5 ml 2% gelatin was injected i.p. 30–60 min before UVA irradiation.
[b] UVB radiation was given once at a total dose of 48.6 kJ/m^2.
[c] Δ = mean of group sensitized with TNCB minus mean of unsensitized group. Five mice per group.
[d] %S = [1-(Δ of test group ÷ Δ of untreated group)] × 100.
[e] p = probability of no difference from untreated group, determined by Student's t-test. Minimum number of mice per group = 5. Only probabilities $p < 0.05$ are recorded.
[f] Mice were treated 5 times/week for 3 weeks with PUVA and sensitized 24 h after last treatment.
[g] Mice were given a single PUVA treatment and sensitized 4–7 days later.

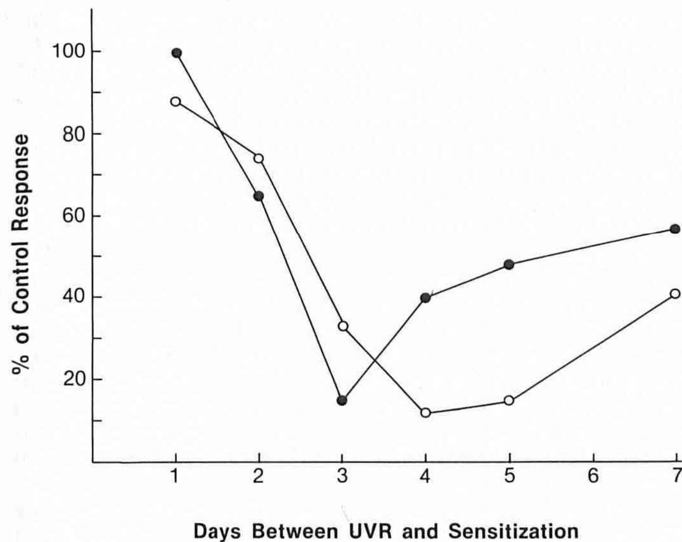


FIG 1. Time course of suppression of CHS following a single PUVA (●—●) or UVB (○—○) treatment. C3H⁻ mice were injected with 0.4 mg 8-MOP i.p. and exposed to 27 kJ/m² UVA (PUVA) or exposed to 48.6 kJ/m² UVB radiation. On successive days thereafter 30 μl of 0.5% DNFB in acetone was applied to the shaved abdomen of half of the mice in each group, and the ears of all the mice were tested 6 days later. Each point represents the mean of two separate experiments each of which contained 5 mice per group. Significant suppression was observed in both the PUVA- and UVB-treated groups from day 3 onward.

to UVA radiation (27 kJ/m²) in combination with 0.4 mg 8-MOP administered i.p. produced approximately the same degree of gross phototoxicity as a single 3-h exposure to FS40 sunlamps (48.6 kJ/m² UVB), i.e., necrosis of the skin along the dorsal ridge of the back about 1 week after the treatment. Because of the comparable phototoxicity produced by these regimens of UV radiation, these doses were used in all subsequent experiments.

Previous studies in BALB/c mice [1] on the time course of the UVB radiation-induced suppression of CHS demonstrated that several days must elapse between the irradiation and the application of the sensitizer in order for suppression to occur. As is illustrated in Fig 1, a similar pattern was observed in C3H⁻ mice after PUVA or UVB radiation treatments. Sensitization of mice 24 h after UVB irradiation or PUVA treatment produced normal levels of reactivity. Minimal reactivity was induced when the sensitizing agent was applied between 3 and 4 days after the irradiation, and it increased slowly thereafter.

Effect of PUVA or UVB Radiation on Elicitation of CHS

We next investigated the stage of the CHS reaction that was affected by PUVA treatments or UVB irradiation. Two types of experiments were performed to determine whether suppression was occurring by means of an effect of these treatments on the elicitation phase of the reaction. In the first, draining lymph node cells (LNC) from DNFB-immunized mice were injected i.v. into animals that had been treated with PUVA or UVB radiation 4 days earlier (passive sensitization). The recipients were challenged by ear painting immediately, and ear swelling was measured 24 h later. As is shown in Table II, experiment 1, the reaction elicited in the passively sensitized animals was not reduced significantly by prior treatment with UVB radiation or PUVA. In contrast, however, mice treated with PUVA or UVB radiation that were actively sensitized by DNFB painting on day 4 exhibited significantly lower reactivity.

