Exposure to Low-Dose Ultraviolet Radiation Suppresses Delayed-Type Hypersensitivity to Herpes Simplex Virus in Mice

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Ultraviolet B (UVB) radiation is reported to induce a defect in epidermal antigen presentation which leads to specific suppression of the delayed-type hypersensitivity (DTH) response to trinitrochlorobenzene. We have used a similar system to examine the murine DTH response to herpes simplex virus type 1 (HSV-1). Mice irradiated with 96 mJ/cm^2 UVB on shaved dorsal skin 3 days before s.c. injection of live HSV-1 in the flank showed 54–92% suppressed DTH responses to challenge with inactivated virus compared with nonirradiated control animals. If irradiation took place 7 days before inoculation with virus, some suppression of DTH occurred; if 14 days before, no suppression was found. The transient nature of the UVB response is further illustrated by the observation that irradiation with the same dose of UVB 5 h before, or 3 days after, inoculation with virus had no effect on DTH. Once induced, some degree of UVB suppression was found to persist for at least 3 months after irradiation. *J Invest Dermatol 86:125–128, 1986*

When herpes simplex virus (HSV) is inoculated s.c. into mice, the virus replicates locally reaching its highest titers between days 2–6 and is undetectable 10 days later [1]. HSV may then invade the sensory nerve endings in the local neurodermatome and establish latency in the sensory ganglia [2]. A delayed-type hypersensitivity (DTH) response can be detected by injection of virus or viral antigen into the ear pinna with measurement of ear swelling 24–48 h later. Delayed-type hypersensitivity is found 4 days after a primary infection, reaches a maximum at 8 days, and lasts for the lifetime of the animal [2]. It is under the control of the I region of the H-2 complex and plays a role in recovery from infection [3,4]. B cells that suppress the DTH response are found in the draining lymph node from 16 days onward [5]. The control of primary HSV infections is complex, but T cells seem to be important in early events, with evidence for both cytotoxic and DTH-mediating T cells being involved (reviewed in [6]).

Several groups, using a variety of systems, have shown that UV irradiation in vivo has a selective suppressive effect on the immune system [7,8]. Epidermal [9,10] and spleen [11] cells from UV-irradiated animals are deficient in antigen-presenting ability and antigen-specific T-suppressor lymphocytes may be generated as a result of this. UV radiation does not penetrate the skin and suppression is not due to local effects on the skin [12]. Although irradiation is known to affect the numbers and morphology of Langerhans cells, and to lead to increased production of epidermal cell-derived activating factor (ETAF) by keratinocytes, it has been shown recently that maximum suppression of DTH occurs at a different wavelength from the one directly affecting the Langerhans cells [13]. Thus, there may be release of an UV-induced photoproduct by epidermal cells which then interacts with Langerhans cells and suppresses their capacity to present antigen.

In the study reported here, infection with an epidermal pathogen, HSV-1, has been used in a mouse model. Measurement has been made of the DTH response to this virus and the effect of UV radiation on this parameter quantitated.

MATERIALS AND METHODS

Animals Female C3HfBu/Kam mice, bred and maintained in the departmental animal house, were used when 10–16 weeks old.

UVB Irradiation Mice were irradiated under a bank of 2 Philips TL20W/12 sunlamps at a distance of 30 cm. These bulbs had an output in the UVB range between 270–350 nm with a peak output at 310 nm. Measurement of output was made inside the perspex box, described below, using an Industrial Light Research Radiometer IL700A with an actinic optical assembly. The output was $0.8 J/m^2/s$. Before irradiation, mice were shaved dorsally and their ears protected by black electrical tape [13]. When tested, this tape did not allow any penetration of UVB radiation. Mice were irradiated in a 5-chambered perspex box with deep walls and no lid, such that they could not turn around or over.

