

IDENTIFICATION OF THE MOLECULAR TARGET FOR THE
SUPPRESSION OF CONTACT HYPERSENSITIVITY
BY ULTRAVIOLET RADIATION

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Ultraviolet radiation (UVR)¹ causes many adverse effects on skin, including sunburn, premature aging, and skin cancer. Even though the skin is an organ of protection that buffers chemical and physical damage from the external environment, the DNA within cells of the epidermis is highly susceptible to injury by mutagenic agents, including UVR. On the molecular level, UVR is known to induce different types of photoproducts in cellular DNA, among which is the cyclobutyl pyrimidine dimer formed by covalent bonding between adjacent pyrimidines on the same DNA strand (1). One of the most recently described effects of UVR on the skin is the alteration of immunological function (2). Immunological alterations occurring after UV irradiation can be divided into two types: (a) distant (systemic) alterations in which UV irradiation at one site alters an immunological reaction induced at an unexposed site, and (b) local alterations that result from a direct effect of UVR on the site of a cutaneous immunological reaction.

The mechanism responsible for distant immune suppression by UVR is not well understood; however, mediators produced by keratinocytes exposed to UVR may be involved. For example, a soluble mediator has been isolated from the culture fluid of murine keratinocytes exposed *in vitro* to UVR (3). Injection of this culture fluid into mice mimicked the effect of UVR, as evidenced by suppression of the contact hypersensitivity (CHS) response. Another factor present in the plasma of UV-irradiated mice also suppressed the induction of the CHS response (4). A cytokine produced by keratinocytes, IL-1, suppresses the induction of CHS upon intravenous injection into mice (5). In addition, a molecule present in the stratum corneum, urocanic acid, has been proposed as the primary chromophore of UVR-induced systemic suppression of CHS (6).

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¹ *Abbreviations used in this paper:* CHS, contact hypersensitivity; MED, minimum erythema doses; PRE, photoreactivating enzyme; PRL, photoreactivating light; UVR, ultraviolet radiation.

1118 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

The cellular basis for local immune suppression after UV irradiation seems to involve an alteration of antigen-presenting Langerhans cells in the epidermis. In 1959 it was first reported that Langerhans cells disappeared from the epidermis of guinea pigs after exposure to UVR (7). Since that time, Langerhans cells have been shown to be part of the immune system, and the disappearance of identifiable Langerhans cells after exposure to UVR in the UVB range (280–320 nm) was found to be accompanied by dysfunction of the immune response to antigens applied epicutaneously to the irradiated site (8, 9). The induction of a CHS response was abrogated when skin-reactive haptens were applied directly to the UVR-exposed skin, and hapten-specific suppressor lymphocytes were induced (10). The mechanism by which UVR alters the appearance and function of Langerhans cells is still unknown. Because loss of antigen-presenting function occurs at doses of UVR that do not affect expression or biosynthesis of class II antigens (11–13), some other structure must be the target for loss of function. DNA is a likely target because it is a primary chromophore for UVR-induced damage to living tissues (14). However, damage to cell membranes (15, 16) could also be involved.

The mechanism for the indirect effects of UVR on immune responses initiated at unexposed sites (systemic alterations) does not correlate with damage to Langerhans cells in the irradiated site, and Langerhans cells in the sites of sensitization and challenge are not altered (17–19). In both local and systemic immunological alterations, the suppressed response is associated with the appearance of antigen-specific suppressor T lymphocytes in the spleens of the UV-irradiated, sensitized animals (10, 20).

The cells of the South American opossum, *Monodelphis domestica*, contain an enzyme that confers the ability to repair UVR-induced pyrimidine dimers in epidermal DNA by a process known as photoreactivation (21). In this process, UVR-induced cyclobutane dimers between adjacent pyrimidines on the same DNA strand are repaired in situ by splitting of the dimers, resulting in restoration of the original pyrimidine bases and thus the integrity of the DNA strand (22). In marsupial cells this process is mediated by an enzyme activated by visible light (photoreactivating enzyme; PRE) and is highly specific for pyrimidine dimers. PRE appears to be effective in marsupials (23, 24) and humans (25–27), but it has not been detected in cells from adult rodents (28, 29). Thus, *Monodelphis domestica* is a useful animal model for investigating the involvement of pyrimidine dimers in photobiologic reactions of mammalian skin (30–36).

Recently, it was shown that the UVR-induced disappearance of ATPase⁺ Langerhans cells from the epidermis of *Monodelphis domestica* was subject to photoreactivation, indicating that this effect resulted from DNA damage (32). Because the reduction in the number of ATPase⁺ Langerhans cells in UV-irradiated skin correlates with altered immunological function, we wished to explore the possible role of DNA damage in the local suppression of immune function in order to help identify the molecular target of UVR in immune suppression. In addition, we have used the opossum to ask whether exposure of these animals to PRL can reverse the systemic suppression of CHS induced by UVR.