In the second type of experiment, mice were first sensitized with DNFB and then treated with UVB radiation or PUVA before elicitation of the reaction. Again, neither treatment reduced the CHS reaction in mice that were already sensitized to DNFB (Table II, experiment 2). Control mice from the same UVB and PUVA treatment groups that were sensitized 5 days after these treatments exhibited only minimal reactivity. These experiments demonstrate that neither PUVA treatment nor UVB irradiation affects the elicitation phase of the response, implying that an earlier step in the CHS reaction is impaired.

Induction of Suppressor Cells

Previous studies demonstrated that the inhibition of CHS by UVB radiation was accompanied by the appearance of T_s cells in the spleens of the irradiated mice [2]. To determine whether T_s cells were also produced as a result of sensitization following PUVA treatment, the following experiment was carried out: Mice were treated with UVB radiation or PUVA, sensitized 4 days later with DNFB, and tested on day 9 for reactivity to demonstrate that their response was impaired. On day 10, spleen cells were taken from these mice and injected i.v. into syngeneic recipients. The recipients were sensitized immediately with DNFB and tested 6 days later to see whether CHS had been induced. The results of a representative experiment are given in the upper portion of Table III. The spleen cell donors that were treated with PUVA or UVB before sensitization with DNFB exhibited 68% and 96% suppression of CHS, respectively, compared with unirradiated control mice. Transfer of 10^8 spleen cells from the suppressed mice resulted in an

TABLE II. *Effect of PUVA or UVB radiation on elicitation of CHS to DNFB in C3H⁻ mice*

Treatment[a]	Sensitization: Agent	Sensitization: Day[b]	Elicitation: Ear swelling ± SEM[c]	Elicitation: Day[b]	Percent suppression[d]
Experiment 1					
UVB	Immune LNC[e]	+4	8.3 ± 0.9	+5	0
UVB	—		1.0 ± 0.3	+5	
PUVA	Immune LNC	+4	7.3 ± 0.5	+5	14
PUVA	—		2.3 ± 0.5	+5	
None	Immune LNC	+4	7.8 ± 0.9	+5	—
None	—		2.0 ± 0.4	+5	
UVB	DNFB[f]	+4	4.3 ± 0.3	+12	76[g]
UVB	—		2.8 ± 0.4	+12	
PUVA	DNFB	+4	5.2 ± 0.7	+12	58[g]
PUVA	—		2.6 ± 0.2	+12	
None	DNFB	+4	9.7 ± 0.5	+12	—
None	—		3.5 ± 0.3	+12	
Experiment 2					
UVB	DNFB	−5	7.2 ± 0.9	+6	0
UVB	—		2.9 ± 0.7	+6	
PUVA	DNFB	−5	6.7 ± 0.8	+6	0
PUVA	—		3.3 ± 0.5	+6	
None	DNFB	−5	6.3 ± 0.5	+6	–
None	—		3.3 ± 0.6	+6	
UVB	DNFB	+5	2.1 ± 0.4	+13	93[g]
UVB	—		1.6 ± 0.5	+13	
PUVA	DNFB	+5	3.5 ± 0.7	+13	87[b]
PUVA	—		2.6 ± 0.3	+13	
None	DNFB	+5	10.0 ± 1.1	+13	—
None	—		3.0 ± 0.4	+13	

[a] UVB = 1 3-h exposure (= 48.6 kJ/m²); PUVA = 0.4 mg 8-MOP i.p. + 90 min (27 kJ/m²) UVA given on day 0.
[b] Number of days after initial treatment.
[c] Mean ± SEM (cm × 10^{-3}) of 5 mice challenged 24 h earlier with DNFB on the ears.
[d] %S = [1-(Δ of test group ÷ Δ of untreated group)] × 100.
[e] Immune LNC = 1.5 × 10^8 draining LNC from mice sensitized 4 days earlier with 30 μl of 0.5% DNFB were injected i.v. Mice receiving cells were challenged immediately after injection.
[f] DNFB = 30 μl of 0.5% DNFB in acetone.
[g] $p < 0.001$ vs unirradiated, DNFB-sensitized control group, as determined by Student's t-test.

impaired response in the recipient animals, indicating that suppressor cells were present in the donor spleens. No significant suppression was transferred with spleen cells from mice treated with PUVA or UVB radiation that were not sensitized with DNFB or with spleen cells from unirradiated sensitized or unsensitized mice. The lower portion of Table III summarizes the results of 5 independent experiments of this kind, indicating that suppressor cells can be detected in the spleens of PUVA-treated or UVB-irradiated mice after sensitization with DNFB.