Virus The virus used was a plaque-purified clinical isolate of HSV-1, the DNA of which has been analyzed using 3 restriction endonucleases. The virus was passaged in vitro in Vero cells. Aliquots were stored at −70°C and sonicated for 60 s in an ultrasonicating water bath immediately before use. The number of plaque-forming units (pfu) of infectious virus present per milliliter of cell suspension was determined on Vero monolayers with 0.25% sea plaque agarose (FMC Corporation) in the overlay. Infectious virus was inactivated by UVB irradiation for 60 min. The stock preparation of virus used throughout had a titer of 10^{10} pfu/ml which was equivalent to 10^3 pfu per Vero cell. In mice, an i.p. injection of 10^7 pfu of this virus strain induced paralysis and death in 90% of animals.

1-chloro-2,4,6-Trinitrobenzene 1-chloro-2,4,6-Trinitrobenzene, obtained from BDH Chemicals Ltd., Poole, England, was made up to 5% (w/v) in acetone immediately before use.

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Abbreviations:
- APC: antigen-presenting cells
- DTH: delayed type hypersensitivity
- HSV: herpes simplex virus
- PBS: phosphate-buffered saline
- pfu: plaque-forming units
- TNCB: 1-chloro-2,4,6-trinitrobenzene
- UVB: ultraviolet B

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DTH Assay The principle of the assay was identical whether HSV-1 or TNCB was used as the sensitizing agent.

TNCB Assay: The abdomens of mice were shaved and then painted with 0.1 ml 5% TNCB in acetone or with acetone alone. Five to 8 days later, the mice were individually marked and their ear thicknesses were measured using a micrometer gauge (Draper Precision Instruments, Japan). They were then challenged by painting 15 μl of 5% TNCB in acetone onto both sides of each ear pinna. Twenty-four hours later, the ear thicknesses were again measured and the increase in ear thickness calculated. A mean of the 2 measurements from each mouse was taken before the mean and SE for each experimental group was calculated.

HSV-1 Assay: Mice were inoculated s.c. in the flank with 10^7 pfu of infectious virus or 10^4 uninfected sonicated Vero cells in phosphate-buffered saline (PBS). Eight days later, they were individually marked and their ear thicknesses were measured as above. They were then injected with 10 μl of PBS containing 10^6 pfu UV-inactivated HSV-1 in both ear pinnae. Twenty-four hours later, the ear thicknesses were again measured and the increase in ear thickness calculated. A mean of the 2 measurements from each mouse was taken before the mean and SE for each experimental group were calculated.

UVB-Induced Suppression of DTH To determine the suppressive effect of UVB on generation of DTH, mice were irradiated as described above 3 days prior to initial sensitization with TNCB or HSV-1 [13]. The net increase in ear thickness of control and UVB-irradiated mice was taken as the difference between the mean ear swelling of sensitized and unsensitized mice. Percentage suppression was calculated according to the formula:

$$\% \text{ suppression} = 100 - \frac{100 \times \text{net increase in UV-irradiated mice}}{\text{Net increase in control mice}}$$

as described by Noonan et al [13].

RESULTS

Calibration of the Suppressive Effect of UVB on DTH Responses Mice were irradiated with increasing amounts of UVB 3 days prior to sensitization with TNCB (UV experimental group) or acetone alone (UV control group) and were compared with control and experimental groups that had not been exposed to UVB. Table I shows that 20-min irradiation with UVB (equivalent to 96 mJ/cm^2) caused 60% suppression of the DTH response. This dose of UVB did not give rise to any observable burning of the skin. Mice irradiated for 30 min did demonstrate burning on the skin of their backs and the decreased suppression of the DTH response seen in this group may be explained by an overriding generalized inflammatory reaction to the epidermal damage. For this reason, it was decided to use 20-min irradiation with UVB in all subsequent experiments.

Induction of Specific DTH to HSV-1 Mice were inoculated with live HSV-1 or an equivalent amount of uninfected Vero cells s.c. in the flank. As described in *Materials and Methods,* the 24-h DTH response was determined after challenge with inactivated virus (cultured in Vero cells) on day 8. The induction of DTH to HSV-1 is shown in Table II, which also indicates that the DTH to the virus is specific in nature and directed against HSV-1 antigens rather than Vero cell antigens.

