Prevention of Ultraviolet Radiation–Induced Suppression of Contact and Delayed Hypersensitivity by *Aloe barbadensis* Gel Extract

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We investigated the ability of *Aloe barbadensis* gel extract to prevent suppression of contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses in mice by ultraviolet (UV) irradiation. Local immune suppression was induced in C3H mice by exposure to four daily doses of 400 J/m² UV-B (280–320 nm) radiation from FS40 sunlamps, followed by sensitization with 0.5% fluorescein isothiocyanate (FITC) through the irradiated skin. Topical application of 0.167–1.67% *Aloe* gel after each irradiation significantly reduced this suppression. *Aloe* treatment partially preserved the number and morphology of Langerhans and Thy-1⁺ dendritic epidermal cells in skin, compared to those in the skin of mice given only UVR or UVR plus the vehicle. Experiments using a single (2 kJ/m²) dose of UVR followed by *Aloe* treatment showed that the effect of *Aloe* was not due to screening of the UVR. Systemic suppression of DTH to *Candida albicans* or CHS to FITC was induced in C3H mice exposed to 5 or 10 kJ/m² UV-B radiation, respectively, on shaved dorsal skin and sensitized 3 d later with a subcutaneous injection of formalin-fixed *Candida* or FITC painted on unirradiated, ventral skin. Treatment of the UV-irradiated skin with *Aloe* immediately after irradiation prevented suppression of both DTH to *Candida* and CHS to FITC. *Aloe* treatment did not prevent the formation of cyclobutyl pyrimidine dimers in the DNA of UV-irradiated skin or accelerate the repair of these lesions. These studies demonstrate that topical application of *Aloe barbadensis* gel extract to the skin of UV-irradiated mice ameliorates UV-induced immune suppression by a mechanism that does not involve DNA damage or repair. *Key words: photo protection*/Candida albicans/*hapten*/*UV-B. J Invest Dermatol 102:197–204, 1994*

Chronic exposure of the skin to ultraviolet radiation (UVR) causes skin cancer in humans and laboratory rodents [1–3]. Furthermore, exposing the skin of experimental animals to wavelengths of UVR in the UV-B (280–320 nm) region of the spectrum impairs their ability to develop immune responses to UV-induced skin cancers, contact-sensitizing haptens, and a variety of infectious microorganisms [4–7]. Several model systems have been developed to study the mechanisms of UV-induced immune suppression. In the local suppression model, exposing C3H mice to low doses of UV-B radiation inhibits the induction of the contact hypersensitivity (CHS) response to haptens applied at the site of irradiation. The depressed immune response is accompanied by a decrease in the numbers of Langerhans and Thy-1⁺ dendritic epidermal cells (DETC) in the skin [8–10] and by the appearance of hapten-specific suppressor T cells in the spleen [10–13]. In the systemic suppression model, higher doses of UV-B are used to suppress the induction of CHS and delayed-type hypersensitivity (DTH) responses to antigens introduced at a non–UV-irradiated site [5]. Systemic suppression of CHS and DTH is also accompanied by the appearance of antigen-specific suppressor T lymphocytes [5,6,11,14].

The precise mechanisms by which UVR induces immune suppression are unclear; however, a picture is emerging of the sequence of events leading to the downregulation of immune responses. Local suppression appears to be mediated by alterations in the activity of epidermal Langerhans cells and involves the release of tumor necrosis factor alpha (TNF-α) [15] and perhaps the formation of *cis*-urocanic acid (*cis*-UCA) [16,17] in UV-irradiated skin. Recent evidence suggests that DNA damage is the initial event that triggers systemic suppression of DTH and CHS [18]. Cytokines such as TNFα [15], IL-10 [19], IL-1 [20], and other soluble factors produced by UV-irradiated keratinocytes [21] have been implicated as mediators of systemic immune suppression and may occupy intermediate steps in the cascade of events leading from DNA damage to immune suppression.

Extracts from a number of *Aloe* species are purported to have therapeutic properties and have been used to alleviate the pain of sunburn and to aid in the healing of thermal burns [22]. *Aloe* extracts, derived primarily from commercially grown *Aloe barbadenis*, have made their way into a myriad of health and cosmetic products, although scientific evidence for their efficacy is limited. Because of its reported anti-inflammatory effects [23], we wanted to determine whether application of an *Aloe* extract to UV-irradiated skin would alter the induction of immune suppression. Therefore, we investigated the ability of a standardized sample of *Aloe* gel extract to modify the effects of UV irradiation in murine models of UV-induced local and systemic immunosuppression.

MATERIALS AND METHODS

Mice Specific-pathogen-free female C3H/HeN (MTV⁻) mice were obtained from the Animal Production Area of the National Cancer Institute-

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Abbreviations: CHS, contact hypersensitivity; *cis*-UCA, *cis*-urocanic acid.

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Frederick Cancer Research Facility (Frederick, MD) and maintained in a pathogen-free barrier facility in accordance with National Institutes of Health and American Association for Accreditation of Laboratory Animal Care guidelines. All procedures were approved by the Institutional Animal Care and Use Committee. Each experiment was performed with age-matched mice 10–12 weeks old. The mice were housed in filter-protected cages. National Institutes of Health open formula mouse chow and sterile water were provided *ad libitum*.

***Aloe Barbadensis* Gel Extract** The *Aloe barbadensis* used in our study was "Standard Gel Sample A for 1991" (ARF91A), prepared by the Aloe Research Foundation using the following procedure. Mature *Aloe barbadensis* leaves were filleted and depulped by passage through a 250 μm screen. The gel extract was lyophilized (Vertis SRC250) within a maximum of 6 h of harvesting the leaves and 4 h of filleting. A detailed description of the properties and chemical constituents of this material may be obtained from The Aloe Research Foundation, Suite 500, 910 Houston St., Fort Worth, TX, 76102. Although this extract was prepared on an industrial scale using standard commercial equipment, it does not correspond to any commercial product currently marketed by any company.