Separations of the spleen cells were carried out to determine whether the suppressor cells induced in PUVA-treated mice belonged to the T-lymphocyte subpopulation, like those induced in UVB-irradiated animals [2]. Removal of the adherent spleen cells by incubation on plastic culture dishes did not decrease the suppressive activity of the cells from either the PUVA-treated or the UVB-irradiated group (Fig 2*A*). Reducing the number of T lymphocytes in the preparation by incubation of the cells with monoclonal anti-Thy 1.2 antibody and C′ removed the suppressive activity from both PUVA-treated and UVB-irradiated groups (Fig 2*B*). The spleen cell preparations treated with C′ alone contained 40–50% T lymphocytes; those treated with antibody plus C′ contained 5–15% T lymphocytes. Each experiment was performed twice with similar results. These results imply that T lymphocytes in the spleen cell preparations are essential for the suppression of CHS in the recipient mice.

To determine which portion of the CHS reaction was affected by the suppressor cells, these cells were injected either immediately before sensitization or immediately before challenge of presensitized mice. As is shown in Table IV, the suppressor cells from the UVB-irradiated or the PUVA-treated groups did not affect the elicitation of the reaction in previously sensitized mice. In contrast, suppressor cells from both groups prevented the induction of CHS in mice painted with DNFB after injection of the suppressor cells.

Results of tests of the antigenic specificity of the suppressor cells are summarized in Table V. Transfer of DNFB-induced suppressor cells from mice treated with PUVA or UVB radiation inhibited sensitization of the recipient mice with DNFB, but not with OXA. In the reciprocal experiment, sensitization of the treated mice with OXA-induced suppressor cells affected the response of the recipients to OXA, but not to DNFB.

TABLE III. *Transfer of suppression with spleen cells*

Treatment of spleen cell donors[a] (%S)[b]	Δ Ear swelling (cm × 10^{-3})[c]	Percent suppression[d]	p[e]
Representative experiment			
PUVA + DNFB (68)	5.3	60	<0.001
PUVA	11.0	16	
UVB + DNFB (96)	5.2	60	<0.001
UVB	14.7	0	
None + DNFB	9.8	25	
None	16.3	0	
No cells	13.1	—	
Totals from 5 separate experiments[f]			
PUVA + DNFB (56.2 ± 5.2)		49.6 ± 6.7	<0.01
UVB + DNFB (68.8 ± 8.7)		55.6 ± 5.7	<0.01
None + DNFB		15.6 ± 4.8	

[a] UVB = 48.6 kJ/m²; PUVA = 0.4 mg 8-MOP i.p. and 27 kJ/m² UVA. Mice were sensitized with 30 μl of 0.5% DNFB 4 days after treatment. Spleen cells were transferred 6 days after sensitization.

[b] %S = [1-(Δ of test group ÷ Δ of untreated group)] × 100, exhibited by the donor mice prior to transfer of their spleen cells.

[c] All recipients (5 mice per group) were sensitized with 30 μl of 0.5% DNFB after i.v. injection of 1 × 10^8 spleen cells from the donor mice. Δ ear swelling = mean swelling in sensitized group minus mean swelling in unsensitized mice.

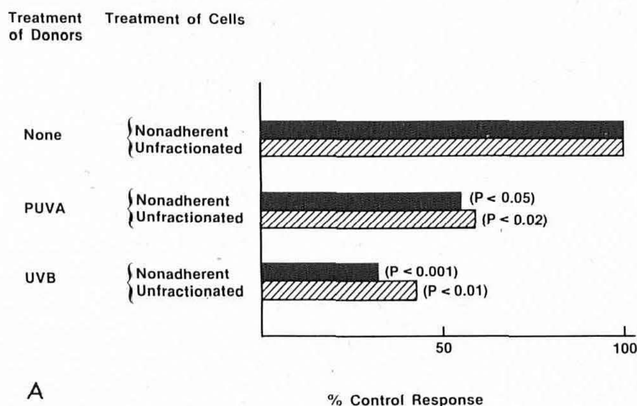
[d] Percent suppression = [1-(Δ of test group ÷ Δ of no cell group)] × 100.

[e] p = probability of no difference from group that did not receive cells, determined by Student's t-test. Only probabilities $p < 0.05$ are recorded.

[f] Numbers represent mean ± SE of the % suppression obtained in 5 separate experiments.