DTH to HSV-1 is Suppressed by UVB Treatment To examine the effect of UVB pretreatment on the generation of DTH to HSV-1, mice were shaved dorsally and UV-irradiated for 0 or 20 min as above. Three days later, they were inoculated with either 10^4 sonicated Vero cells or 10^7 pfu of HSV-1 as above. The 24-h DTH response to challenge 8 days later with 10^6 UV-inactivated pfu of HSV-1 was determined as above. Table III shows that this single exposure to a suberythemal dose of UVB irradiation induced 54–92% suppression of the DTH response to HSV-1

UVB-Induced Alteration in the Generation of DTH is Transient and Short-Term in Nature To determine the relationship between the time of exposure to UVB and the time of sensitization with virus, mice were irradiated at several time points before and after infection with HSV-1. Fig 1 shows that mice irradiated 14 days before sensitization to virus demonstrated no suppression of DTH, whereas mice irradiated 7, 3, and 2 days before sensitization were suppressed by 40, 70, and 82%, respectively. Irradiation on the same day as (5 h before) or 3 days after, sensitization to virus had no effect on DTH responses. This indicates that the UVB-induced generation of DTH suppression is transient, short-term in nature, and that it takes up to 2 days to be effective.

However, in contrast to this result, mice that had been UVB suppressed for DTH to HSV-1 remained suppressed for as long as tested. This was up to 3 months after UV-induced DTH suppression had been demonstrated (data not shown). Thus the suppressed state, once generated, is not transient.

DISCUSSION

Knowledge of the immunologic mechanisms that control HSV infections is crucial to an understanding of HSV pathogenesis. This is important both for the prevention of spread of primary disease and for attempts to modify the pattern of subsequent episodes of epidermal recrudescence.

We have examined how a well-characterized in vivo immune response, DTH, to experimental HSV-1 infection [4,5,14] is altered by UV irradiation, a stimulus known to affect both viral pathogenesis [15] and the correct functioning of the immune system itself [9,13].

Table I. UVB-Induced Suppression of DTH to TNCB

Sensitized with	UVB Irradiation	24-h Increase in Ear Thickness (units × 10^2 mm ± SEM[a])	% Suppression of DTH
Acetone	Nil	8.2 ± 1.14	—
TNCB	Nil	27.3 ± 2.01	—
TNCB	10 min	20.2 ± 1.74[b]	37
TNCB	20 min	15.8 ± 0.84[b]	60
TNCB	30 min	19.0 ± 1.94[b]	44

All mice were challenged with 15 μl TNCB in acetone on both sides of both ear pinnae.
[a]Five mice per group.
[b]Significantly different from the experimental group sensitized with TNCB without prior UVB exposure, $p < 0.001$ in all cases (Student's *t*-test).

Table II. Induction and Specificity of DTH to HSV-1

Sensitized with	Challenged with	Increase in Ear Thickness (units × 10^{-2} mm ± SEM)
Vero	HSV-1	6.8 ± 0.92 (n = 5)[a]
HSV-1	HSV-1	17.7 ± 0.5 (n = 5)
Vero	TNCB	9.5 ± 0.92 (n = 5)
HSV-1	TNCB	8.1 ± 0.42 (n = 5)
Acetone	TNCB	9.3 ± 1.0 (n = 5)
TNCB	TNCB	23.7 ± 0.68 (n = 6)
Acetone	HSV-1	4.9 ± 0.52 (n = 5)
TNCB	HSV-1	5.4 ± 0.76 (n = 5)

Mice sensitized on day 0 with 0.1 ml 5% TNCB in acetone, acetone alone, 10^7 pfu infectious HSV-1 grown in Vero cells or 10^4 Vero cells alone (see *Materials and Methods* for inoculation details).
Mice challenged on day 8 with either 15 μl 5% TNCB in acetone on both sides of each ear pinna or by injection of 10^6 pfu UV-inactivated HSV-1 into each ear pinna.
[a]n = Number of mice.