Compounding of Agent The lyophilized ARF91A Crude Gel was reconstituted by sprinkling the dried powder onto stirred, chilled "high-performance liquid chromatography (HPLC) grade" water (Aldrich Chemical Co., Milwaukee, WI) at a concentration of 5 gm/100 ml. After stirring overnight at 4°C, the remaining hydrated lumps of gel were dispersed by homogenizing for 60–120 seconds in a chilled blender. The extract was diluted with HPLC grade water to twice the final concentration and was manually emulsified into an equal volume of Aquaphor, using a spatula and a glass plate. Aquaphor consists of petrolatum, mineral oil, and crude lanolin (wool wax) and is widely employed for the incorporation of experimental compounds into creams for clinical testing. Three concentrations of *Aloe* in Aquaphor were prepared for use in this study. The highest concentration, 1.67% (w/v) *Aloe* extract, corresponds to three times the concentration of native gel (approximately 0.5% solids). In terms of materials commercially available, the concentrations of *Aloe* used in our study — 1.67%, 0.5%, and 0.167% (w/v) — would correspond to 300%, 100%, and 33% *Aloe* on packaging labels. A control cream was prepared that contained only water and Aquaphor in a 1:1 ratio. After compounding, creams were stored at 4°C.

UV Irradiation Ultraviolet radiation was administered using a bank of six unfiltered FS40 sunlamps (National Biological Corp., Twinsburg, OH). Approximately 65% of the energy emitted from these lamps is within the UV-B range (280–320 nm) and the peak emission is at 313 nm [7]. The average irradiance of the source was approximately 5 W/m² at a 20-cm distance, as measured by an IL700 radiometer with an SEE240 detector fitted with an SES280 filter and a W quartz diffuser (International Light Inc., Newburyport, MA). For local suppression of CHS, the abdominal fur of the mice was shaved using electric clippers, and the animals were sedated with methoxyfluorane. The ears were protected from irradiation by covering the heads of the mice with aluminum foil. Their shaved ventral skin was exposed to 400 J/m² UV-B radiation daily for four consecutive days. In some experiments, the mice were shaved and anesthetized as above and given 2 kJ/m² as a single (approximately 6-min) exposure.

Systemic suppression of DTH or CHS was induced using a single exposure of UV-B as follows. The dorsal fur of mice was shaved with electric clippers and the ears covered with opaque tape. The animals were put into cages with plexiglas dividers, one mouse per chamber, and the cage was covered with a wire lid. The incident light received by the animals under these conditions was reduced to 2.6 W/m², by the shielding from the wire cage top. The 5 kJ/m² (for suppression of DTH) or 10 kJ/m² dose of UV-B radiation (for systemic suppression of CHS) was delivered in approximately 30 min or 1 h of exposure, respectively. Control mice were treated in an identical fashion, but were not UV irradiated.

Contact Sensitization Mice were sensitized on their shaved abdominal skin with a solution of 0.5% fluorescein isothiocyanate (FITC; isomer I, Aldrich Chemical Co.) in acetone:dibutylphthalate (1:1 v/v). UV-irradiated mice were sensitized either 6 h after the last UV treatment (4 × 400 J/m² protocol) or 3 d after the single dose of UVR. Five days after sensitization, the mice were challenged by painting 5 μl of 0.5% FITC in 50% (v:v) acetone:dibutylphthalate (Aldrich Chemical Co.) on both the dorsal and ventral surfaces of each ear. Ear thickness was measured using an engineers' micrometer (Production Tools, Houston, TX) immediately before challenge and 24 h later. Specific ear swelling was determined by subtracting values obtained from mice challenged but not sensitized. Each treatment group contained five mice.

Delayed Hypersensitivity to *Candida albicans* Groups of five mice were injected subcutaneously in each flank with 10^7 formalin-fixed *Candida albicans* cells. Ten days later, the mice were challenged with 50 μl of commercially prepared *Candida* antigen, supplied as a 1:100 dilution (Berkley Biologicals, Berkeley, CA) in each rear footpad. Footpad thickness (dorsal to plantar aspect) was measured immediately before challenge and 24 h later. Control mice were not sensitized with yeast cells but were challenged in both hind footpads with the *Candida* antigen. Specific footpad swelling was determined by subtracting the average values obtained from mice challenged but not sensitized.

***Aloe* Treatment** Within 5 min after UV irradiation, the irradiated skin was treated with *Aloe* in Aquaphor or Aquaphor vehicle alone. Unirradiated control groups of mice were also shaved, anesthetized, and topically treated with *Aloe* extracts in Aquaphor. Approximately 75 mg of vehicle or *Aloe* in vehicle was applied to the shaved skin of each mouse.

Sunscreen Testing The ability of *Aloe* or vehicle to reduce the dose of UV-B radiation received by the skin was tested using the ear swelling method of Cole *et al* [24]. Prior to irradiation, the thickness of each ear was measured with a micrometer. Fifteen minutes before irradiation, *Aloe* in vehicle or vehicle alone was manually applied to both sides of each ear. The amount applied to the ears (30 mg/mouse) approximated the degree of coating obtained in CHS experiments in which the *Aloe* was applied to abdominal skin. The mice were placed in cages with plexiglas dividers, covered with a wire lid, and exposed to a single dose of 5 kJ/m² UV-B radiation, as described above. The ear thickness for each animal was measured 24, 48, and 72 h later and changes in ear thickness calculated by subtracting the values at each time point from their initial values.

Identification of Ia^+, ATPase$^+$, and Thy-1^+ Dendritic Epidermal Cells (DETC) Three mice from each experimental group and an untreated control group were killed and their ventral skin was excised. The connective tissue was removed and the skin was cut into 1-cm squares and incubated in a 20-mM ethylenediamine tetraacetic acid (EDTA)/phosphate-buffered saline solution at 37°C for 2 h, as described [8]. The epidermis was separated from the dermis and epidermal sheets were washed in normal saline, fixed in 4% paraformaldehyde at 4°C for 24 h and stained for ATPase activity according to the method of Mackenzie and Squier [25]. Ia^+ cells were detected by fixing epidermal sheets in acetone for 30 min and incubating them with mouse anti-Ia^k antibody (Becton Dickinson, San Jose, CA) DETC were detected in a similar manner using rat anti-Thy-1 antibody and rhodamine-labeled goat anti-rat $(Fab')^2$ second antibody (Organon Teknika Corp., West Chester, PA). The ATPase$^+$, Ia^+, and Thy-1^+ cells were observed at 400× using light and dual epifluorescence microscopes (Nikon Inc., Garden City, NJ) with an ocular grid of known area. The numbers of cells reported represent the mean ± the standard deviation of 20 randomly selected fields in each group.

Statistical Analysis The significance of differences between treatment groups was analyzed using analyses of variance, as performed using STATVIEW 512 software (Abacus Concepts, Berkeley, CA) on a MacIntosh SE microcomputer. Each experiment was performed at least twice. $p < 0.05$ was considered statistically significant.

