High Doses of UVA Suppress Contact Hypersensitivity

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Contact hypersensitivity(CH) responsiveness to 2-4-dinitro-1-fluorobenzene(DNFB)is depressed in mice sensitized through unexposed skin sites after exposure to high dose of ultraviolet B radiation(UVB). Exposure of mice to ultraviolet A(UVA) radiation in combination with 8-methoxypsoralen(8-MOP) also results in a systemic suppression of CH. Our study was designed to determine whether a high dose of UVA radiation alone can induce a systemic suppression of CH, and if so, which phase of CH response is influenced by UVA radiation.

Relatively large doses of UVA(400, 600, 800J/cm^2) induced significant systemic suppression of CH when DNFB was applied to UVA-unirradiated abdominal skin. The duration of the rest period after UVA exposure did not cause any significant change in systemic suppresion of CH. Functional analyses showed that lymph node cells(LNCs) obtained from donors that were sensitized on the unirradiated skin site with DNFB 5 days after UVA treatment transferred normal ear-swelling responsiveness to non-primed recipients, thus implying that high doses of UVA can induce systemic suppression which is not affected in the induction phase of CH but affected in the elicitation phase of CH. UVA irradiation decreased Langerhans cell(LC) numbers significantly with a dose of 100J/cm^2 or greater. LNCs obtained from donors that were sensitized on the irradiated skin site with DNFB 5 days after UVA treatment did not transfer normal ear-swelling responsiveness to non-primed recipients. This phenomenon may be related to the decreased number of LC after UV treatment. To look for possible mediators impairing the elicitation phase of the CH reaction, we checked prostaglandin E(PGE) levels in serum after 800J/cm^2 irradiation. A high dose of UVA did not increase the serum PGE level in mice as much as UVB irradiation, in which a significant increase of PGE may affect CH response. (Ann Dermatol 3:(2) 96−106, 1991)

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Mice exposed to ultraviolet radiation(UVR) display a marked depression in their ability to mount a contact hypersensitivity(CH) response to topically applied contact-sensitizing agents.[1] These change can be divided into two forms depending on the dose of ultraviolet radiation that is administered to the animal before skin sensitization.[1-3] These have been referred to as local and systemic suppression of CH. Local suppression of CH is induced by a low UVR dose(ap-

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proximately 2-5kJ/m^2), and it is manifested as a reduced CH reaction in the UVR-treated animal that is contact-sensitized directly through UVR-exposed skin sites.[1-3] In contrast, systemic suppression of CH is induced by high doses(15-90kJ/m^2) of UVR and represents a generalized decrease in CH responsiveness, regardless of whether the animals are sensitized through UVR-exposed or nonexposed skin.[2-4]

Toews et al[1] suggested that UVR exposure caused a localized inactivation of epidermal Langerhans cell(LC) capacity within exposed skin sites. When antigens are introduced through UVR-exposed skin, the diminished immune response observed appears to be due to an impaired antigen presenting LC activity within the epidermis. In contrast, animals exposed to high doses of UVR fail to mount normal CH responses, whether sensitized through UVR-exposed or nonexposed skin, thus suggesting that a mechanism independent of antigen presenting LC inactivations is responsible for the depression in CH. This suggested that a soluble mediator might be involved in the mechanism or mechanisms responsible for systemic suppression of CH.[5-6] Jun et al[7] reported that prostaglandin dependent mechanisms influence the elicitation phase of CH responses by inhibiting the normal activity of lymph node CH effector cells in UVB induced systemic CH depression. However, Swartz[8] suggested that serum from UVB-irradiated donors was sufficient to induce splenic suppressor cells in recipient mice which inhibit the afferent pathway of CH response.

Elmet et al[9] reported that the action spectrum for suppression of CH in mice is in the ultraviolet B(UVB) range(280−320nm). In addition, there is clear evidence that exposure of animals to UVA radiation in combination with 8-methoxypsoralen(8-MOP) also results in a sysemic depression of CH and that this is always accompanied by the appearance of antigen-specific T-lymphocytes, and they prevent the induction, but not the elicitation, of CH in mice.[10] Concerning UVA alone, Morison et al[4] reported that 700J/cm^2 UVA-treated mice had a 31-105% enhancement of CH, and LCs were completely eliminated from the epidermis as judged by ATPase staining and ultrastructural examination. Aberer et al[11] also reported that cumulative doses of 100−200J/cm^2 UVA resulted in a dramatic reduction of LCs exhibiting ATPase and Ia-reactivity.

