Inhibition of Contact Hypersensitivity by PUVA Treatment

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Normal C3H/HeN strain mice exposed to topical 8-methoxypsoralen plus long wave ultraviolet (PUVA) showed a reduction in contact hypersensitivity (CH) which was localized to the skin in the area of PUVA treatment (local suppression), whereas systemic PUVA treatment caused diffuse suppression of CH reaction, regardless of the application site of 2,4-dinitro-1-fluorobenzene (DNFB).

There seem to be two different mechanisms responsible for CH reduction by PUVA. Local suppression by topical PUVA treatment was thought to be a result of blocking the afferent phase of immune response, it was associated with a lack of CH effector cells in the peripheral lymph nodes and could not be reversed by indomethacin treatment. Diffuse suppression induced by systemic PUVA treatment seemed to be associated with blocking of egress of effector cells from the regional lymph nodes, this depressed CH response was prevented when indomethacin was administered before PUVA treatment.

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Ultraviolet B (280-320 mm) radiation (UVR) is an environmental agent that is known to induce a large number of changes in the skin of humans and animals[1]. Acute UVR exposure induces the migration of inflammatory cells into the exposed area and results in cutaneous erythema and edema (sunburn); chronically, it leads to the gradual disappearance of the inflammatory cell infiltrate and the development of epidermal hyperplasia (premature cutaneous aging)[2] and can result in the induction of skin cancer. Recently, UVR has been shown to alter many parameters of the immunologic reactivity of the host[3,4]. Changes in immune responses that occur following UVR exposure of animals include the functional inactivation of Langerhans cells (LC)[5,6], the depressed ability to respond to contact sensitizer[7,8], the generation of a tumor susceptable state, the alternation, in lymphocyte trafficking patterns and a modification of splenic antigen-presenting cells (APC) function[9,10], a condition caused by their migration from the spleen to peripheral sites[11]. Contact hypersensitivity (CH) represents an example of antigen-specific delayed type hypersensitivity (DTH). CH is best induced by epicutaneous application the sensitizer to intact skin; this epicutaneous sensitization closely resembles the clinical situation in humans. Elicitation is carried out by an epicutaneous challenge of the skin and the reaction is read 24-72 hours later. It has been shown that the ability of a chemical contactant to induce sensitization is related to its ability to couple covalently to protein and the data suggest that

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the actual immunogen recognized by immune system is some self component which has been modified by its reaction with the sensitizer[8]. Macher and Chase[12] reported the importance of the skin, even after the application of sensitizer, in the appropriate induction of CH.

Recent studies addressing the question of how UV radiation induces suppression of CH 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[13]. 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 CH. Many researcher's approach to testing this hypothesis was to select another agent that also produced DNA damage in the skin and to determine whether treatment with this agent would produce similar immunologic alternations. The chemical photosensitizer 8-methoxypsoralen (8-MOP), in combination with longwave ultraviolet radiation (UVA, 320-340 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 responses include sunburn, melanization, damage to Langerhans cells and, probably, the induction of cancer. Thus, it was not unreasonable to suppose that PUVA treatment might also alter immunologic functions in a manner similar to that described for sunlamp irradiation[14-16].

The purpose of this study was to determine whether treatment of mice with PUVA produces local and systemic suppression of CH and, if so, whether the cellullar mechanisms are similar to those associated with suppression of CH by UVB radiation. This issue is of 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 potential immunologic alternation that may accompany PUVA treatment.

MATERIALS AND METHODS

Animals: Normal 8-to-12 week old C3H/HeN mice obtained from the breeding colony maintained at the Department of Microbiology, Wonkwang University School of Medicine. All mice were housed at a maximum density of six animals per 18×28 cm cage and maintained on Wayne Sterizable lab blox and acidified Water ad libitum. Animals were sex matched and between 8-and-12-week old at the onset of any given experiment. Five mice were used for each experiment.

PUVA Treatment: 8-MOP was administered by intraperitoneal (i.p.) injection of 0.4 mg (Sigma, St. Louis, Missouri, U.S.A.) in 0.5 ml of a 2% gelatin solution. Before treatment, the dorsal fur was removed using electric hair clippers and the mice were exposure to UVA radiation. The UVA radiation (320-400 nm) was delivered from a bank of 3 fluorescent bulbs (Oriel Corp., Long Beach, CA, U.S.A.) filtered through a 0.05 mm sheet of Mylar to eliminate wavelengths in the UVB region. The output of the filtered light source was measured with an IL 700 UV radiometer (International Light, Inc., Newburyport, MA, U.S.A.), using a WBS 350 filter (International Light, Inc.) and SEE 400 detector (International Light, Inc.), which provides a measure of the irradiation of the integrated waveband between 320-400 nm. The irradiance at the level of the animals' backs veraged 5W/m^2. During the irradiation, the mice were placed in individual compartments on a shelf 21 cm below the radiation sources to prevent shielding by cagemates. Black electrical tape was used to shield the ear during the irradiation period.