Measurement of Pyrimidine Dimers The number of pyrimidine dimers in epidermal DNA was kindly measured by Dr. Daniel Yarosh (Applied Genetics, Inc., Freeport, NY) using the endonuclease sensitive-site assay and alkaline agarose gels [26]. The treatment of murine epidermis and details of the assay have been published elsewhere [18]. Briefly, the dorsal skin of groups of three C3H/HeN mice was shaved with electric clippers. One day later the mice were exposed to 5 or 10 kJ/m² UV-B. Control mice were shaved but not irradiated. Six hours later the mice were killed, their dorsal skin was removed, and the fur was completely removed with a razor. The skins were placed into a 0.25% trypsin solution and sent on ice to Dr. Yarosh for processing and analysis of the DNA. Briefly, the DNA was purified by proteinase K digestion followed by chloroform extraction and ethanol precipitation. The purified DNA was then treated with T4 endonuclease V to produce breaks at all dimer sites. The single strands were separated by alkaline agarose gel electrophoresis, and the frequency of dimers in DNA was measured by the change in average single-stranded DNA length.

RESULTS

Effect of *Aloe barbadensis* Gel Extract on the CHS Response and on UVR-Induced Local Suppression of CHS to FITC Vehicle alone or three concentrations of *Aloe* gel (1.67%, 0.5%, and 0.167%, w/v) compounded in vehicle were applied topically to the shaved ventral skin of mice once per day for 4 consecutive days. Six

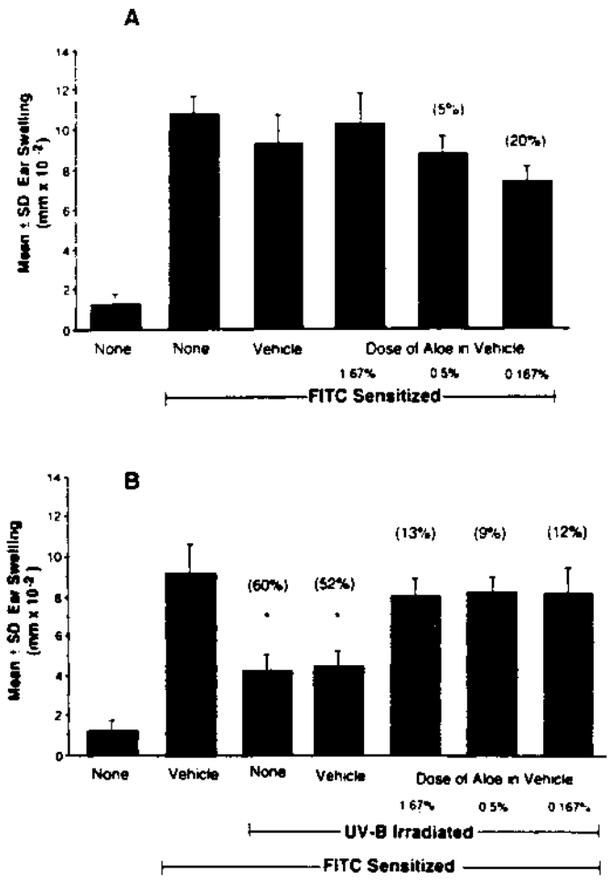

Figure 1. Local effect of *Aloe barbadensis* gel extract on CHS to FITC. *A)* The shaved ventral skin of C3H mice was treated daily for 4 consecutive days with topical applications of three concentrations of *Aloe barbadensis* gel extract in vehicle or vehicle alone. Six hours after the last treatment, the mice were sensitized with 0.5% FITC on their ventral skin. Five days later their CHS response was measured by painting 10 ml 0.5% FITC on each ear and measuring ear swelling 24 h later. *B)* C3H/HeN mice were exposed daily for 4 consecutive days to 400 J/m² UV-B radiation on their shaved ventral skin. Immediately after each exposure, *Aloe barbadensis* gel extract in vehicle or vehicle alone was topically applied to the irradiated site. The mice were sensitized with FITC and challenged as described above. Numbers in parentheses represent percent reduction compared with the matching positive control group. The data illustrated are pooled from two experiments. Statistical analysis by two-way ANOVA (factorial design) indicated that there was no difference between experiments, thus allowing combining of the data. The data are expressed as the mean ± standard deviation from 10 mice. *Asterisk*, statistical significance ($p < 0.0125$) between UV-B–treated groups and their matching, unirradiated controls.

hours after the final treatment, CHS was induced by painting 0.5% FITC on the ventral skin. The animals were challenged on their ears 5 days later. Mice sensitized with FITC showed a strong ear swelling response (Fig 1*A*). Treatment with vehicle alone reduced the CHS response by about 20%, but the reduction was not statistically significant ($p = 0.12$). The CHS response in the *Aloe*-treated groups did not differ significantly from the vehicle control.

We next investigated the effect of *Aloe* on UV-B–induced suppression of CHS. Groups of mice were exposed to 400 J/m² UV-B radiation on their shaved, ventral skin, followed immediately by topical application of vehicle or *Aloe*. The treatment was repeated daily for 4 consecutive days and was followed by sensitization with FITC 6 h after the last UV irradiation. As shown in Fig 1*B*, UV irradiation suppressed the response by 60%. *Aloe*, but not vehicle, prevented UV-B–induced suppression. With ARF91A gel, protection was observed even at the lowest (0.167g *Aloe*/100 ml cream) dose. The response in the mice given UVR followed by *Aloe* was not statistically different from the mice given *Aloe* or vehicle but no UV-B ($p > 0.05$). These data indicate that topical application of *Aloe barbadensis* gel extract does not suppress CHS in unirradiated mice and prevents low-dose UV-B–induced suppression of CHS to the hapten FITC.

Additional control experiments were performed to determine whether vehicle alone suppressed CHS and whether *Aloe* non-specifically increased the ear swelling response. Mice were treated with vehicle or 0.5% *Aloe* in vehicle on their shaved abdominal skin but were not sensitized. After 5 d, their ears were painted with FITC, and ear swelling was measured 24 h later. Vehicle-treated mice showed $1.2 \pm 0.76 \times 10^{-2}$ mm ear swelling, whereas the *Aloe*-treated group had a $0.5 \pm 0.65 \times 10^{-2}$ mm response (not shown). These data show that neither vehicle nor *Aloe* had non-specific suppressive or stimulatory effects on the ear swelling response by themselves.