DISCUSSION

Our studies demonstrate that PUVA treatment of mice can suppress CHS reactions much as UVB radiation does, i.e., by a delayed time course after irradiation, inhibition of the afferent arm of the CHS reaction, and induction of antigen-specific T_s cells. These results suggest that UVB irradiation and PUVA treatment might initiate the same sequence of cellular events, which culminates in activation of the suppressor cell pathway. There is some evidence that this sequence of events involves an alteration in the antigen-processing or antigen-presenting activity of splenic macrophages in UVB-irradiated animals [1,4], but this possibility remains to be tested in PUVA-treated mice. Also, the induction of the suppressor cell pathway in UVB-irradiated animals is selective for certain antigens. For example, although the immune response to contact allergens is impaired in UV-irradiated mice, these animals exhibit normal reactivity to alloantigens [14,15] and produce normal amounts of antibody to sheep erythrocytes and polyvinylpyrrolidone [14,16]. Whether PUVA-treated mice show a similar pattern of immune responsiveness also remains to be determined. Nonetheless, the results presented here are consistent with the hypothesis that the same target molecule in skin may serve as the initiator of immunosuppression induced by either UVB radiation or PUVA treatment.



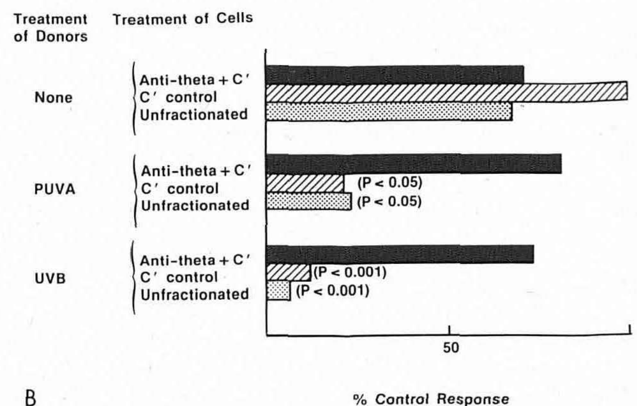


FIG 2. Effect of fractionation of donor spleen cells on their suppressive activity by adherence to plastic culture dishes (*A*) and by T lymphocyte deletion (*B*).

TABLE IV. *Suppressor cells do not affect elicitation of CHS*

Recipients sensitized	Treatment of spleen cell donors[a] (%S)[b]	Δ Ear swelling (cm × 10^{-3})[c]	Percent suppression[d]	p[e]
Six days before cell transfer	PUVA + DNFB (56)	14.1	0	
	UVB + DNFB (71)	14.3	0	
	None + DNFB	12.4	5	
	No cells	13.6	—	
Immediately after cell transfer	PUVA + DNFB (56)	2.2	63	<0.001
	UVB + DNFB (71)	3.8	36	<0.01
	None + DNFB	9.9	0	
	No cells	5.9	—	

[a] UVB = 48.6 kJ/m^2; PUVA = 0.4 mg 8-MOP i.p. and 27 kJ/m^2 UVA. Mice were sensitized with 30 μl of 0.5% DNFB 4 days after treatment. Spleen cells were transferred 6 days after sensitization.
[b] %S = [1-(Δ of test group ÷ Δ of untreated group)] × 100, exhibited by the donor mice prior to transfer of their spleen cells.
[c] All recipients (5 mice per group) were sensitized with 30 μl of 0.5% DNFB after i.v. injection of 1 × 10^8 spleen cells from the donor mice. Δ Ear swelling = mean swelling in sensitized group minus mean swelling in unsensitized mice.
[d] Percent suppression = [1-(Δ of test group ÷ Δ of no cell group)] × 100.
[e] p = probability of no difference from group that did not receive cells, determined by Student's t-test. Only probabilities $p < 0.05$ are recorded.

TABLE V. *Specificity of suppressor cells*

Treatment of spleen cell donors[a] (%S)[b]	Recipients[c] sensitized with: DNFB			OXA		
	Δ Ear swelling (cm × 10^{-3})[c]	Percent suppression[d]	p	Δ Ear swelling (cm × 10^{-3})	Percent suppression	p
PUVA + DNFB (66)	6.0	44	<0.03	17.3	4	—
UVB + DNFB (57)	4.1	62	<0.001	18.3	0	—
None + DNFB	9.6	62	—	16.2	10	—
No cells	10.8	—	—	18.1	—	—
PUVA + OXA (37)	9.4	0	—	9.7	26	<0.05
UVB + OXA (93)	8.5	0	—	10.0	29	<0.05
None + OXA	9.7	0	—	13.8	0	—
No cells	8.1	—	—	13.6	—	—

[a] UVB = 48.6 kJ/m^2; PUVA = 0.4 mg 8-MOP i.p. and 27 kJ/m^2 UVA. Mice were sensitized 4 days after treatment with 30 μl of 0.5% DNFB in acetone or 100 μl of 3% OXA in ethanol. Spleen cells were transferred 6 days after sensitization.
[b] %S = [1-(Δ of test group ÷ Δ of untreated group)] × 100, exhibited by the donor mice prior to transfer of their spleen cells.
[c] All recipients (5 mice per group) were sensitized as above, immediately after i.v. injection of 1 × 10^8 spleen cells from the donor mice. Δ Ear swelling = mean swelling in sensitized group minus mean swelling in unsensitized mice.
[d] Percent suppression = [1-(Δ of test group ÷ Δ of no cell group)] × 100.