Materials and Methods

Experimental Animals. *Monodelphis domestica*, the South American, gray, short-tailed opossums were obtained from the Lovelace Medical Foundation animal facility. Animals were housed

in the M. D. Anderson animal facility according to methods adapted from Fadem et al. (37) and fed a diet of dried fox food (reproduction diet; Milk Specialties, New Holstein, WI) and water ad libitum. They were housed under yellow fluorescent lights (to avoid photoreactivating wavelengths of light) on 12-h light-dark cycles. The rooms were maintained at 25°C and 40% humidity. Animals were used for experimentation at 4–5 mo of age. The animal facilities are accredited by the AAALAC and all procedures were approved by the Institutional Animal Care and Use Committee.

Specific pathogen-free C3H/HeN(MTV⁻) mice were purchased from the National Cancer Institute, Frederick Cancer Research Facility Animal Production Area (Frederick, MD). The mice were 12-wk-old females and received NIH-31 open formula mouse chow and sterile water ad libitum.

Radiation Sources. UVR was provided by a bank of Westinghouse FS-40 sunlamps that emit a continuous spectrum between 280 and 400 nm. 60% of the energy is emitted between 280 and 320 nm with a peak emission at 313 nm and relative emissions of 0.04, 0.27, 0.69, 1.0, and 0.09 at 280, 290, 300, 313, and 360 nm, respectively (38). The dose rate from the FS-40 sunlamps was 12.0 W/m² (250–400 nm); therefore, a total dose of 1500 J/m² was delivered in 125 s. The amount of radiation at wavelengths >320 nm administered during this period (~600 J/m²) is insufficient to cause detectable photoreactivation. PRL was obtained from a bank of Westinghouse BLB fluorescent lamps filtered through 3 mm of window glass to remove wavelengths below 320 nm (>90% between 320 and 400 nm). The dose rate of the filtered BLB source was 10 W/m², which provided a total dose of 72 kJ/m² in a 120-min exposure. Dose rates of all light sources were monitored with a calibrated Optronic model 742 spectroradiometer (Optronic Laboratories, Inc., Orlando, FL). The scanning spectroradiometer measures the spectral emissions at 1-nm intervals and integrates the emitted energies over the entire emission spectrum of the lamps.

Induction of Contact Hypersensitivity. Hair was removed with animal clippers (Model A2, Oster Corp.) followed by clean shaving with a Remington Microscreen electric razor the day before hapten application. The hapten was titrated on opossum dorsal epidermis to determine the concentration that produced the optimal CHS response. DNFB (1-fluoro-2,4-dinitrobenzene) was diluted in acetone and olive oil (4:1), and 200 μ l was applied to a 9-cm² area of the shaved dorsal skin. Control animals received the vehicle alone. Baseline ear thickness values were determined using an engineer's spring-loaded micrometer (model no. 7309; Mitutoyo, Tokyo, Japan). At either 4 or 6 d after sensitization, 20 μ l of the same concentration of hapten (challenge antigen) was applied to the ears of each opossum and to animals receiving the vehicle alone. At 12-h intervals thereafter, the ears were measured to determine the amount of swelling. The net ear swelling response was determined by subtracting the baseline ear thickness from the ear thickness measured at each time interval after challenge. The specific ear swelling was determined by subtracting the swelling observed in opossums that were challenged but not sensitized.

Effects of Ultraviolet Radiation and Photoreactivating Light Treatment on Systemic Suppression of Contact Hypersensitivity. The clean-shaved dorsal skin of groups of three to four opossums was exposed to 1,500 J/m² (~2 minimum erythema doses [MED]), or 4,500 J/m² (given in three separate doses of 1,500 J/m² on Monday, Wednesday, and Friday) from the FS-40 sunlamp. 3 d later, the animals were sensitized on unexposed skin to determine whether UV irradiation altered the CHS response induced at a distant site. After establishing the dose of UVR required for systemic suppression of CHS, other groups of opossums received 120 min of PRL (72 kJ/m²) before or immediately after UVR exposure. This exposure to PRL was estimated to be able to repair the majority of the pyrimidine dimers induced by 1,500 J/m² UVB radiation, based on previous studies (24, 30, 32). During UVR and PRL exposures the ears were covered. 3 d after UVR and PRL treatments, the opossums were tested for their ability to develop CHS to DNFB applied to unirradiated, abdominal skin as described above with ear challenge occurring 6 d after sensitization. The treatment groups (three to four opossums per group) were as follows: (a) UVR alone (1,500 J/m², FS-40 sunlamp); (b) PRL alone (120-min exposure, BLB fluorescent lamps, glass filtered); (c) UVR/PRL; (d) PRL/UVR; and (e) shaved alone.

Effects of Ultraviolet Radiation and Photoreactivating Light Treatment on Local Suppression of Contact Hypersensitivity. The shaved dorsal skin of groups of three to six opossums was exposed to

1120 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

1,500 J/m² (~2 MED), 3,000 J/m², or 4,500 J/m² from the FS-40 sunlamp to determine whether UV irradiation altered the CHS response to haptens applied to the irradiated site. After the dose of UVR required for local suppression of CHS was determined, other groups of opossums received 120 min of photoreactivating light (PRL) before or immediately after the UVR exposure. During UVR and PRL exposures, the ears were covered. 5 d after the UVR and PRL treatments, opossums were assessed for their ability to develop CHS to DNFB applied to the irradiated skin, as described above. The treatment groups (three to six opossums per group) were the same as for systemic suppression of CHS as described above. In addition, unirradiated opossums with and without sensitization were tested for their ear swelling response to DNFB, and the ears were removed for histological analysis.