Effect of *Aloe* on ATPase⁺, Ia⁺, and Thy-1⁺ Dendritic Epidermal Cells in Mouse Skin In C3H mice, reduced CHS responsiveness correlates with reduced numbers of dendritic cells in skin following UV irradiation. Therefore, we examined the numbers and morphology of Langerhans and Thy-1⁺ dendritic epidermal T cells (DETC) in skin treated with *Aloe* and low-dose UVR. Groups of three C3H mice were treated as described above with four daily doses of 400 J/m² UV-B radiation, followed within 5 min by topical application of *Aloe* or vehicle to the irradiated skin. Non-irradiated control groups received vehicle or *Aloe* on their ventral skin. The epidermal sheets were analyzed for numbers of ATPase⁺, Ia⁺, and Thy-1⁺ cells.

The results from two experiments are presented in Table I. Unirradiated mice treated with vehicle alone or *Aloe* had numbers of ATPase⁺ cells similar to those in normal controls. UV-B irradiation reduced the number of ATPase⁺ cells in skin by 90% compared with the untreated control group. *Aloe* treatment of UV-irradiated skin increased the number of ATPase⁺ cells by 77 to 112%, compared with the UV-irradiated group. Vehicle alone failed to prevent the loss of ATPase⁺ cells by UVR. The number of Ia⁺ cells in epidermal sheets was similar to that of ATPase⁺ Langerhans cells (Table I). Mice treated with vehicle alone or *Aloe* showed a similar number of Ia⁺ cells to that of untreated control skin. UV irradiation alone or UVR followed by vehicle treatment reduced the number of Ia⁺ cells by approximately 80%. UV-irradiated skin treated with *Aloe* had approximately 2.5 times more Ia⁺ cells than UV-irradiated skin.

We also examined the effects of UVR on DETC. Treatment of unirradiated skin with vehicle alone or *Aloe* in vehicle reduced the numbers of Thy-1⁺ DETC by 10 to 37% compared with naive controls; however, this reduction was not statistically significant. UV irradiation reduced the number of DETC cells by 78–90% compared with that in normal skin. *Aloe* treatment following UV irradiation significantly increased the numbers of DETC cells compared with that in the UV-irradiated control group in experiment 2, but in experiment 1 the increase was not statistically significant.

The effect of these treatments on the morphology of the dendritic epidermal cells was examined in whole mounts of epidermal sheets. Untreated skin had numerous ATPase⁺ cells evenly distributed throughout the epidermis. The ATPase⁺ cells in untreated (Fig 2*A*) or *Aloe*-treated skin (Fig 2*B*) exhibited an intensely stained central body, which had multiple, branching dendrites. The morphology of the ATPase⁺ cells in vehicle-treated skin was indistinguishable from that of cells in untreated epidermis (not shown). UVR injury greatly reduced the number of ATPase⁺ Langerhans cells in skin (Fig 2*C*). The few remaining cells stained poorly, and their dendritic processes were absent or blunted. In contrast, ATPase⁺ cells were more numerous in UV-irradiated skin treated with *Aloe* (Fig 2*D*). The ATPase⁺ cell bodies were more intensely stained and

Table I. Effect of *Aloe* Gel Extract on Numbers of Dendritic Epidermal Cells in Low-Dose UVR-Treated C3H Mice

Cell Types Detected in Epidermal Sheets	Treatment Groups[a]					
	None	Vehicle	Aloe	UV-B	UV-B + Vehicle	UV-B + Aloe
Experiment 1						
ATPase$^+$	797 ± 64	1014 ± 54 (127)	1059 ± 79 (133)	148 ± 30 (19)[b]	146 ± 17 (18)	314 ± 10 (39)[c]
Ia$^+$	781 ± 98	700 ± 27 (90)	780 ± 57 (100)	145 ± 44 (19)[b]	179 ± 35 (23)	359 ± 27 (46)[c]
Thy1$^+$	632 ± 48	566 ± 18 (90)	534 ± 53 (84)	142 ± 12 (22)[b]	127 ± 39 (21)	179 ± 39 (28)[d]
Experiment 2						
ATPase$^+$	945 ± 45	825 ± 50 (87)	908 ± 130 (96)	200 ± 29 (20)[b]	190 ± 5 (21)	355 ± 45 (38)[c]
Ia$^+$	834 ± 74	780 ± 72 (94)	794 ± 6 (95)	140 ± 38 (17)[b]	211 ± 26 (25)	345 ± 44 (41)[c]
Thy1$^+$	727 ± 90	569 ± 113 (78)	461 ± 47 (63)	71 ± 15 (10)[b]	84 ± 39 (12)	210 ± 37 (29)[c]

[a] Groups of three C3H mice received 400 J/m² UV-B on shaved ventral skin each day for four consecutive days. 1.67% *Aloe* in vehicle or vehicle alone was applied to the irradiated skin immediately after each irradiation. Control groups were shaved and sedated but not irradiated. Six hours after the last treatment the mice were killed and their skins removed, and epidermal sheets were prepared for staining with antibodies to Ia^k and Thy-1, or for detection of ATPase. The numbers of ATPase$^+$, Ia$^+$, and Thy-1$^+$ cells represent the mean ± SD cells/mm² from 20 fields per group (four different epidermal sheets, five fields per sheet). The percent of the untreated control value is shown in parenthesis.

[b] $p < 0.001$ versus untreated group.

[c] $p < 0.01$ versus UV-B + vehicle group and < 0.001 versus UV-B group.

[d] $p > 0.05$ versus UV-B + vehicle or UV-B groups.

possessed multiple dendrites, although the dendrites were less branched and numerous than those of cells in normal skin. Similar morphologic changes were observed in preparations stained for Ia (not shown).

The effect of *Aloe* on the morphology of DETC was examined in untreated and UV-irradiated ventral skin (Fig 3). Like the ATPase$^+$ and Ia$^+$ cells, Thy-1$^+$ cells were numerous and evenly distributed throughout normal and *Aloe*-treated skin (Fig 3*A,B*). The Thy-1 marker was found predominantly on the plasma membranes of positively stained cells. UV irradiation greatly reduced the numbers of DETCs and altered the appearance of the cell bodies to a rounded shape that lacked dendrites (Fig 3*C*). Topical application of *Aloe* following UV irradiation resulted in partial preservation of Thy-1$^+$ cells. Unlike the ATPase$^+$ and Ia$^+$ cells, the remaining DETCs in the skin still appeared rounded and lacking in dendrites (Fig 3*D*). These results demonstrate that UV-B irradiation of ventral skin reduced the numbers of ATPase$^+$, Ia$^+$, and Thy-1$^+$ cells and that *Aloe*, but not vehicle alone, partially preserves the number and morphology of the dendritic cells in skin receiving low doses of UVR.