Our study was designed to determine whether a high dose of UVA radiation alone can induce a systemic depression of CH and if so, which phase of CH response is influenced by UVA radiation.

MATERIALS AND METHODS

Mice

Syngeneic C3H/HeN strain of mice of both sexes were obtained from the Genetic Engineering Center of KAIST(Korea Advanced Institute of Science and Technology). The animals were 8-12 weeks old at the start of the experiment. Five animals were employed within each experimental group. All experiments were repeated at least twice with similar results.

UVA treatment

For the high doses of UVA exposure to the animals, Sellas UVA(Dr. Sellmeier Co., Gevelaberg, Germany) lamps served as the source of UVA irradiation. The energy emission spectrum from these lamps was between 320 and 400nm with the peak wavelength of 365nm. At a lamp to target distance of 20cm, the energy flux from these lamps was 50mW/cm^2. To eliminate UVB wavelengths, the UVA radiation was filtered through a sheet of Mylar. The output of the filtered light source was measured with an IL442 UV spectroradiometer system(Inter-national Light, Inc., Newburyport, Mass-achusetts, U.S.A.), using a SEE 015 detector, which provides a measure of the irradi-

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ance for the integrated waveband between 320 −400nm. The irradiance at the level of the animals' backs averaged 50mW/㎠. During the irradiation, the mice were anesthesized with chloral hydrate(0.18㎖ of a 4% chloral hydrate sloution injected i.p) to immobilize them. Before initiation of the UVA treatments the dorsal hairs were completely removed by electric clippers and treatment with a depilatory agent. Black electrical tape was used to shield the ears during the irradiation period.

Sensitization and elicitation of CH

Mice were sensitized through a shaved back or shaved abdominal skin site on days 0 and 1 by the topical application of 30㎕ of 0.5% 2-4-dinitro-1-fluorobenzene(DNFB)(Sigma Chemical Co., St. Louis Missouri, U.S.A.) in a vehicle consisting of a 4:1 acetone/olive oil solution. On day 5, the animals were challenged by the topical application of 10㎕ of 0.25% DNFB to the right ear pinna. Twenty-four hours later, ear swelling was measured with an engineer's micrometer(Mitutoyo, Tokyo, Ja-pan). The increment in ear thickness of a constant area of the challenged right ear pinna compared with the unchallenged left ear is expressed in ear swelling units of 10^{-3}㎝. Measurements were made under ethyl ether anesthesia, and each was measured at least twice per time point. The results are reported as the mean±SD units of ear swelling. The percentage of depression of CH responses in UVA-exposed animals was calculated according to the following formula.

$$\% \text{ depression} = \left(1 - \frac{\text{experimental} - \text{nagative}}{\text{positive} - \text{negative}}\right) \times 100$$

Where experimental is the ear swelling unit of UVA-treated, sensitized, and challenged group;negative is the ear swelling unit of the challenged-only group;and positive is the ear swelling unit of the normal sensitized and challenged group.

Adoptive transfer of CH effector cells

Groups of non-treated control mice, as well as high-dose UVA(800J/㎠)-exposed, donor mice were sensitized with 30㎕ of 0.5% DNFB, topically applied to the shaved, UVA-unexposed abdominal skin or UVA-exposed back skin on 2 consecutive days. Four days after sensitization, inguinal, axillary, and brachial lymph nodes were excised and gently dissociated in RPMI 1640 media(Dutchland Laboratory, Inc., Denver, Pennsylvania, U.S.A.) supplemented with 5% fetal calf serum(Hy-Clone;Sterile System, Inc., Logan, Utah U.S.A.). The cells were washed by centrifugation and resuspended in phosphate buffered saline(PBS) at 1.5×10^{8} cells/㎖. The resultant single cell suspension was transferred by intravenous injection into the lateral tail vein in groups of normal syngeneic recipient mice. Each animal received a total of 3×10^{7}cells. The recipient mice, along with the negative control groups, were then challenged on the right ear with 10㎕ of a 0.25% DNFB solution immediately. Ear swelling was measured 24 hours after the challenge with an engineer's micrometer.