Antigen: 2,4-dinitro-1-fluorobenzene (DNFB) was obtained from Sigma.

Induction of Contact Hypersensitivity: Mice were sensitized on their shaved abdomen or back with 25μl of 0.25% DNFB in a vehicle of 4:1 acetone: olive oil on day 0 and day 1. The animals were challenged by the application of 10μl of 0.25% DNFB in 4:1 acetone: olive oil their right ear lobes on day 5. Ear swelling was measured by using an

engineer's micrometer (Mitutoyo, Japan) at 24 hr after challenge. The increment in ear thickeness of a constant area of the challenged ear pina compared with the unchallenged ear is expressed in units of 10^{-4} inches. The measurements were made under ethyl ether anesthesia and each ear measured at least twice per point. The average increment of increase for each mouse was averaged with the others in the group and the mean±standard error. The percent depression of CH responses in the UV irradiated animal was calculated according to the formula:

$$\% \text{ depression} = \frac{\text{normal-experimental}}{\text{normal-challenge only}} \times 100$$

Adoptive Transfer of Effector Cells: One group was given a topical application of 1.0 ml of a 0.1% solution of 8-MOP in 30% acetone and 70% ethanol, 30 minutes prior to UVA exposure. Another group received 27 KJ/m^2 of UVA over a 90 minute period. Ears were protected with black electrical tape. Experimental animals were sensitized on either their shaved dorsal or ventral surface by the topical application of 24μl of 0.25% solution of DNFB dissolved in acetone: olive oil (4:1) on day 5 and experimental animals used for adoptive transfer into tail vein. Normal animals received 3×10^7 lymph node cells from the aforementioned treatment group. The recipient were immediately ear challenged with 10μl of a 0.25% DNFB solution and extent of ear swelling was measured 24 hours later.

Indomethacin Treatment: At the initiation of the experiment, animals were implanted with a indomethacin-containing pellet designed to administer its contents over 20 day-perod. Pellets which contained specified doses (2.5μg/day) of indomethacin were obtained from Innovative Research of America (Rockville, MD, U.S.A.). These pellets were inserted subcutaneously with a trocar. To prevent the indomethacin treated animals from dying of gastrointestinal bleeding and/or ulceration due to a lack of prostaglandins, all indomethacin treated mice were given 1μg of prostagladin E^2 by way of a gastric lavage needle on alternative days. These indomethacin pellets which release 2.5μg/day were administered subcutaneously 2 days prior to the UVA treatment.

Staining of Langerhans Cells: Five-mm punch biopsies were taken from the depilated midback of the mice before and 5 days after the topical PUVA treatment. Additional C3H/HeN mice received only 8-MOP on one side of their back and only UVA on the other side. Biopsy specimens were taken from both sides 5 days later. Slides were prepared and stained with adenosine triphosphatase (ATPase) with the procedure by Juhlin and Shelly[18]. Briefly the epidermis was seperated from the dermis by incubation in EDTA and fixed in cacodylate-formaldehyde solution. Then, it was incubated in APT-lead nitrate solution for 20 minutes and immersed in ammonium sulfide solution. The specimens were mounted in glycerine jelly. The ATPase positive cells were counted using a reticle fitted into the eyepiece of the microscope at a magnification of 400x. One field outlined in the reticle corresponded to an area of 0.000625 mm^2. In each specimen, cells were counted in 5 to 30 interfollicular areas. The cell populations were expressed as average number of cells per mm^2.

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 preparation, this cell suspension was injected intravenously (i.v.) at a dose of 1×10^8 viable nucleated cells per 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 hr 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.

To test for suppressor cell activity, mice were injected with various spleen cell preparations and contact sensitized with 25μl of 0.25% DNFB on day 0 and 1 and challenged 6 days later. Ear swelling was measured at 24 hr after challenge. The % 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 i.v.; B, not sensitized but given spleen cells i.v.; C, sensitized but not given spleen cells.