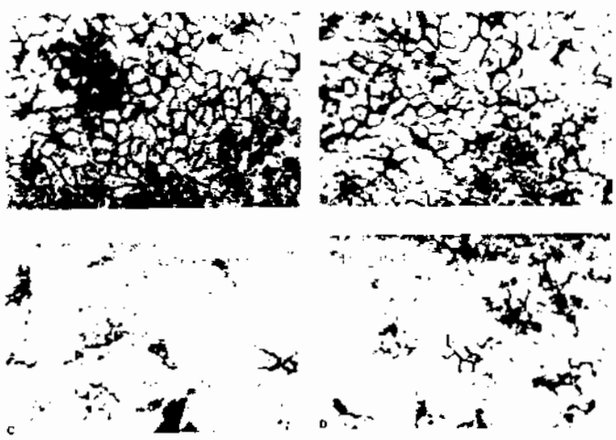

Figure 2. Effect of *Aloe barbadensis* gel extract on the number of ATPase$^+$ Langerhans cells in murine skin. *A)* Untreated ventral skin of C3H/HeN mice stained for ATPase activity; *B)* 1.67% *Aloe* in vehicle was applied daily for four consecutive days; *C)* skin exposed to four daily doses of 400 J/m² UV-B radiation; *D)* skin treated with *Aloe* after each of the four daily exposures to 400 J/m² UV-B radiation. Note depletion and loss of dendrites in ATPase$^+$ Langerhans cells in UV-B–treated skin and partial preservation of numbers and morphology of these cells in irradiated skin treated with *Aloe* (×400).

Efficacy of *Aloe* and Aquaphor Vehicle as Sunscreens The ability of vehicle and *Aloe* to act as sunscreens was also tested directly in mice by measuring their ability to reduce edema in UV-irradiated ears. The ability of agents to prevent UV-induced edema has been used by other investigators as a measure of sunscreen efficacy and correlates well with SPF values for commercial sunscreens obtained using human subjects [24]. To test whether *Aloe* or vehicle reduced the irradiance received by skin, Aquaphor vehicle or 0.5% *Aloe* in vehicle was applied to the ears of mice 15 min before exposure to a 5 kJ/m² dose of UV-B. The edema was quantitated by measuring the change in ear thickness at 24-h intervals for three consecutive days. The data are presented in Table II. The maximum ear swelling occurred by 48 h after UV irradiation. Neither vehicle nor *Aloe* significantly affected ear swelling in UV-irradiated mice. Non-irradiated control groups, included to determine whether vehicle or *Aloe* by themselves affected ear thickness, also showed no effect. These data demonstrate that the effect on UV-induced reduction of CHS was not due to sunscreening activity by the *Aloe* or vehicle.

One possible explanation for the ability of *Aloe*'s application to prevent suppression of CHS might be that it blocks UV-B penetration, thereby decreasing the effective dose of UVR received. To

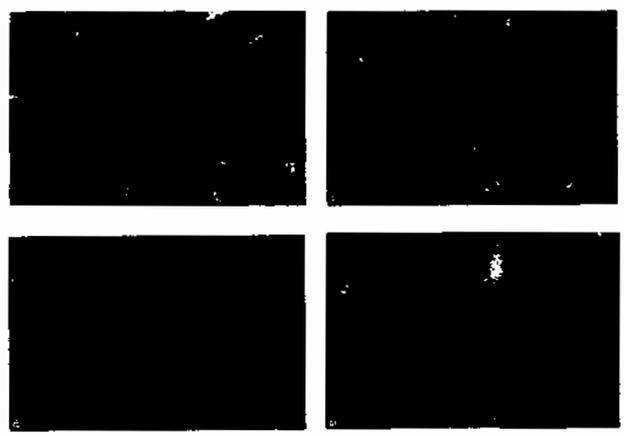

Figure 3. Effect of *Aloe barbadensis* treatment on Thy-1$^+$ DETC in C3H/HeN skin. *A)* Normal C3H/HeN skin stained with rabbit anti–Thy-1 and FITC-goat anti-rabbit second antibody. *B)* *Aloe*-treated skin *C)* exposed to four daily doses of 400 J/m² UV-B radiation; *D)* treated with *Aloe* after each of the four daily exposures to 400 J/m² UV-B radiation. Note the partial preservation of cell numbers but not morphology in the UV-*Aloe* group compared with UV alone (×400).

Table II. Effect of *Aloe* or Vehicle on UV-Induced Edema[a]

Treatment	Change in Ear Thickness (Mean ± SD)		
	24 h	48 h	72 h
UV-B	0.7 ± 0.7	7.9 ± 2.1	5.5 ± 1.4
UV-B + Vehicle	1.2 ± 0.8	8.0 ± 5.0	7.6 ± 3.6
UV-B + *Aloe*	2.9 ± 1.7	6.6 ± 1.5	6.0 ± 2.2
Vehicle	0.0 ± 0	0.8 ± 1.5	0.7 ± 1.3
Aloe	0.1 ± 0.2	0.2 ± 0.3	0.0 ± 0

[a] *Aloe* in Aquaphor vehicle or Aquaphor alone was applied to the ears of groups of 5 C3H/HeN mice 15 min before their exposure to 5 kJ/m² UV-B. The change in ear thickness was determined by subtracting the thickness of each ear at 24, 48, and 72 h after treatment from its value before treatment. Values are expressed as the mean ± SD. Mean ± SD ear thickness of all groups before treatment was 25.4 ± 1.1. No statistically significant differences were found between the untreated UV-irradiated group and UV-irradiated/vehicle or *Aloe*/vehicle-treated mice at any timepoint ($p > 0.05$ by Student *t* test).

determine whether *Aloe* was acting as a UV screening or a therapeutic agent, a single exposure to UV-B radiation was administered to mice, followed by topical application of *Aloe* in vehicle or vehicle alone. The dose of UV-B used, 2000 J/m², is approximately one minimal erythemal dose for a C3H mouse. The combined results of three experiments are presented in Fig 4. Vehicle alone slightly (29%) but significantly ($p < 0.01$) reduced the CHS response when compared with sensitized controls. *Aloe* in vehicle also slightly (27%) reduced sensitization but the reduction was not statistically significant. *Aloe* treatment after UV-B irradiation, but not vehicle alone, restored the response to FITC to levels comparable to those in mice given only *Aloe* application. There was no statistically significant difference between the CHS response of mice treated with *Aloe*