Histochemical staining of epidermal sheets

Biopsy specimens of back and abdominal skin were taken under chloral hydrate anesthesia(0.18㎖ of a 4% chloral hydrate solution injected i.p.) on days 1 and 4 after termination of UVA exposure for ATPase staining, and on days 1,4 and 10 after termination of UVA exposure for immunoperoxidase staining. Subcutaneous tissue was mechanically scraped away from the dermis of the biopsy specimen with a scalpel blade. The skin was incubated, dermal side down, in an isotonic 25 mM EDTA/PBS solution.[12] After 2−3h at 37℃, the epidermis was separated as an intact sheet from the underlying dermis with a fine forceps and cut into 4×4㎜ pieces. The epidermal pieces were histochemically stained for the identification of

ATPase and Ia expressing epidermal cells.

For the identification of cells possessing ATPase activity, the McKenzie and Squier method,[13] with minor modification, was used. Briefly, the epidermal sheets were washed in cold, 0.2M Tris buffer(pH 7.3)for 20 min. They were incubated at 37℃ for 20 min in a substrate consisiting of 10㎎ ATP(Sigma), 5㎖ 5 % $MgSO_4$, and 3㎖ 2% $PbNO_3$ in 42㎖ Tris buffer(pH 7.3). After a thorough wash in Tris buffer, the sheets were treated with a 5% ammonium sulfide solution for 5 min. The sheets were then mounted on microscope slides in glycerol. For the identification of Ia expressing cells, the epidermal pieces were fixed in acetone, dehydrated with PBS, and incubated in either tissue culture medium(control) or I-A and I-E specific monoclonal antibody-containing hybridoma tissue culture supernatants. The monoclonal antibodies(obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A.) that were used in this study included 14-4-4s, specific for the Iak determinant associated antibody and was assessed by an indirect immunoperoxidase method performed with the Vectastain avidin-biotin immunoperoxidase staining procedure, and reagents(Vector Laboratories, Burlingame, California, U.S.A.) with 3-amino-9-ethylcarbazole as the developing substrate. Stained sheets were mounted flat on slides in glycerol and were examined by light microscopy. The numbner of ATPase and Ia positive epidermal cells was determined by randomly counting 10 fields at 400× with and ocular grid of a known area.

From these values the mean number of positive epidermal cells per square millimeter was calculated. The reported number of positive epidermal cells represents the combined mean± SD of all 10 samplings from all the pieces analyzed within a particular treatment group.

Measurement of PGE plasma levels

Prostaglandin E(PGE) levels were assayed according to a modification of the method of Maclouf et al.[14] Briefly, 500㎕ plasma samples were added to Bond-Elut C-18 extraction columns(Analytichem International, Harbor City, California, U.S.A.). The columns were subsequently washed, then dried under a gentle stream of gaseous nitrogen and reconsitituted with assay buffer(0.9% NaCl, 0.01 M EDTA, 0.3% bovine-γ-globulin, 0.005% Triton X-100) and 0.05% sodium azide in 50nM phosphate buffer(pH 6.8). Reconstituted samples were then analyzed by radioimmunoassay performed with PGE-specific antisera and 3H-labeled PGE obtained from New England Nuclear(Boston, Masachusetts, U.S.A.). Radioactivity was measured by a β-liquid scintillation counter(Packard, Tri-CArb 300, Chicago, Illinois, U.S.A.). Results are expressed as mean level of mean± SD.

RESULTS

Suppression of CH with UVA

A summary of the results of several experiments with the different doses of UVA exposures, different sites of sensitization and different duration of rest period are presented in Table 1. Relatively small doses of UVA(50, 100, 200J/㎠) induced local suppression of CH. The duration of the rest period after UVA exposure did not cause a singificant change in systemic suppression of CH. This means that several days need not elapse between the irradiation and the application of sensitizer in order for systemic suppression to occur.