Ultraviolet Irradiation and Photoreactivation of ATPase⁺ Langerhans Cells. Opossums were anesthetized by inhalation of methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ) in a closed chamber system. A group of four, clean-shaved opossums were irradiated with 1,500 J/m² of UVB radiation on half of the dorsal skin while the other half was shielded with aluminum foil. This dose of UVB is equivalent to ~2 MED in opossum skin. Immediately before irradiation treatments, and on days 1, 3, 5, 10, 12, and 15 after UVR, biopsies (4 × 6 mm ovals) were taken from irradiated and unirradiated dorsal skin.

To determine the effect of PRL on ATPase⁺ Langerhans cells, the shaved dorsal skin of a group of three opossums was marked into five equal areas: (a) UVR alone (1,500 J/m², FS-40 sunlamp); (b) PRL alone (120-min illumination, BLB fluorescent lamps, glass filtered); (c) PRL/UVR; (d) UVR/PRL; and (e) no treatment. PRL treatment was given immediately before (PRL/UVR) or after (UVR/PRL) the UVR exposure. The areas not being irradiated or illuminated with PRL were appropriately masked during treatment with aluminum foil. On day 5, skin biopsies (4 × 6 mm ovals) were taken from each area of the back.

Identification of ATPase⁺ Langerhans Cells. Biopsies were placed in vials of PBS on ice and then incubated in PBS-EDTA at 37°C for 2 h, after which the epidermis was separated from the dermis with jeweler's forceps. The epidermal specimens were washed three times in 0.15 M NaCl (saline), fixed for 20 min in cacodylate-buffered 4% formaldehyde, washed three times with saline, incubated for 15 min in ATP-PB(NO₃)₂ substrate for ATPase, washed three times with saline, developed for 20 min in dilute (NH₄)₂S, washed three times in saline, and mounted, internal-side up, on microscope slides with glycerol/PBS (9:1, vol/vol). ATPase⁺ Langerhans cells were counted in 15–25 fields per specimen using a calibrated microscope grid at ×400 magnification. The results were expressed as the number of ATPase⁺ Langerhans cells/mm² epidermis for each specimen.

CHS and Langerhans Cell Density in Mice. The dorsal hair of the mice was removed with electric clippers, and before irradiation, the ears were covered with an opaque tape. Mice were irradiated with the same dose and in the same treatment groups as described above for opossums. 5 d later, mice from each group were killed, the dorsal (UV-irradiated) skin was removed, and the epidermis was separated from the dermis and stained for ATPase activity. Mice from the treated and untreated groups were also sensitized on the UV-irradiated skin with 50 μl of 0.3% DNFB in acetone (vol/vol). The mice were tested for CHS 6 d later by applying 5 μl of 0.2% DNFB on each surface of both ears. Ear thickness was measured with a spring-loaded micrometer before and 24 h after application of the challenge dose of DNFB. The specific ear swelling was obtained by subtracting the amount of swelling produced in mice that were challenged on the ears but were not sensitized.

In Vivo Radiolabeling, Biopsies, and DNA Extractions. Opossums were anesthetized, clean-shaved, and the epidermal DNA was radiolabeled in vivo by six subcutaneous injections of 50 μCi each of [³H]thymidine (20 Ci/mmol; New England Nuclear, Boston, MA) along the dorsal shaved skin at sites where biopsies were to be taken. 12 h after injection, animals were exposed to a dose of 1,500 J/m² of UVR from an FS-40 sunlamp. PRL was administered immediately thereafter by illuminating the UV-irradiated skin for 60 or 120 min with the glass-filtered BLB fluorescent lamp. After the irradiations, 1-cm² biopsies were taken along the mid-dorsal regions of the animals. Biopsies and DNA extraction procedures were routinely carried out under yellow safelights (General Electric F40G0) and subdued lighting conditions. The samples were placed in ice-cold, distilled water and then subjected to heat-cold shock by submersion for 30 s in a 60°C water bath followed immediately by 3 min in 0°C distilled water. After treatment, the epidermis was separated from the dermis by mild scraping with forceps into 2 ml of Tris (0.1 M)/EDTA (0.01 M)/NaCl (0.2 M) buffer (TEN buffer,

pH 8.0). The epidermal cells were lysed by adding 25 μ l of 10% SDS and incubating at 37°C for 30 min. RNA was degraded by adding RNase (100 μ g/ml) and incubating at 37°C for 30 min. Pronase was then added at 200 μ g/ml, and the preparations were incubated for 60 min at 37°C. Lysates were then brought to 1 M NaCl by the dropwise addition of 5 M NaCl. After 15 min at 37°C, an equal volume of Sevag's reagent (24 parts chloroform to 1 part isoamyl-alcohol) (39) was added to each sample, and the solutions were lightly mixed to form emulsions. Samples were incubated for 60 min at 37°C and mixed every 10 min, and centrifuged at 1,200 rpm for 30 min. The supernatants were pipetted into a clean centrifuge tube and treated again with Sevag's reagent. The deproteinized solution of DNA was dialyzed against 0.05 M phosphate buffer (pH 7.0) for 48 h at 4°C with three changes of buffer.