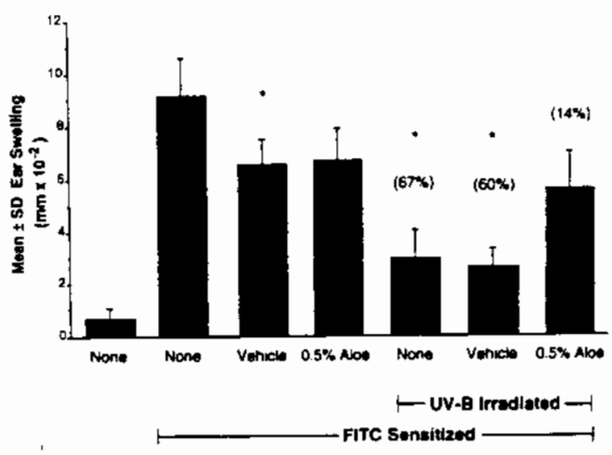

Figure 4. Effects of *Aloe* treatment on UVB-induced local suppression of CHS to FITC. Groups of five C3H/HeN mice were given 2 kJ/m² in a single exposure on their shaved ventral skin followed immediately by topical application of 0.5% *Aloe* in vehicle or vehicle alone. Unirradiated control groups were shaved and treated with *Aloe* or vehicle. Three days later the mice were sensitized with 0.5% FITC through their ventral skin. Five days after sensitization the mice were challenged on their ears with 0.5% FITC and swelling measured 24 h later. These data are the mean ± standard deviation of 15 mice from three separate experiments. As in Fig 1, data were analyzed by ANOVA. Experiment-to-experiment variability was not significant, thus permitting the pooling of data. Percent suppression compared with the appropriate sensitized group is given in parenthesis. Difference between vehicle and UV-B + vehicle: $p = 0.001$; difference between *Aloe* and UV-B + *Aloe* (NS); * $p < 0.01$ determined by two-way ANOVA.

alone and mice treated with UV-B plus *Aloe*, suggesting that complete restoration occurred. Because *Aloe* was applied only after exposure to UVR, it could not have been acting by decreasing the incident dose of UV-B.

In the experiments measuring the effect of *Aloe* on CHS described above, *Aloe* was always applied immediately after UV irradiation. In the following experiment, we determined the efficacy of *Aloe* when applied to skin 1 d before, or 1–2 d after UV irradiation. The data, from a single experiment, are presented in Table III. *Aloe* was only effective at preserving the CHS response to FITC when applied after UV irradiation. The protective activity peaked at 24 h after exposure to UV and was absent after 48 h. Taken together, these data demonstrate that *Aloe* is not acting as a sunscreen but rather prevents events occurring within the first 24 h after UV irradiation that lead to the induction of immunosuppression.

Local versus Systemic Action of *Aloe* *Aloe*, applied topically, could be acting locally or it may have a systemic action. To distinguish between these two possibilities, *Aloe* was applied to abdominal skin after a single exposure to 2 kJ/m² UV-B or a non-irradiated site on the animal's back. Three days after exposure, the mice were epicutaneously sensitized on the abdominal skin. The data are presented in Table IV. UV irradiation suppressed the CHS response to FITC by 53–59% compared with unirradiated matching control groups. Treatment of UV-irradiated skin with *Aloe* partially preserved the CHS response (group VI versus IV and V). This protection was highly significant ($p = 0.001$ versus UV-irradiated, vehicle-treated group IV). In contrast, application of *Aloe* to a distant, non-irradiated site failed to protect against UV-induced suppression (groups VIII versus IV, $p =$ NS; VIII versus VI, $p = 0.03$). These findings demonstrate that *Aloe* acts at the site of irradiation to block induction of immune suppression.

The ability of *Aloe* to block induction of systemic suppression of the CHS response by UVR was examined. Higher doses of UVR suppress CHS to hapten in C3H mice sensitized epicutaneously on unirradiated skin. The effect of topical administration of *Aloe* on systemic suppression was examined in mice given a single dose of 10 kJ/m² UV-B on their dorsal skin. Vehicle or 1.67% *Aloe* was topically applied to the dorsal skin immediately after irradiation. Three days later, the irradiated mice and unirradiated control groups were sensitized with 0.5% FITC on their unirradiated, ventral skin and challenged on their ears 5 d after sensitization. The results from a representative experiment are shown in Fig 5. Neither the vehicle nor *Aloe* alone significantly affected sensitization ($p > 0.05$). Treatment with UVR suppressed CHS to FITC by 71%. UVR followed by vehicle resulted in 55% suppression of CHS compared with the matching unirradiated vehicle control. The difference between the untreated and vehicle-treated UV-irradiated groups was not statistically significant ($p > 0.05$). Treatment of UV-irradiated skin with *Aloe* completely prevented suppression in these mice, compared to both the unirradiated and vehicle control groups. These data indicate that treatment of UV-irradiated skin with *Aloe barbadensis* gel extract can prevent systemic as well as local suppression of CHS.

Effect of *Aloe* on UVR-Induced Systemic Suppression of DTH to *Candida albicans* Ultraviolet-B irradiation of murine skin has been shown to suppress systemically the induction of delayed type hypersensitivity (DTH) to *Candida albicans* [6]. We examined the effect of topical application of *Aloe* on systemic suppression of DTH in mice given a single 5-kJ/m² dose of UV-B on their dorsal skin. *Aloe* (1.67%) in vehicle was topically applied to the dorsal skin immediately after UV-B exposure. Three days later the mice were sensitized with 10^7 formalin-fixed *Candida albicans* cells, injected subcutaneously into each flank. The data presented in Fig 6 show that UV irradiation suppressed the DTH response by 52% ($p < 0.01$). In contrast, treatment of UV-irradiated skin with *Aloe* completely prevented the UV-induced suppression. Therefore, treatment of UV-irradiated skin with *Aloe* prevents the induction of

Table III. Relationship Between Time of UV Irradiation and Efficacy of *Aloe barbadensis* in Preserving CHS Responses

Group	UV-B	Treatment	Time of Application[a]	Specific Ear Swelling[b] (Mean ± SD)	Suppression[c]	Significance (p)[d] Versus Group I	Versus Group II
I	−	None		13.1 ± 3.8			
II	+	None		5.4 ± 1.9	59%	0.004	
III	+	Vehicle	−24 h	4.1 ± 0.9	69%	0.002	NS
IV	+	Vehicle	0 h	3.8 ± 1.4	71%	0.006	NS
V	+	Aloe	−24 h	3.2 ± 3.1	76%	0.006	NS
VI	+	Aloe	0 h	7.1 ± 0.5	46%	0.02	NS
VII	+	Aloe	+24 h	9.4 ± 1.7	28%	NS	0.03
VIII	+	Aloe	+48 h	3.7 ± 1.7	72%	0.004	NS