Histological recovery of ATPase- and Ia-positive epidermal LCs in mouse skin after exposure to UVA radiation

The following experiments were conducted to evaluate the recovery of ATPase and Ia positive epidermal LCs in the skin of C3H mice exposed to UVA radiation. Mice were exposed to various doses of UVA at one time. After expo-

Table 1. Effect of Ultraviolet A(UVA) on the Suppression of Contact Hypersensitivity in Mice

UVA(J/cm^2) treatment[a]	Rest(day) after UVA exposure[b]	Sensitization site	Ear swelling (cm $\times 10^{-3}$)	% Depression
Control(positive)			11.3±1.3[c]	
Control(negative)			1.3±0.5[d]	
50	1	back	11.8±2.4	−5
	4	back	0.8±2.6	33
100	1	back	5.3±1.1	59
	4	back	4.8±0.3	65
200	1	back	4.8±0.4	66
	4	back	6.1±1.0	51
	1	abdomen	9.7±2.0	16
	4	abdomen	8.3±1.5	30
400	1	abdomen	3.3±1.7	80
	3	abdomen	4.2±0.3	71
	5	abdomen	4.3±0.6	70
600	1	abdomen	2.3±1.6	90
	3	abdomen	3.0±2.0	83
	5	abdomen	3.3±1.0	80
800	1	abdomen	1.8±0.7	95
	3	abdomen	1.6±1.1	97
	5	abdomen	2.8±0.8	85

[a] The UVA-treated animals received a single dose of UVA given at 50mW/cm^2 per second on their backs. Before UVR treatment, the dorsal hair was completely removed with electric clippers and treatment with a depilatory agent. The hair of normal control animals was removed, but they were not exposed to UVR.

[b] After completion of the UVA treatments, the animals were rested for the indicated amount of time before sensitization.

[c] Ear swelling(mean±SD) of the normal sensitized and challenged mice minus that of unchallenged mice.

[d] Ear swelling(mean±SD) of the challenge only group minus that of unchallenged mice.

sure, the mice were divided into 3 experimental groups for ATPase staining and 4 experimental groups for immunoperoxidase staining. Each group had a rest period for a specified amount of time before skin biopsy specimens were taken for analysis. The density of ATPase and Ia positive LCs within the epidermis of these animals was compared to that in normal non-UVA-treated control mice(Tables, 2, 3, 4). One day after UVA exposures(more than 100J/cm^2), the density of ATPase- and Ia-expressing epidermal LCs on the irradiated back was significantly decreased and the decrease in densities was sustained at 10 days after UVA esposures. In contrast, however, there were no changes in the density of ATPase- and Ia-expressing epidermal LCs on the unirradiated abdomen.

These experiments shows that UVA can directly decrease the number of ATPase- and Ia-positive epidermal LCs, but even a high dose of UVA can not decrease the cells of the unirradiated abdomen indirectly.

Effect of UVA radiation on epidermal antigen-presenting cell activity

The stage of the CH reaction that was affected by UVA was investigated. Two types of experiments were performed to determine whether suppression was occurring through an effect of these treatments on the elicitation phase of

Table 2. Numeric Change of ATPase-positive Epidermal Cells and Ia-positive Epidermal Cells 1 day after Ultraviolet A Irradiation on the Backs and Ears of Mice

UVA(J/cm²) treatment[a]	Rest(day) after UVA exposure[b]	ATPase-positive epidermal cells (No./mm²)[c]			Ia-positive epidermal cells (No./mm²)[c]		
		Back	Ear	Abdomen	Back	Ear	Abdomen
−	−	1102±264	1201±140	1084±190	1260±128	1100±170	1258±154
50	1	902± 51[d]	925± 50[d]	ND[f]	1050±125[d]	1007±145[d]	ND
100	1	672± 25[e]	730±100[e]	ND	890± 74[e]	755± 85[e]	ND
200	1	533± 95[e]	610± 75[e]	ND	780±175[e]	710± 25[e]	ND
400	1	510± 75[e]	590±130[e]	ND	630± 80[e]	710±150[e]	ND
800	1	472± 15[e]	520±125[e]	1198±207[d]	450± 65[e]	525± 98[e]	1298±152[d]

[a] The UVA-treated animals received a single dose of UVA given at 50mW/cm² per second on their back. Dorsal hair was removed from the animals with electric clippers before the UVA treatment was delivered. The dorsal hair of the normal control animals(−) was removed, but they were not exposed to UVR.

[b] After completion of the UVA treatments, the animals were rested for the indicated amount of time before skin biopsies were taken.

[c] The number of ATPase and Ia expressing cell per square millimeter of epidermis is presented as the mean±SD, calculated from counting 100mm² grid for each sample.