Table III. Suppression of DTH to HSV-1 by UVB Irradiation 3 Days Before Sensitization

UVB Irradiation (min)	Sensitized with	Increase in Ear Thickness (units × 10^{-2} mm ± SEM)		
		Experiment 1	Experiment 2	Experiment 3
0	10^4 Vero	3.4 ± 0.7 (n = 5)[a]	3.53 ± 0.36 (n = 8)	2.9 ± 0.51 (n = 5)
0	10^7 HSV-1	13.31 ± 0.84 (n = 5)	9.3 ± 0.73 (n = 5)	14.0 ± 0.90 (n = 6)
20	10^4 Vero	1.45 ± 0.65 (n = 5)	3.75 ± 0.53 (n = 6)	3.5 ± 0.65 (n = 8)
20	10^7 HSV-1	2.2 ± 1.02 (n = 5)	4.4 ± 0.47 (n = 6)	8.6 ± 0.92 (n = 5)
% suppression of DTH		92%	88%	54%

All mice challenged on day 8 (after sensitization) with 10^6 UV-inactivated pfu HSV-1 in both ear pinnae.
[a]n = Number of mice.

The results described demonstrate that exposure to a single suberythemal dose of 96 mJ/cm² UVB irradiation at a critical time (2–7 days) prior to s.c. inoculation with infectious virus suppressed the DTH response to HSV-1. Significant suppression persisted for as long as tested (1 month) after UVB irradiation. No suppression was generated if virus was injected immediately, or 3 days prior to irradiation with the same dose of UVB. This suggests that skin exposed to UVB is altered over a period of time such that immune responses against antigens encountered up to 7 days subsequently, do not follow the "normal" pattern.

UVB irradiation is known to affect the antigen-presenting capacity of the skin [9,10] and other organs [11]. It also causes an alteration in antigen handling such that foreign antigens subsequently encountered induce the systemic generation of specific suppressor cells. This phenomenon has been described for contact sensitizing agents [13] and also for tumor antigens [7]. The murine skin is reported to contain 2 functional sets of antigen-presenting cells (APC), one sensitive to UVB irradiation and inducing positive immunity, and the other resistant to UVB and inducing specific suppressor cells [16]. The balance between these sets must be delicately controlled and any factor that alters this will have far-reaching effects on the immune response to antigens subsequently encountered. Recently, it has been reported that antibody responses to topically applied antigens are also suppressed by UV irradiation; in this case, failure to prime due to a defect in antigen presentation was the main mechanism postulated [17]. The effects of UV irradiation on the antibody responses to HSV-1 are currently being investigated.

As suppression was found to be long-lasting, it is unlikely to be due to a loss of APC from the epidermis as it is known that skin depleted of Langerhans cells by tape stripping is repopulated within 24–48 h [18] and that animals first exposed to an antigen 2 weeks after UVB irradiation have normal DTH responses [19].

Adoptive transfer experiments are in progress to characterize any population of immune cells induced by UVB irradiation. In addition, the effect of irradiation on the function of epidermal cells and other APC after HSV-1 infection is being investigated.

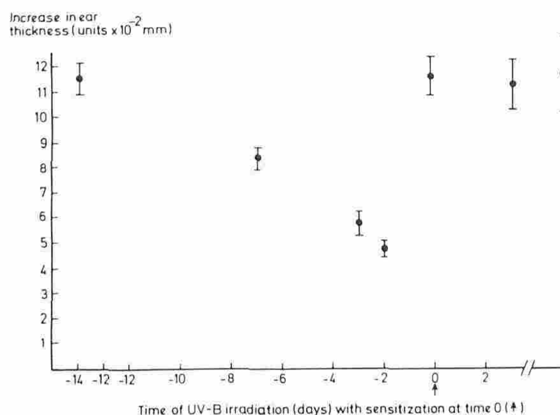

Figure 1. Kinetics of UVB induction of DTH suppression. HSV-1 (10^7 pfu) was used for sensitization and challenge was 8 days later with 10^6 UV-inactivated pfu HSV-1 in both ear pinnae. At least 5 mice were present in each group and SEM for each point are shown. (The increase in ear thickness of an equivalent group of nonirradiated mice is shown, ○).

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