Pyrimidine dimers were measured using damage-specific endonucleases from *Micrococcus luteus* in conjunction with sedimentation of DNA through 5–20% alkaline sucrose gradients (40). Previous studies have shown that >90% of the endonuclease-sensitive sites induced with the FS-40 sunlamp are pyrimidine dimers (41).

Results

Contact Hypersensitivity Response in *Monodelphis Domestica*. Because the opossum has not been used previously for immunological studies, it was necessary to define the conditions for induction and elicitation of CHS. Various concentrations of DNFB were used to induce and elicit a CHS response; concentrations below 1.25% DNFB did not produce a measurable CHS response when used for sensitization of dorsal skin and challenge on the ears 6 d later (data not shown). However, 1.25, 1.88, and 2.5% DNFB applied to dorsal skin, sensitized the animals for ear swelling reactions elicited 6 d later with the same concentration of DNFB; 1.25% DNFB induced the greatest response (Fig. 1) and caused no irritation of the dorsal or ear skin. In addition, the CHS response was determined in opossums challenged 4 or 6 d after sensitization on dorsal skin. The ear swelling response peaked at 72 h regardless of whether 4 or 6 d elapsed between dorsal sensitization and challenge (data not shown). In opossums sensitized on the dorsum 4 d before challenge, the ear swelling response was dramatic but no ulceration of the ear skin was observed. However, when 6 d elapsed between dorsal sensitization and challenge, the ear swelling was even greater but ulceration occurred at the challenge site. Thereafter, the challenge dose of DNFB was routinely applied 4 d after dorsal sensitization. When the animals were sensitized on abdominal skin and challenged 6 d later, the ear swelling response peaked at 24 h and decreased slowly from 48 to 96 h (Fig. 2). No ulceration of the ear skin was observed using these conditions; therefore, the challenge dose was routinely applied 6 d after abdominal sensitization. Examination of histological sections of ears

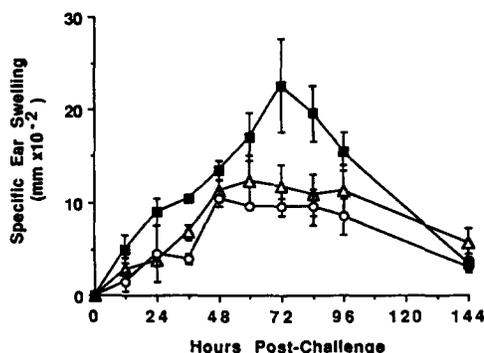


FIGURE 1. Specific ear swelling response in *Monodelphis domestica* as a function of hours after challenge in animals sensitized on dorsal skin with 2.5% (Δ), 1.88% (\circ), or 1.25% (\blacksquare) DNFB and challenged 6 d later with the same concentration of DNFB. Values represent mean ear swelling response measured at various times after ear challenge and are an average of four determinations with associated SEM.

1122 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

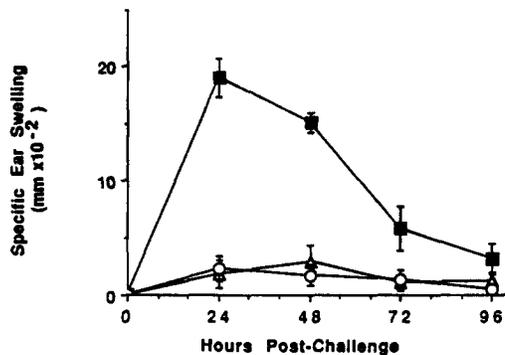


FIGURE 2. Effect of UVR on the induction of immunologic unresponsiveness. Groups of *Monodelphis domestica* were irradiated with 1,500 J/m² (Δ), 4,500 J/m² (○) (three separate doses of 1,500 J/m² on Monday, Wednesday, and Friday), on dorsal, clean-shaved skin or no UVR exposure (■) from the FS-40 sunlamp. 3 d later, DNFB (1.25%) was applied to unirradiated abdominal skin, and opossums were ear challenged with DNFB 6 d later. Points represent mean ear swelling response measured at various times after ear challenge and are an average of six determinations ± SEM.

resected 24–72 h after application of the challenge hapten revealed extensive inflammatory infiltrates consisting mainly of mononuclear cells and a few granulocytes.