RESULTS

Depression of Contact Hypersensivity in Topical PUVA Treated Animal is Not Affected by Indomethacin Treatment: These data are shown in Table 1. Most interestingly, animals that were sensitized through PUVA and treated with indomethacin animals were indistinguishable in their level of responsiveness to the contact sensitizer from those that were sensitized through PUVA only. As shown in Table 1, we confirmed that the local depression in contact hypersensitivity response cause by the topical application of 8-MOP followed by exposure is not affected by indomethacin treatment. When the animals had been sensitized the UVR skin sites, CH was depressed in PUVA treated mice and also PUVA and indomethacin treated mice.

Indomethacin Treatment Inhibits the Development of Diffuse Suppression of Contact hypersensitivity Response Normally Observed in Mice Treated with the Systemic PUVA: The percentage change was related to the positive control. Most importantly, indomethacin treated animals were found to exhibit completely normal response when they were sensitized through the ventral site. However, indomethacin did not reverse the depressed CH when sensitization was performed through the dorsal site (Table 2). Indomethacin treatment inhibits the development of a diffuse suppression in contact hypersensitivity response normally observed in mice given an intraperitoneal injection of 8-MOP followed by UVA exposure.

The Capacity of Topically Applied Haptens to Stimulate Lymph Node Effector Cell Generation Differs between the Systemic PUVA-and Topical PUVA-Treated Groups: To find whether effector cells were generated in the PUVA treated mice that showed depressed CH. We transferred only the stimulated peripheral lymph node cells from topical 8-MOP+UVA treated group and systemic PUVA treated group (Table 3). It was observed that effector cells against the contact sensitizer in the PUVA treated animals, regardless of whether the PUVA was topical or systemic, were induced through intact skin sites. However, no effector cells were generated even in the topical

Table 1. Contact hypersensitivity responses causes by the topical PUVA therapy

Group Treatment of Animals	Skin Site of Sensitization[a]	Contact Hypersensitivity Response±SEM[b]
1. None	Dorsal	77.7±1.5
2. None	Ventral	75.0±2.9
3. 8-MOP[c]+UVA[d]	Dorsal	31.0±4.3 (60)[f]
4. 8-MOP+UVA	Ventral	70.4±2.8 (6)
5. 8-MOP+UVA+Indomethacin[e]	Dorsal	31.6±3.9 (59)
6. 8-MOP+UVA+Indomethacin	Ventral	73.7±2.3 (2)
7. Indomethacin	Dorsal	75.6±2.5 (1)
8. Indomethacin	Vertral	74.2±2.3 (1)

a Animals were sensitized by the topical application of 25μl of a 0.25% solution of DNFB in 4:1 acetone: olive oil on days 0 and 1. Animals were ear challenged with 10μl of the same DNFB solution on day 5.
b Twenty four hours following ear challenge, the extent of ear swelling was measured with an engineer's micrometer (10^{-4} inches). Results are expressed as the difference between the challenged and unchallenged ear.
c Animals were given a topical application of 1.0 ml of 0.1% solution of 8-MOP in30% acetone and 70% ethanol 30 minutes prior to UVA exposure.
d Animals received 27 KJ/m^2 UVA over a 90 minute period. Ears were protected with black electrical tape.
e Indomethacin pellets which release 2.5μ/day were administered subcutaneously 2 days prior to the UVA treatment.
f % depression related to the normal, untreated control.

Table 2. Contact hypersensitivity responses in mice treated with systemic PUVA

Group	Treatment of Mice			Sensitization Site[c]	Contact Hypersensitivity Response±SEM[d]
	8-MOP[a]	UVA[b]	Indomethacin		
Experiment I					
1.	–	–	–	Ventral	65.0±3.5
2.	+	–	–	Ventral	63.2±2.3
3.	–	+	–	Ventral	65.0±0.9
4.	–	–	+	Ventral	62.7±1.3
5.	+	+	–	Ventral	32.2±2.0 (50)[3]
6.	+	+	+	Ventral	64.0±3.1
Experiment II					
1.	–	–	–	Dorsal	67.3±1.48
2.	–	–	–	Ventral	67.7±0.90
3.	+	+	–	Dorsal	29.0±2.10 (59)
4.	+	+	–	Ventral	26.7±1.40 (61)
5.	+	+	+	Dorsal	61.6±3.10 (8)
6.	+	+	+	Ventral	63.3±3.10 (6)

a Experimental animals were given an intraperitoneal injection of 0.5 ml of a 0.8% solution of 8-methoxypsoralen in 2% gelantin-PBS 60 minutes prior to UVA exposure.
b Animals receive 27 KJ/m^2 of unfiltered UVA over a 90 minute period. Black electrical tape was used to cover the ears during the UVR exposure.
c Sensitization was achived by the topical application of 0.25% DNFB in 4:1 acetone: olive oil to the shaved skin surface on day 0 and day 1.
d Animals were ear challenged on day 4. Twenty-four hours later, ear swelling was read using an engineer's micrometer and the results expressed as the difference between the challenged and unchallenged ear in 10^{-4} inches.
e % depression as related to the positive control.