[d] P〉0.05, as compared with Wilcoxon Matched Pairs Signed-ranks test

[e] P〈0.05, as comared with Wilcoxon Matched Pairs Signed-ranks test

[f] not done

Table 3. Numeric Change of ATPase-positive Epidermal Cells and Ia-positive Epidermal Cells 4 days after Ultraviolet A(UVA) Irradiation on the Backs and Ears of Mice

UVA(J/cm²) treatment[a]	Rest(day) after UVA exposure[b]	ATPase-positive epidermal cells (No./mm²)[c]			Ia-positive epidermal cells (No./mm²)[c]		
		Back	Ear	Abdomen	Back	Ear	Abdomen
−	−	1102±264	1201±140	1084±190	1260±128	1100±170	1258±154
50	4	790± 63[d]	816± 75[d]	ND[f]	765± 52[d]	785± 51[d]	ND
100	4	620± 79[d]	650± 85[d]	ND	700±110[d]	650± 60[d]	ND
200	4	480±110[d]	515± 30[d]	ND	650± 88[d]	560± 75[d]	ND
400	4	420± 73[d]	415± 60[d]	ND	500± 79[d]	450± 75[d]	ND
800	4	420±125[d]	315± 60[d]	1115±120[e]	550± 80[d]	475± 65[d]	1312±198[e]

[a] The UVA-treated animals received a single dose of UVA given at 50mW/cm² per second on their back. Dorsal hair was removed from the animals with electric clippers before the UVA treatment was delivered. The dorsal hair of the normal control animals(−) was removed, but they were not exposed to UVR.

[b] After completion of the UVA treatments, the animals were rested for the indicated amount of time before skin biopsies were taken.

[c] The number of ATPase and Ia expressing cell per square millimeter of epidermis is presented as the mean±SD, calculated from counting 100mm² grid for each sample.

[d] P〉0.05, as compared with Wilcoxon Matched Pairs Signed-ranks test

[e] P〈0.05, as compared with Wilcoxon Matched Pairs Signed-ranks test

[f] not done

the reaction. In the first, draining lymph node cells(LNC) from mice sensitized through the irradiated back were injected i.v. into normal mice. In the second type of experiment, draining LNCs from mice sensitized through the unirradiated abdomen were injected i.v. into normal mice. The recipients were challenged by ear painting immediately, and ear swelling was

Table 4. Numeric Change of Ia-positive Epidermal Cells 10 days after Ultraviolet A(UVA) Irradiation on the Backs and Ears of Mice

UVA(J/cm²) treatment[a]	Rest(day) after UVA exposure[b]	Ia-positive epidermal cells (No./mm²)[c]		
		Back	Ear	Abdomen
−	−	1260±128	1100±170[d]	1258±154
50	10	785±150[d]	593±110[d]	ND[f]
100	10	535± 75[d]	590± 70[d]	ND
200	10	550±480[d]	540± 90[d]	ND
400	10	575± 50[d]	525± 86[d]	ND
800	10	615± 80[d]	620± 90[d]	1115± 89[e]

[a] The UVA-treated animals received a single dose of UVA given at 50mW/cm² per second on their back. Dorsal hair was removed from the animals with electric clippers before the UVA treatment was delivered. The dorsal hair of the normal control animals(−) was removed, but they were not exposed to UVR.

[b] After completion of the UVA treatments, the animals were rested for the indicated amount of time before skin biopsies were taken.

[c] The number of Ia expressing cell per square millimeter of epidermis is presented as the mean±SD, calculated from counting 100mm² grid for each sample.

[d] P>0.05, as compared with Wilcoxon Matched Pairs Signed-ranks test

[e] P<0.05, as compared with Wilcoxon Matched Pairs Signed-ranks test

[f] not done

Table 5. Adoptive Transfer of 2-4-Dinitro-1-fluorobenzene(DNFB) Primed Lymph Node Cells from Donors Exposed to Ultraviolet A(UVA) Irradiation

UVA(J/cm²) treatment on back[a]	Rest(day) after UVA exposure[b]	Sensitization site	Adoptive transfer of DNFB primed cells[c]	Ear swelling cm × 10^{-3}	% Depression
−	−	−	−	1.3±0.5[d]	−
−	−	back	+	3.3±0.7[e]	−
−	−	abdomen	+	3.2±0.9[e]	−
+	5	back	+	1.1±0.4[e]	110
+	5	abdomen	+	3.4±0.7[e]	−10

[a] The UVA-treated animals(+) received a single 800J/cm² dose of UVA given at 50mW/cm² per second on their backs. Dorsal hair was removed from the animals with electric clippers and treatment with a depilatory The hair wes removed from the normal control amimals(−), but they were not exposed to UVR.