UVR-induced Systemic Suppression of CHS and Photoreactivation. To determine whether UVR could suppress CHS to hapten applied at an unexposed site, doses of 1,500 J/m² or 4,500 J/m² of UVR were administered to the dorsal skin of opossums 3 d before sensitization with DNFB on abdominal, unexposed skin. As shown in Fig. 2, a single exposure of 1,500 J/m² of UVR was sufficient to produce systemic suppression of CHS in *Monodelphis domestica*. Two additional exposures of 1,500 J/m² of UVR (total dose = 4,500 J/m²) also abrogated the CHS response. To test whether the UVR-induced immune suppression could be reversed by photoreactivation, opossums were given a single exposure of 1,500 J/m² UVR followed by PRL. Exposure of opossums to 120 min of PRL alone had no effect on the CHS response; ear swelling in the PRL alone group was similar to that of the shaved controls (Fig. 3). Moreover, PRL administered immediately before UVR exposure (PRL/UVR) had no effect on UVR-induced suppression of CHS. In this group, the ear swelling response was suppressed and equivalent to that seen with UVR alone. In contrast, a 120 min PRL treatment given immediately after UVR completely reversed the suppression of CHS by UVR. This result is consistent with the fact that in enzymatic photoreactivation, the reversal of dimers can occur only when PRL is given after the dimers are induced by UVR.

UVR-induced Local Suppression of CHS and Photoreactivation. We next examined

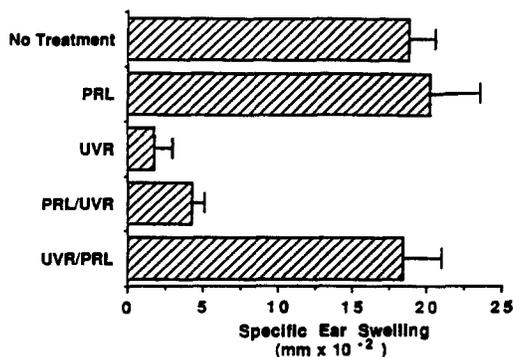


FIGURE 3. Specific ear swelling response in *Monodelphis domestica* 24 h after challenge with 1.25% DNFB. Opossums were either shaved only or were exposed to: UVR alone, 1,500 J/m²; a 2-h PRL treatment, BLB fluorescent lamps; a 2-h PRL treatment followed by UVR; or UVR followed by a 2-h PRL treatment. 3 d later, DNFB (1.25%) was applied to unirradiated abdominal skin, and opossums were ear challenged with DNFB 6 d later. Bar graphs are an average of six determinations of mean ear swelling response with associated SEM ($p < 0.05$ for UVR and PRL/UVR versus shaved or PRL alone groups).

whether local suppression of CHS by UVR was reversible by PRL treatment. Because suppression of CHS at a distant site was photoreversible, we could examine the ability of PRL to restore both local and distant immune suppression after application of hapten onto the irradiated site. The opossums were exposed to $1,500 \text{ J/m}^2$ (2 MED) of UVR and sensitized 5 d later by applying DNFB onto the treated skin. Fig. 4 illustrates that CHS was suppressed by the UVR treatment, as expected, since this dose of UVR also suppressed CHS induced at a distant site. Exposure to 120 min of PRL alone had no effect on the CHS response; ear swelling in the PRL group was similar to that of the shaved controls (Fig. 4). PRL given immediately before UVR exposure had no effect on UVR-induced suppression of CHS and mimicked the suppression seen with UVR alone. In contrast, PRL administered immediately after UVR reduced the suppression of CHS caused by UVR by 75–100% at 72 and 48 h after challenge, respectively ($p < 0.05$, PRL or shaved group versus UVB group). This result demonstrates that both the local and distant forms of immune suppression produced by UV irradiation are inhibited by exposure to PRL.

UVR-induced Disappearance and PRL Treatment of ATPase⁺ Epidermal Langerhans Cells. To confirm that the UVR caused local alterations in epidermal Langerhans cells, opossums were treated with $1,500 \text{ J/m}^2$ from the FS-40 sunlamp, and the number and morphology of the Langerhans cells in the irradiated skin were monitored on various days after UVR exposure. Half of each opossum's back was covered with aluminum foil, while the other half received a single dose of UVR; biopsies from both halves were obtained. In this way, we were able to determine whether multiple skin biopsies on the same animal altered the number of ATPase⁺ Langerhans cells. The numbers of ATPase⁺ Langerhans cells in unirradiated skin did not fluctuate significantly, as depicted in Table I. In adjacent skin exposed to UVR, the number of ATPase⁺ cells decreased within 24 h after UVR, and none were detectable on

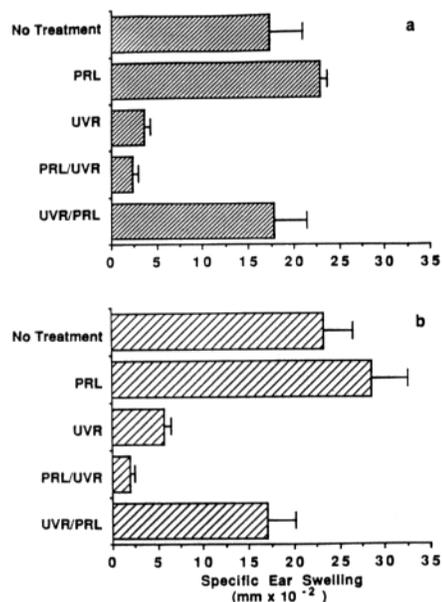


FIGURE 4. Specific ear swelling response in *Monodelphis domestica* 48 h (a) and 72 h (b) after challenge with 1.25% DNFB. Opossums were either shaved only or were exposed to UVR alone, $1,500 \text{ J/m}^2$; a 2-h PRL treatment; a 2-h PRL treatment followed by UVR; UVR followed by a 2-h PRL treatment. 3 d later, DNFB (1.25%) was applied directly to irradiated, dorsal skin and opossums were ear challenged 4 d later. Bar graphs are an average of six to eight determinations of mean ear swelling response with associated SEM ($p < 0.05$ for UVB versus shaved or PRL alone groups).