Table 3. Lymph node effector cell generation between mice treated with either systemically or topical 8-methoxypsoralen followed by UVA-exposure

Group	Treatment of Donor Animals	Site of Donor Contact Sensitization[a]	Contact Hypersensitivity Response of Normal Recipients ±SEM 1 Day Following Adoptive Transfer[b]
1.	None	Dorsal	33.3± 1.9
2.	None	Ventral	34.5±1.0
3.	Topical 8-MOP+UVA[c]	Dorsal	16.0±0.9 (52)[e]
4.	Topical 8-MOP+UVA	Ventral	33.0±2.9
5.	Systemic 8-MOP+UVA[d]	Dorsal	32.0±1.7
6.	Systemic 8-MOP+UVA	Ventral	31.3±1.3

a Experimental animals were sensitized on either their shaved dorsal or ventral surfaces by the topical application of 25μl of a 0.25% solution of DNFB dissolved in acetone; olive oil (4:1) on day 0 and day 1. Lymph nodes were coilected on day 5 and used for adoptive transfer.
b Normal animals received 3×10^7 lymph node lymphocytes from the described treatment groups. All mice were immediately enr challenged with 10μl of a 0.25% DNFB solution and the extent of enr swe!ling measured 24 hours later (See b., Table 1).
c See c and d, Table 1.
d See a and b, Table 3.
e % depression as related to positive control.

Table 4. Langerhans cell density in the skin of animals treated with systemic PUVA

Group	Treatment of Animals[a]	Skin Site Analyzed	Cells/mm^2±SEM[b]
1.	Normal Control	Dorsal	1081±51.0
2.	Systemic 8-MOP+UVA (day 1)	Dorsal	1044±46.0 (3)[c]
		Ventral	997±15.3
3.	Systemic 8-MOP+UVA (day 2)	Dorsal	1015±34.0 (6)
		Ventral	996±19.0
4.	Systemic 8-MOP+UVA (day 3)	Dorsal	1010±36.0 (7)

a Experimental animals received an intraperitoneal injection of 0.5 ml of a 0.08% solution of 8-methoxypsoralen described in 2% gelatin-PBS 60 minutes prior to receiving 27 KJ/m^2 of unfiltered UVA over a 90 minute period.
b See b, Table 2.
c See c, Table 2.

Table 5. Generation of suppressor cells in the spleen after systemic PUVA

Treatment of Spleen Cell Donor[a]	Contact Hypersensitivity Response±SEM[b] (10^{-4} inches)	% Suppression[c]
Normal+DNFB	38.58±0.3	25±0.2
Normal	64.17±0.2	0
PUVA+DNFB	20.86±0.2	60±0.5
PUVA	43.30±2.0	16±0.3

a PUVA=0.5 ml of 0.08% solution of 8-methoxypsoralen i.p. and 27 KJ/m^2 UVA. Mice were sensitized with 25μl of DNFB 0 and 1 day after treatment. Spleen cells were transfered 4 days after sensitization.
b All recipients (5 mice per group) were sensitized with 25μl of 0.25% DNFB after i.v. injection of 1×10^8 spleen cells from the donor mice.
c % supression $= (1 - \frac{\text{test group}}{\text{no cell group}}) \times 100$

PUVA treated animals when sensitization was induced through PUVA treated skin.

PUVA Effects on Langerhans Cells: The number ATPase positive cells were not affected by systemic PUVA treatment. The average densities of Langerhands cells in normal control and PUVA treatment group were shown in Table 4. The sparse Langerhans cells (LC) in PUVA-treated skin exhibited morphological changes. Dendritic processes were shortened or not apparent and the central cell body was more heavily stained than that in normal skin. Neither 8-MOP alone nor UVA alone influenced the ATPase positive cells.

Transfer of Suppressor Cells: Previous studies[7] demonstrated that the inhibition of CH was due to or was associated with the appearance of suppressor T (Ts) cells in the spleens of the irradiated mice. To determine whether Ts cells were also produced as a result of sensitization following PUVA treatment, the following experiment was carried out. Mice were treated with PUVA, sensitized 4 days later with DNFB, and tested on day 9 for their reaction to demonstrate that their response was impaired. On day 10, spleen cells were taken from these mice and injected i.v. into syngenic recipients. The recipients were sensitized immediately with DNFB and tested 6 day later to see whether CH had been induced. The results of a representative experiment are given in Table 5. This result showed that Ts cells were generated as a result of sensitization after PUVA treatment.