[b] After completion of the UVA treatments, the animals were rested for the indicated amount of time before sensitization.

[c] Normal syngeneric animal received a transfer of 30×10^6 DNFB-primed donor lymphocytes(+) immediately before DNFB challenge. The DNFB-challenged only control animals(−) did not receive an adoptive transfer of primed lymphocytes. The ears of the adoptive transfer recipients and control mice were challenged by topical application of 10u ℓ of 0.25% DNFB solution.

[d] Ear swelling(mean±SD) of the challenge only mice minus that of unchallenged mice

[e] Ear swelling(mean±SD) of the adoptive transfered and challenged minus that of unchallenged mice

measured 24 hours later. As is shown in Table 5, experiment 1, the CH reaction elicited in the passively sensitized animal was not seen when we transfered LNCs from mice sensitized through the irradiated back. In contrast, however, recipient mice of experiment 2 showed a similar CH response compared with the normal control.

These experiments demonstrate that even with high dose irradiation with UVA, CH effector cells can be produced when sen-sitization is done through unirradiated skin and LCs are essential to produce CH effector cells. From these results, we may suggest that UVA irradiation affects the induction phase of CH when sensitization is done through irradiated skin, while a high dose level of UVA in mice, much the same as UVB irradiation, affects the elicitation phase rather than the induction phase of CH when sensitization is done through unirradiated skin.

Measurement of prostaglandin E level in serum after UVA irradiation

The prostaglandin E level in serum was measured 1, 5 and 10 days after 800J/cm^2 of UVA irradiation. The serum PGE level increased slightly 1 day after irradiation and maintained this incresed state until 10 days after irradiation(Table 6). The results of these experiments indicate that a high dose of UVA does not increase the serum PGE level in mice as much as UVB irradiation, in which a significant increase of PGE may affect CH response.[5]

DISCUSSION

Under the appropriate conditions, UVR can function as a potent modulator of certain types of immune responses. These include decreased immune surveillance for the rejection of highly immunogenic skin tumors,[15] reduced severity of graft-vs-host disease[16] and depressed capacity for eliciting CH reactions.[1,2] Elmet et al[9] reported that UV in the UVB range and not in the UVA range is required for the generation of suppressor cells to DNFB sensitization and immunosuppression. Exposure of mice to UVB radiation reduces CH reactions to DNFB that are applied subsequently to irradiated skin. Although the exact mechanism or mechanisms responsible for the UVB-induced depression associated with each of these distinct immune responses are unknown, numerous studies suggest that UVB exposure favors the development of antigen-specific suppressor T lymphocyte(Ts-cell)-dominated immune responses.[9,17] This is especially true of CH responses in which hapten-specific Ts-cells are readily identified in

Table 6. Sequential change of prostaglandin E(PGE) level in plasma after ultraviolet A(UVA) irradiation on the backs of mice

Indomethacin treatment[a]	UVA(800J/cm^2) treatment[b]	Rest(day) after UVA esposure[c]	Plasma lavel of PGE(ng/mℓ)[d]
−	−	−	2.5±0.2
+	−	−	0.7±0.1[e]
+	+	1	0.9±0.2[e]
−	+	1	3.5±0.2[e]
−	+	5	3.3±0.3[e]
−	+	10	2.8±0.3[e]

[a] Individual indomethacin-treated animals(+) received a subcutaneous implant of a drug-containing pellet that released 1.25−2.5 μg of active compound/day, 2 days before UVA treatments began.
Control animals(−) did not receive a pellet implant.

[b] Same as Table 2.

[c] After completion of the UVA treatments, the animals were rested for the indicated amount of time before PGE measurement.

[d] Mice were bled and plasma was obtained 24hr after UV irradiation.
Plasma concentration of PGE was determined by radioimmunoassay after extraction(described in Material and Method). Results are expressed as mean±SD.