1124 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

TABLE I
The Effect of Ultraviolet Radiation on ATPase⁺ Epidermal Cells in
Monodelphis domestica

Days after UVR*	Mean number of ATPase ⁺ Langerhans cells/mm ² epidermis (± SEM)		Percent of control [‡]	p value [§]
	- UVR	+ UVR		
1	627 ± 42	222 ± 26	34	0.001
3	683 ± 58	0	0	<0.001
5	609 ± 32	22 ± 22	3	<0.001
10	639 ± 39	104 ± 13	16	<0.001
12	661 ± 35	114 ± 34	18	<0.001
15	669 ± 22	369 ± 73	56	0.001

* A single exposure of 1,500 J/m² (2 MED) from the FS-40 sunlamp.

‡ Control value is the average number of ATPase⁺ Langerhans cells/mm² epidermis from all of the - UVR samples combined.

§ Probability of no difference between - UVR and + UVR groups determined by Student's *t*-test.

day 3 after irradiation. ATPase⁺ cells were detectable again on day 5 after UVR and continued to increase thereafter. A 120-min exposure to photoreactivating light immediately after treatment with 1,500 J/m² UVR prevented the disappearance of ATPase⁺ Langerhans cells in the epidermis when examined 5 d later (Fig. 5). Untreated and PRL-treated epidermis contained the same number of ATPase⁺ cells. When PRL was given immediately before UVR, the disappearance of ATPase⁺ cells was similar to that observed after UVR alone. The number of ATPase⁺ cells in the skin of opossums given UVR alone and PRL/UVR differed significantly from that in the unirradiated control and PRL alone groups ($p < 0.001$).

Effects of PRL Treatment on UVR-induced Disappearance of ATPase⁺ Langerhans Cells and Local Suppression of CHS in Mice. If the effects of PRL treatment were due to some mechanism other than activation of an enzyme that cleaves pyrimidine dimers, then PRL treatment of animals lacking a photoreactivating enzyme should also abrogate the effects of UVR. To test this hypothesis, the effects of PRL were examined in adult mice, which are known to lack photoreactivating enzyme (29) and are susceptible to UVR-induced immune suppression (8, 17, 19). The same protocols for the UVB and PRL treatments of opossums were used for the study in mice. Groups

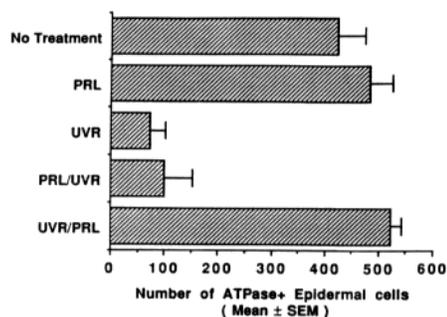


FIGURE 5. Number of ATPase⁺ Langerhans cells per mm² of epidermis in *Monodelphis domestica* either not treated or exposed to: PRL alone (2 h, BLB fluorescent lamps, glass filtered); UVR alone (1,500 J/m², FS-40 sunlamp); PRL followed by UVR; or UVR followed by PRL. Biopsies were taken 5 d after treatments, stained for ATPase activity, and counted. Points are the mean ± SEM of three determinations.

TABLE II
The Effect of Ultraviolet Radiation and Photoreactivation on
ATPase⁺ Langerhans Cells and CHS in C3H Mice

Treatment*	ATPase ⁺ cells/mm ² mean ± SEM [†]	Percent of control	Sensitization [§]	Ear swelling cm × 10 ⁻³	Percent suppression [¶]
None	1,167 ± 53		None	1.7 ± 0.4	
None			+	12.5 ± 0.8	
UVB	605 ± 84	52	+	4.1 ± 0.6	77
UVB/PRL	567 ± 67	49	+	3.6 ± 0.7	82
PRL/UVB	477 ± 36	41	+	4.6 ± 0.5	73
PRL	1,125 ± 63	96	+	12.8 ± 1.1	0

* Treatment consisted of a single exposure of 1,500 J/m² UVB radiation. PRL was given for 2 h either before or after UVB radiation.

† All numbers of ATPase⁺ cells were significantly different ($p < 0.001$) when compared with the no treatment group, except the PRL alone group, which was not significantly different ($p > 0.05$) using Student's two-tailed t test.

§ Mice were sensitized on the treated skin with 50 μ l of 0.3% DNFB in acetone 5 d after irradiation. Challenge was performed 6 d later by applying 5 μ l of 0.2% DNFB on each surface of both ears.

|| Probability of no difference calculated by Student's two-tailed t -test for all groups compared with the untreated, sensitized group at $p < 0.001$ except for the PRL alone group, where $p > 0.05$.