[a] 0.5% ARF93A lot of *Aloe* in Aquaphor vehicle or Aquaphor alone was applied to the abdomens of C3H/HeN mice 24 h before (−24), 5 min after (0 h), and 24 h or 48 h after exposure of the skin to a single dose of 2 kJ/m² UV-B. Three days after UV irradiation, the animals were sensitized with 0.5% FITC through the abdominal skin.
[b] Values are expressed as the mean ± standard deviation of groups of five mice.
[c] Suppression is calculated as 1 − (ear swelling of test mice − swelling of no UV-B control group) × 100%.
[d] p value by Student t test. NS, not significant ($p > 0.05$).

systemic suppression of both the DTH response to *Candida* and CHS to FITC, as shown above.

Measurement of DNA Damage DNA damage has been implicated as the triggering event in the systemic suppression of CHS and DTH in mice [18]. To determine whether *Aloe* was acting by increasing DNA repair, we examined the effect of *Aloe* on the number of cyclobutyl pyrimidine dimers in UV-B–treated murine skin. The dorsal fur was removed from the skin of groups of three C3H mice using electric clippers. The mice were exposed to 5 or 10 kJ/m² UV-B. Following exposure, the irradiated skins or an unirradiated control were immediately treated with either 1.67% *Aloe* in vehicle or vehicle alone. No pyrimidine dimers were detectable in normal or vehicle-treated skin. Irradiation with 5 kJ/m² UV-B resulted in the formation of an average of 40 dimers per million bp and 56 dimers per million bp in the skin of mice given 10 kJ/m² UV-B. Treatment of irradiated skin with *Aloe* in vehicle or vehicle alone failed to reduce the numbers of pyrimidine dimers formed (not shown) under conditions similar to those in which treatment of skin with T4N5 endonuclease-containing liposomes repaired 40–50% of the dimers formed [18].

DISCUSSION

The major environmental source of UVR is the sun. Because of reported decreases in the concentration of stratospheric ozone [1] and the expected increases in ambient UV-B radiation, the impact of UVR on human health has become a matter of growing public concern. There is a close association between the development of skin cancers and UV-induced immune suppression [3,4,27], and UVR has been shown to decrease immunity to infectious diseases in animal models [6,7]. Therefore, it is important to understand the mechanisms by which UVR induces photoimmunosuppression and to provide new approaches for its prevention and treatment. The development of probes that inhibit some portion of the UV-B–triggered suppression pathway may assist in dissecting these mechanisms.

In studies presented here, we found that a standardized extract of *Aloe barbadensis* gel (ARF'91A and ARF'93A) ameliorated some of the immunosuppressive effects of UVR. The rationale for using *Aloe* was based on its reported anti-inflammatory and burn-healing properties [22,23] and on its widespread empirical acceptance as a palliative treatment for sunburn. Two different protocols of low-dose UV irradiation were used to explore the effects of *Aloe*. UVR administered over 4 consecutive days or given as a single dose of 2 kJ/m² impairs the induction of the CHS response to hapten applied through the UV-irradiated skin of susceptible mouse strains. *Aloe* was equally effective in preventing immune suppression by both regimens. Direct measurement of the efficacy of *Aloe* and Aquaphor vehicle as sunscreens showed that neither agent reduced inflammatory edema following UV irradiation. Taken together, these data demonstrate that *Aloe* acts as a therapeutic agent rather than by reducing the incident dose of UVR.

Langerhans cells have been shown to be important for induction of CHS to antigens encountered through skin and are depleted by UV-B irradiation [8]. Applying *Aloe* to the skin immediately after UV irradiation partially preserved the numbers of dendritic epidermal cells. Not only were $ATPase^+$ and Ia^+ Langerhans cells more numerous in *Aloe*-treated, UV-irradiated skin, but their morphology was nearly normal as well. Dendritic epidermal T-cell numbers

Table IV. Systemic Versus Local Action of *Aloe:* Efficacy of *Aloe* Applied to UV-Irradiated or Non-Irradiated Skin

Group	Site of UV-B Exposure	Site of Treatment[a]	Treatment	Specific Ear Swelling (Mean ± SD)[b]	Suppression Versus Homologous Control[c]
I	None	None	None	13.1 ± 3.8	
II	Abdomen	None	None	5.4 ± 1.9	59%
III	None	Abdomen	Vehicle	8.1 ± 1.1	
IV	Abdomen	Abdomen	Vehicle	3.8 ± 1.4	53%
V	None	Abdomen	Aloe	10.0 ± 1.0	
VI	Abdomen	Abdomen	Aloe	7.1 ± 0.5[d]	29%
VII	None	Back	Aloe	11.6 ± 3.1	
VIII	Abdomen	Back	Aloe	5.2 ± 1.8[e]	55%

[a] 0.5% *Aloe* in Aquaphor vehicle or Aquaphor alone was applied to the skin of normal or UV-irradiated mice (within 5 min after exposure of the skin to 2kJ/m² UV-B).
[b] Groups of five animals.
[c] Suppression is calculated as 1 − (specific ear swelling of test mice − specific swelling of the homologous non-irradiated control group) × 100%.
[d] Difference between UV-B-irradiated, *Aloe*-treated, and vehicle-treated animals (groups VI versus IV) is significant ($p = 0.001$). Effect of site of treatment (*Aloe* on back versus *Aloe* on abdomen, groups VI versus VIII) after UV-B injury of abdominal skin is significant ($p = 0.03$).
[e] *Aloe* treatment of a site distant from injury has no significant effect ($p > 0.05$, group VIII versus IV).