DISCUSSION

It is known that UVR induces inflammation of skin acutely by the production of arachidonic acid

metabolites and there is a report[19] that has shown that intradermal injection of cyclooxygenase inhibitors could protect the skin from damage by UVR. Recently, Gahrling et al[20]. reported that UVR induces the production of interleukin-1 (IL-1) by the epidermal keratinocytes. Bernheim et al[21]. showed that IL-1 induces prostaglandin (PG) release from the dermal fibroblasts and Chung et al[22]. showed that PG is involved in the immune suppression induced by low dose as well as high dose UVB irradiation. These results suggest that local and systemic suppression of CH responses by UVR are due to similar mechanisms. However our results (Table 1 and Table 2) showed that topical PUVA treatment suppresses the CH locally due to the inactivation of what? in the UV-irradiated skin site and systemic PUVA treatment suppresses CH systemically due to the production of arachidonic acid metabolites. Systemic PUVA can induce the effector cell generation (Table 3) and does not decrease the number of Langerhans cells. (Table 4). Support for this concept came from our finding that indomethacin treatment for animals could totally abrogate the depression in CH response observed in systemic PUVA-treated animals sensitized through the protected dorsal skin sites. Animals sensitized with hapten through irradiated skin sites, however, exhibited markedly depressed CH responses and this depression was not reversed by indomethacin treatment.

Establishing that systemic PUVA treated animals developed normal CH responses to hapten applied to non-irradiated skin sites following treatment with indomethacin suggested that condition might actually result from a quantitative increase in the PUVA-mediated alterations observed in the topical PUVA-treated animals. Under PUVA treatment conditions, peripherally stimulated effector lymphocytes having CH inducing potential remain sequestered within the draining lymph nodes and therefore are unavailable for infiltration into the tissue sites of antigen challenge.

Based on the probability that PUVA-induced inflammation and the associated stimulation of PG synthesis was causing a protracted splenocytes compartmentalization of CH effector cells following epicutaneous hapten application, we hypothesized that systemic PUVA treated animals that were contact-sensitized on their ventral surface would demonstrate a presence of splenic suppressor cell activity. Our finding that splenocytes from both PUVA-treated and normal animals that were hapten sensitized through non-irradiated skin sites were equally capable of adoptively transferring a CH response to naive recipients provided support for this hypothesis. The existence of this Ts cell activity in spleen was demonstrated in spite of the presence of a significant suppressor cell potential in the spleens of those same donors. These results are in indirect contrast to the conclusions reached by others[10,11], which state that a preferential suppressor cell induction is responsible for the immunologically mediated hyporesponsiveness to contact sensitization observed in systemic PUVA treated animals.

The importance of LC in the afferent phases of immune responses to epicutaneously applied antingens is well recognized[5,8,17,23]. The observations made in this study are consistent with the known antigen presenting role of LC. First, inability to adoptively transfer CH treated donors that had been contact sensitized through the irradiated skin sites support earlier findings that PUVA inhibits LC function. Second, splenocytes taken from mouse were fully capable of adoptively transferring HC responses to naive recipients. This indicates that topical PUVA adversely affects normal LC function only in directly exposed skin sites.

These experimental results have allowed us to formulate a number of conclusions concerning the effects of UVR-exposure on the expression of contact hypersensitivity responses in mice. Each is completely in agreement with the probability that both effector and regulatory responsiveness are initiated simultaneously following the topical application of contact sensitizing agents to skin sites on either normal or PUVA treated animals. When hapten is directly applied to a PUVA-treated skin site devoid of normal LC function, the capacity to stimulate the induction of effector lymphocytes within the draining LN is markedly decreased, but normal regulatory responsiveness in the spleen is preserved as evidenced by suppressor cell generation. This consequence implicates the importance of functional LC activity to stimulate the generation of effector cell, but not suppressor cell domi-

nated responses.

Both suppressor cell induction as well as effector cell induction were found to be independent of stimulation of the cyclooxygenase pathway[24]. PG stimulation by UVR, however, did have a significant impact upon the capacity of appropriately sensitized, through LC containing, skin sites protected from UVR exposure animals to functionally mediated a CH response in situ. This alteration in responsiveness is apparently mediated through a forced sequestration, by PGs, of the sensitized CH effector cells in peripheral lymph nodes. Such findings provide additional support of our recent finding that cell mediated immune responses might be effectively controlled, not only through suppressor cell circuits, but also by processes which regulate the capacity of the appropriately sensitized T-cell to gain access to tissue sites of antigen deposition[24].

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