¶ Percent suppression = $[1 - \text{specific ear swelling of treated mice} / \text{specific ear swelling of control mice}] \times 100$.

of mice were exposed to 1,500 J/m² UVR and sensitized 5 d later with DNFB through the dorsal (treated) skin. This treatment suppressed the CHS response, and at the time of sensitization, the UV-irradiated skin had a decreased number of ATPase⁺ dendritic cells (Table II). Exposure of the mice to 120 min of PRL before or immediately after UVR radiation did not abrogate the depletion of ATPase⁺ cells induced by UVR, nor did it reduce the UVR-induced suppression of CHS (Table II).

Photoreactivation of Pyrimidine Dimers in DNA. DNA was extracted and purified from UV-irradiated and PRL-treated skin samples from the opossums and analyzed for pyrimidine dimer content. Exposure of opossums to the dose of UVR capable of suppressing of CHS (1,500 J/m²) induced ~ 22 pyrimidine dimers per 10⁸ daltons of DNA in cells of the epidermis. The kinetics of photoreactivation repair were measured following various doses of PRL. The results obtained with 120 min of PRL treatment are illustrated in Fig. 6 and indicate that 86% of the pyrimidine dimers were removed by a 120-min exposure to photoreactivating light used in these experiments.

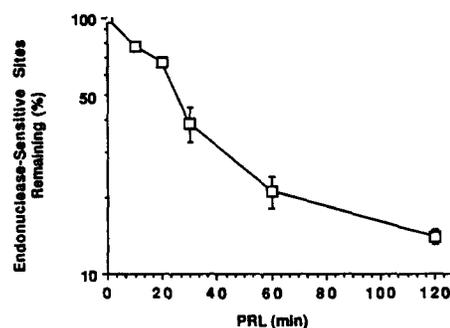


FIGURE 6. Percentage of endonuclease-sensitive sites (pyrimidine dimers) remaining in the epidermal DNA of *Monodelphis domestica* as a function of min of PRL treatment after exposure to 1,500 J/m² UVB (~ 2 MED) from the FS-40 sunlamp. Points are an average of three to five determinations with associated SEM.

1126 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

Discussion

Systemic suppression of the CHS response and the induction of hapten-specific suppressor cells are observed when the skin of mice (20) or guinea pigs (42) is exposed to UVR at one site and a hapten is administered on unexposed skin. In these studies, we demonstrated that systemic suppression of CHS can also be produced by UVR in the opossum, although the lack of an inbred strain, at present, precludes investigation of the role of suppressor cells in this model. The low dose of UVR required to suppress CHS to hapten applied to unirradiated opossum skin ($1,500 \text{ J/m}^2$) may seem surprising in comparison with earlier studies in mice, which used much higher doses ($40\text{--}50 \text{ kJ/m}^2$) of UVR (17–20). However, more recent studies have indicated that $1,000\text{--}2,000 \text{ J/m}^2$ is sufficient to induce systemic immune suppression in some strains of mice (43, 44).

In spite of extensive studies on the cellular events associated with suppression of CHS at an unirradiated site (2–6, 17–20), the molecular basis for the initial effect of UVR has remained controversial. Previous studies in mice indicated that UVR-induced systemic suppression of CHS must be initiated by the absorption of UVR by a component in the exposed skin because UVR suppressed CHS in mice whose dorsal fur is clipped, but only marginally suppressed CHS in unclipped mice (20). In addition, an action spectrum for systemic suppression of CHS in mice (6) indicated that the most effective wavelengths were between 265 and 275 nm. This result implied that the chromophore must be located in the superficial layers of the skin because there was little interference from absorption of UVR by proteins or nucleic acids. Based on this action spectrum, there are two likely molecules in skin that could serve as the initial target of the UV radiation because of their UV absorption characteristics: urocanic acid and DNA (6). Urocanic acid is located in the stratum corneum and undergoes photoisomerization when exposed to UVR (6). Injection of UV-irradiated urocanic acid into mice has been shown to decrease the activity of splenic APCs (45) and to prevent the induction of a delayed hypersensitivity response to HSV (46). Attempts to remove urocanic acid from the skin of mice by tape stripping before exposure to UVR have produced conflicting results (6, 47).

We chose to investigate the possibility that DNA is the primary target of UVR in systemic suppression of CHS. The availability of an experimental animal model in which the primary UV-induced lesion in DNA can be repaired with high efficiency and exquisite specificity (21–24) afforded us the opportunity to determine whether or not DNA is the initial target of the UVR in systemic immunosuppression. As demonstrated in this study, repairing UV-induced pyrimidine dimers in DNA with a highly specific enzyme that is activated by visible light prevented UVR-induced systemic immune suppression. It is highly unlikely that exposure to PRL could reverse the UV-induced isomerization of urocanic acid because neither isomer absorbs appreciable amounts of energy at wavelengths greater than 320 nm (48). Therefore, our data provide direct evidence that DNA is the primary chromophore for immune suppression in the opossum and that the specific photoproduct involved in systemic suppression of contact hypersensitivity is likely to be the pyrimidine dimer. In support of this conclusion, it has been shown that agents that interact with DNA, such as psoralens in conjunction with UVA radiation and superficial X-ray, produce systemic suppression of CHS, similar to UVR, whereas other phototoxic agents that do not interact with DNA, such as eosin and rose bengal in combination with UVA

exposure, do not produce systemic suppression of CHS associated with suppressor cells (49).