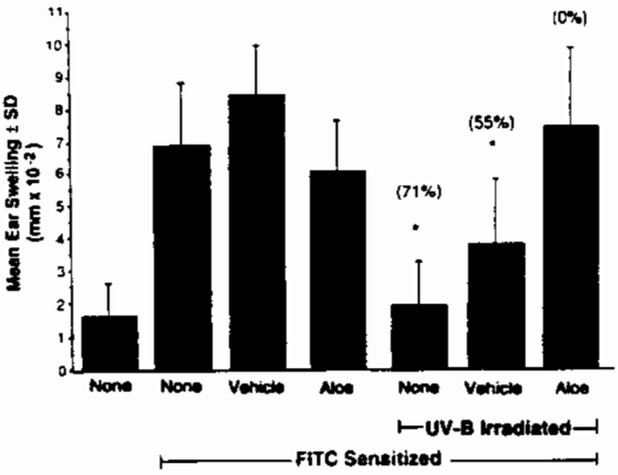

Figure 5. Effects of *Aloe* treatment on UVB-induced systemic suppression of CHS to FITC. C3H mice were exposed to a single dose of 10 kJ/m² UV-B on their shaved dorsal skin followed immediately by topical application of 1.67% *Aloe* in vehicle or vehicle alone. Unirradiated control groups were shaved and treated with *Aloe* or vehicle. Three days later the mice were sensitized with 0.5% FITC through their untreated ventral skin. Five days after sensitization the mice were challenged on their ears with 0.5% FITC and swelling measured 24 h later. These data are representative of three separate experiments and are the mean ± SD of groups of five mice. Percent suppression compared with the matching, unirradiated control group is given in parentheses. $p = 0.26$, untreated positive control versus vehicle treatment; $p = 0.1$, UV versus UV + vehicle. * $p < 0.01$ determined by two-way ANOVA.

were also partially preserved by *Aloe* treatment following UV irradiation but their dendritic morphology was not preserved. However, treatment of UV-irradiated skin with *Aloe* preserved the level of immune function beyond what would be expected by the Langerhans cell numbers in these mice [8]. It is well known that there is not a perfect correlation between morphologic alterations in epidermal dendritic cells and immune function, as measured by CHS [28]. This is probably due to the fact that cells in the dermis can also act as antigen-presenting cells for CHS, particularly when a high dose of antigen is used [29]. Alternatively, *Aloe* may be acting at a later stage of the immune suppressive pathway and have little influence on the morphologic alterations of Langerhans cells.

Besides preserving local immune function in mice given less than 1 minimum erythemal dose of UVR, systemic suppression of DTH and CHS by higher doses of UVR (5 and 10 kJ/m², respectively) was also inhibited by treatment of the UV-irradiated skin with *Aloe*. Previous studies suggested that both effects of UVR are triggered primarily by the formation of cyclobutyl pyrimidine dimers in the DNA of cells in the UV-irradiated skin [18]. In those experiments, application of liposomes containing T4 endonuclease V to UV-irradiated murine skin, a procedure that increases the repair of pyrimidine dimers, prevented systemic photoimmunosuppression. Although *Aloe* treatment had a similar effect on systemic immune suppression, it did not alter the number of pyrimidine dimers in UV-irradiated skin under conditions in which liposome treatment decreased the number of dimers by 40–50%. We therefore propose that *Aloe* influences a later step in the sequence of events leading to immune suppression. For example, soluble factors such as TNF-α, IL-10, and *cis*-UCA, released by UV-irradiated skin, can mediate suppression of different T-cell–mediated immune responses [15,16,19,30]. It is possible that *Aloe* acts by inhibiting the formation or release of one or more of these factors.

Our experiments demonstrate that a crude *Aloe* extract can have potent and reproducible biologic activity. Unfortunately, previous studies involving *Aloe* preparations have not produced consistent results [22]. This problem can probably be attributed to variability in the source of the *Aloe* plants, variability in the production process, the presence in the preparations of multiple components with varying, and even opposing, biologic activities, and the use of different vehicles for *Aloe* administration. Within the past year, the Aloe Research Foundation has attempted to remedy some of these problems by preparing standard reference samples of *Aloe barbadensis* gel. The materials used in this study (ARF'91A, ARF93A) have been characterized extensively in terms of biologic and physical properties, chemical constituents such as salts, proteins, polysaccharides, and assorted small molecules, and microbial contaminants.

The Aloe Research Foundation materials used in these studies do not correspond to any currently available commercial product. The various processes involved in producing commercial "Aloe" have the potential for significantly altering the chemical composition of the type of *Aloe* extract we describe herein. The bacteriology of *Aloe* extracts is complex and the material we used in these studies may be considered somewhat atypical of commercially produced materials in that the starting substance had an extremely low bacterial content. The *Aloe barbadensis* gel extract content of most commercial materials may be highly variable, the nature of the processing may not be indicated, and the bacteriology of starting materials unspecified. These caveats are well described in the "trade" literature [31–33] but not in the scientific literature. Previous biologic investigations have employed either uncharacterized raw materials prepared in the laboratory or undefined commercial material. It is therefore not surprising that results from various investigators on the noncathartic biologic properties of various *Aloe* species do not always agree. Needless to say, the results of our study cannot constitute endorsement of any commercial product. Clearly, purification of the component(s) responsible for amelioration of photoimmunosuppression would be highly desirable and would permit more detailed studies of the mechanism of action of *Aloe* in this system.

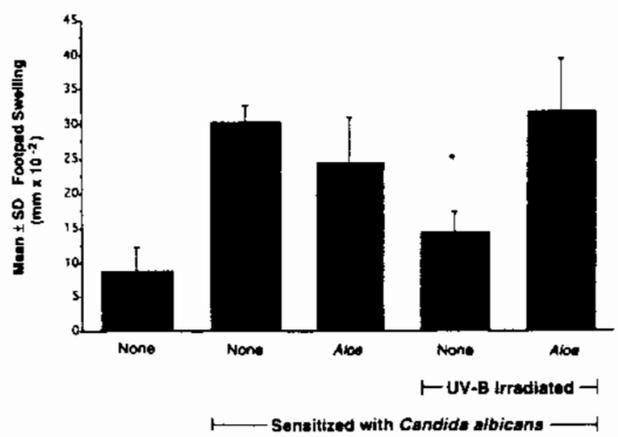

Figure 6. Effects of *Aloe* treatment on UVB-induced systemic suppression of DTH to *Candida albicans*. C3H mice were exposed to a single dose of 5 kJ/m² UV-B on their shaved dorsal skin followed immediately by topical application of 1.67% *Aloe* in vehicle or vehicle alone. Unirradiated control groups were shaved and treated with *Aloe* or vehicle. Three days later the mice were sensitized with 10^7 formalin-fixed *C. albicans* injected subcutaneously into each flank. Ten days after sensitization the mice were challenged with 50 μl soluble *Candida* antigen in each rear footpad and swelling measured 24 h later. These data are representative of two separate experiments and are the mean ± SD of groups of five mice. * $p < 0.01$ determined by two-way ANOVA.

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