[e] $P<0.05$, as compared with Kruskal-Wallis 1-way ANOVA test

UVB-exposed, contact-sensitized animals.[9]

The activation of antigen-specific Ts-cells occurs as a consequence of the absence of I-A$^+$LC function after UV radiation and Ts-cells inhibit the afferent arm of the CH reaction.[18] UVB irradiation induces a population of suppressor-inducers with specificity for a modified skin antigen and this antigen serves as a carrier molecule for haptens that induce contact hypersensitivity and for tumor-specific transplantation antigens on UVB-induced tumors.[19] Our experiment with UVA irradiation also showed immunosuppression when DNFB was applied to the back, in which case the number of LCs decreased. LCs decreased in a UVA dose-dependent manner. We could suggest that local UVA-induced immunosuppression is associated with epidermal LC depletion.

In the case of systemic suppression, Daynes and Spellman[20] reported that systemic UVB-induced immunosuppression was associated with generation of antigen specific Ts cells. However, the skin provides an optical barrier against UV radiation and UVB irradiation cannot directly affect cells outside the epidermis in mice. One may therefore speculate that epidermal cells release, in addition to immunomodulating cytokines such as epidermal cell-derived thymocyte-activating factor(ET-AF) and epidermal cell-derived interleukin 3, other mediators that are responsible for UV-induced immunosuppression.[21,22,23] Schwartz et al[24] reported that UVB radiation may induce epidermal cells to produce an inhibitor of CH which is distinct from PG and leukotrienes and may participate in the regulation of UV-mediated local as well as systemic immunosuppression by blocking the afferent arm of CH not interfering with the elicitation phase. In contrast, however, Jun et al[7] reported that a PG dependent mechanism is responsible for many of the persistent and systemic effects that cause a depression in the CH responsiveness of mice treated with UVB radiation, and PG-dependent mechanisms influence the elicitation phase of CH responses by inhibiting the normal activity of lymph node CH effector cells.

Concerning UVA, treatment of mice UVA radiation eliminated detectable LCs from the exposed skin, based on ATPase staining and electron microscopy,[11] and the CH reaction was enhanced in mice when DNFB was applied to unirradiated skin.[4] Our studies demonstrate that a high dose of UVA irradiation of mice can induce systemic suppression of the CH reaction, unlike Morison's experiment.[4] We think that the difference between our results and Morison's results may be due to the irradiation method. We irradiated with a high dose of UVA for 3 hours;however, Morison irradiated with a high dose of UVA for 72 hours. The backs of our experimental mice were destroyed, devoloping bullae due to the irradiation with high energy of UVA in a short period of time.

Sensitization of mice 24 hours after UVB irradiation or PUVA treatment produced normal levels of reactivity and several days had to elapse between the UVB irradiation or PUVA treatment and the application of sensitizer in order for systemic suppression to occur.[18] Our results suggest that the immunodepression with a high dose of UVA irradiation might not be involved in the activation of antigen-specific Ts cells, which need several days for suppression to occur.

In the results of the lymph node cell(LNC) transfer test, LNCs from mice sensitized on unirradiated abdominal skin were as active as LNCs from normal controls. From these results, we may suppose that the production of CH effector cell in not inhibited by only UVA irradiation, and UVA does not influence the induction phase of CH by the production of antigenpresenting spleen cells which leads to the generation of antigen specific Ts-cells.

Although it is not clear which mechanism is responsible for systemic UVA-induced immunosuppression, a high dose of UVA can induce

systemic immunosuppression which may be affected in the elicitation phase of CH. It might be argued that the immunosuppression we observed was due to general debilitation as a consequence of 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 CH induced by UVB radiation.[18] However, such a separation between skin damage and suppression of CH has not been made for UVA treatment. The deep infiltration of a high dose of UVA could affect the vascular components, such as lymphocytes directly, or produce mediators from the dermis to cause a generalized immunologic debilitation which induces immunodepression soon after UVA irradiation.

To look for possible mediators, we checked serum PGE to determine whether this substance increases after UVA irradiation. There was no remarkable increase of PGE to a level high enough to cause a generalized immunologic change as in the UVB study.[5] Other possible soluble factors need to be studied.

We think the mechanism of immunosupprssion caused by high-dose UVA irradiation may be different from UVB and PUVA since UVA has different characteristics, such as deep infiltration into the dermis, so that a high dose of UVA may cause damage not only to the epidermis but also to the entire dermis, including dermal capillaries and other dermal components.

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