UV irradiation also inhibits the CHS response in mice (8-11) and guinea pigs (50) to haptens applied locally to the irradiated site. The cellular mechanism of this immune suppression seems to involve a UVR-induced alteration of cutaneous, antigen-presenting, Langerhans cells because the reduction in the number of ATPase⁺ Langerhans cells in UV-irradiated skin correlates with altered immunological function (8, 51). Our studies in the opossum support this view since PRL treatment abrogated both the UV-induced alterations in Langerhans cells and the local suppression of CHS. Under our experimental conditions, 85% of the pyrimidine dimers induced in DNA by UVR were removed by the PRL treatment, while the effect of UVR on Langerhans cells was reduced by 80-85%, and local suppression of CHS was reduced by 75-100%. Our studies also indicate that DNA is the primary chromophore for these effects of UVR. As evidence of the specificity of the PRL treatment for enzymatic photoreactivation, the UVR-induced effects on Langerhans cells and CHS in mice, which do not possess active PRE in the skin, were not inhibited by exposure to PRL. These results make it highly unlikely that the ability of PRL to reverse the effects of UVR was due to an activity other than enzymatic repair of lesions in DNA, such as reversing the isomerization of urocanic acid. Thus, these data imply that DNA damage, in the form of pyrimidine dimers, is the initiating lesion in the pathway for local immune suppression in *Monodelphis domestica*. At present, it is not clear whether the DNA damage occurs in the Langerhans cells themselves or in other cells of the epidermis. It is possible that the effects of UVR on Langerhans cells result secondarily from damage to other epidermal cells.

The specificity of the photoreactivation repair pathway has been used to determine that DNA damage and, in particular, the formation of pyrimidine dimers, is involved in the induction of a number of pathological changes in UVR-exposed skin (30-34) and eyes (35, 36) in *Monodelphis domestica*. However, a caveat should be made when interpreting photoreactivation experiments that based on the specificity of the PRE, appear to identify pyrimidine dimers as the lesion responsible for the induction of a biological endpoint. If the cells in which dimers are being photoreactivated are capable of repairing DNA by excision repair, the amelioration of the biological effect by photoreactivation could result from an increase in excision repair of other lesions in DNA. Because the dimers are being repaired by PRE this might increase the availability of enzymes involved in excision repair, with the net result that other lesions in DNA are repaired more efficiently and thus could be responsible for the biological effect. For example, Mitchell et al. (52) reported that photoreactivation of dimers in cultured frog cells resulted in a more efficient removal of pyrimidine (6-4)pyrimidone photoproducts. Even if this were the case, however, successful photoreactivation still identifies DNA as the chromophore for the UVR-induced effect.

Thus, our studies demonstrate that in *Monodelphis domestica* DNA is the target molecule for both the local and systemic immunosuppressive effects of UVR. Based on studies demonstrating that UV irradiation of keratinocytes in vitro results in the release of immunomodulatory factors (3), we can speculate that DNA damage to these cells may trigger the cascade of events that ultimately result in the suppression of CHS at sites distant from the UV irradiation.

1128 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

Summary

This study was conducted to explore the involvement of DNA damage in the suppression of contact hypersensitivity (CHS) by UV irradiation. The opossum, *Monodelphis domestica*, was used because cells of these marsupials have an enzyme that is activated by visible light (photoreactivating enzyme) and repairs ultraviolet radiation (UVR)-induced pyrimidine dimers in DNA. A single dose of 1,500 J/m² of UVB (280–320 nm) radiation, representing 2 minimal erythema doses, was administered to the dorsal skin of opossums. This treatment prevented the opossums from developing a CHS response to dinitrofluorobenzene (DNFB) applied either at the site of irradiation or an unirradiated site. In addition, this dose of UVR decreased the number of ATPase⁺ epidermal Langerhans cells in the dorsal epidermis to ~3% of that in unirradiated skin at the time of DNFB application. Treatment of the animals with wavelengths that activate the repair enzyme (320–500 nm, photoreactivating light, PRL) for 120 min immediately after UV irradiation inhibited the UVR-induced suppression of CHS almost completely. Exposure to PRL before UVR did not prevent UVR-induced suppression of CHS. PRL treatment after UV irradiation also prevented the decrease in the number of ATPase⁺ Langerhans cells. Measurements of lesions in DNA indicated that PRL treatment removed around 85% of the UVR-induced pyrimidine dimers. These data provide direct evidence that DNA, and most likely, the pyrimidine dimer, is the primary molecular target for the UVB-induced suppression of contact hypersensitivity to haptens applied to irradiated or unexposed skin.

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1130 CHROMOPHORE FOR SUPPRESSION OF CONTACT HYPERSENSITIVITY

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