One possible candidate for such a target molecule is DNA, since both UVB radiation and PUVA induce DNA damage. 8-MOP intercalates into DNA and absorbs long-wave UV radiation (> 300 nm). This energy is utilized to form covalent bonds between the psoralen and the DNA strands, resulting in the formation of monoadducts and cross-links in the DNA. Like the thymine dimers produce in DNA by the shorter UV wavelengths, these lesions can be removed by cellular repair mechanisms, but in sufficient quantity they interfere with DNA replication [17]. With both PUVA and UVB radiation, the formation of DNA damage in vivo is limited by the depth of penetration of the radiation. The penetration of UV radiation increases somewhat with longer wavelengths [18]; however, the DNA damage that occurs following systemic administration of 8-MOP and UVA radiation or of UVB radiation alone still is confined mainly to the skin and would not be expected to occur in cells of the deeper internal organs. Although DNA is a likely candidate for the target of PUVA- and UVB radiation-induced immunosuppression based on the action spectrum for this effect [7], other possibilities are not ruled out by these studies. Both treatments are likely to cause alterations of other cellular constituents in addition to inducing DNA damage, and it is possible that another, yet unidentified, target may be responsible for initiating the immunosuppressive effects. Additional studies with other photosensitizing and DNA-damaging agents are required to resolve this point.

Previous studies by others have examined the effects of PUVA treatment on the induction and/or elicitation of CHS reactions at the site of irradiation. Using C3H mice, Lynch et al [9] found that topical 8-MOP plus a 60-min UVA exposure given for 7 days resulted in a reduction of CHS to DNFB that was applied on the treated site for sensitization. Similarly, Horio and Okamoto [19] reported that in guinea pigs, sensitization through PUVA-treated skin also reduces the CHS reaction, although this was not found in a previous study by Morison et al [20]. Other studies have suggested that PUVA treatment of guinea pigs at the site of elicitation of CHS reduces the reaction [20,21], but it is not clear from these experiments whether PUVA treatment was affecting the afferent or the efferent phase of the reaction, or both.

In our experiments, PUVA treatment was shown to affect the afferent phase of the reaction by means of a systemic alteration, since neither the sensitization nor the elicitation sites were exposed to the radiation. The doses of PUVA and UVB used in these studies generally were sufficient to produce gross phototoxicity, particularly in the experiments in which a single treatment was employed. In fact, the PUVA doses were selected to produce approximately the same degree of phototoxicity as that produced in the mice by a 3-h exposure to the UVB radiation source. Sufficient damage was produced by this treatment to result eventually in necrosis and scarring of the skin along the dorsal ridge. It might be argued that the immunosuppression we observed was due to general debilitation as a consequence of an acute inflammatory response. However, previous studies addressing this point have established that there is no correlation between the degree of skin damage and the amount of suppression of CHS induced by UVB radiation. For example, fractionating the dose of radiation into small increments pro-

duces less skin damage than a single treatment with the same total dose, yet both produce the same level of suppression of CHS [2]. In addition, monochromatic 270-nm UV radiation produces no visible alterations in mouse skin at doses that are, nonetheless, highly immunosuppressive [22]. Although such a separation between skin damage and suppression of CHS has not been made for PUVA treatment, the selectivity of its effect for the afferent portion of the CHS response suggests that it is not producing generalized immunologic debilitation.

It is clear that PUVA treatment, like UVB irradiation, can cause systemic suppression of CHS reactions in the mouse, which is associated with the production of antigen-specific T_s cells. At present, both of these treatments are widely used in the therapy of skin diseases in humans. Both treatments have beneficial therapeutic effects, but they may also be associated with the adverse effect of increasing the risk of skin cancer [10]. The mechanisms of both the therapeutic and the adverse effects of these treatments may involve alterations of immune function; in fact, there is some evidence that PUVA treatment can alter immune reactivity in humans. Suppression of CHS to DNCB has been reported in patients with psoriasis treated with PUVA therapy [23,24]; however, there is no information on the specificity of this effect or its mechanism, nor is it known whether UVB phototherapy has a similar effect. Further studies in suitable animal models and in humans are necessary to explore the possibility that immunologic factors are involved in the responses to phototherapy.

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