Effect of Psoralens and Ultraviolet Radiation on Murine Dendritic Epidermal Cells

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Monofunctional psoralens produce less phototoxicity than bifunctional psoralens after ultraviolet A (UVA) irradiation. We investigated the effect of repetitive treatments with angelicin (isopsoralen), a monofunctional psoralen, plus UVA radiation (IPUVA) on the number and morphology of dendritic epidermal cells (dEC). This effect was compared with that of 8-methoxypsoralen plus UVA radiation (PUVA), UVA alone, and UVB radiation. C3H/HeN mice were treated topically with the drugs three times/wk for 4 consecutive wk; followed each time by 1 or 2.5 J/cm^2 of UVA radiation. Other groups of mice were treated with the drugs alone, UVA alone, or 0.81 J/cm^2 of UVB. Epidermal sheets were stained for ATPase, Ia, and Thy-1 markers. Mice treated with PUVA and UVB exhibited severe phototoxicity, whereas no overt phototoxicity was observed in mice treated with IPUVA, UVA alone, or the drugs alone. Early during the PUVA and UVA treatments the ATPase marker was lost from dEC, followed by loss of the Ia marker; the Ia marker was lost before the ATPase marker from dEC in animals treated with IPUVA. At the end of the treatment, however, nearly total depletion of ATPase$^+$, Ia$^+$, and Thy-1$^+$ dEC was observed in mice treated with PUVA and IPUVA. UVB radiation caused rapid depletion of Thy-1$^+$ dEC as well as ATPase$^+$ and Ia$^+$ cells. During treatments with IPUVA, PUVA, UVA, and UVB, the Langerhans cells became rounded and lost their dendrites. These changes were quantitated by image analysis. We conclude that alterations of cutaneous immune cells can occur in the absence of overt phototoxicity, and that monofunctional and bifunctional psoralens plus low dose of UVA radiation may have different effects on dEC markers. *J Invest Dermatol 92:657–662, 1989.*

During the last decade it has been established that ultraviolet (UV) radiation causes local and systemic alterations of the immune system [1–3]. These alterations play a role both in the evolution of skin cancers in mice [4–6] and in the induction of specific unresponsiveness to contact allergens in mice and humans [7–10]. It has also been established that the immune system activity extends to the epidermis. This concept arose mainly from the recognition that epidermal Langerhans cells (LC) bear an Ia antigen on their surface and serve as antigen-presenting cells of the immune system [11]. These findings raised the question of whether damage to LC by UV radiation would result in an impaired immune response to contact allergens. Studies by Toews et al [7] and Elmets et al [12] demonstrated that UVB-induced changes in the number and morphology of LC correlated with an impaired contact hypersensitivity response to haptens applied on the irradiated skin and the induction of hapten-specific suppressor lymphocytes.

8-Methoxypsoralen (8-MOP) plus UVA radiation (PUVA), in addition to UVB radiation alone, depletes adenosine triphosphatase$^+$ (ATPase$^+$) LC in mice, guinea pigs, and humans [13–15]. In mice and guinea pigs, the depletion is also associated with an impaired contact hypersensitivity response [14]. In humans bifunctional psoralens, such as 8-MOP, often used with UVA radiation in the treatment of various dermatologic diseases, may have serious acute and chronic side effects, such as cutaneous phototoxicity and an increased risk of skin cancer [16]. Therefore, attempts have been made in recent years to assess the beneficial effect of other psoralens, particularly monofunctional psoralens. These are angular furocoumarins that form monoadducts with DNA but not interstrand cross-links [17], and they are nonerythemogenic and noninflammatory [18].

The purpose of this study was to assess the effects of a monofunctional psoralen plus UVA radiation (IPUVA) on the number and morphology of LC and to compare them with the effects of a bifunctional psoralen plus UVA radiation. In addition, we examined the effects of these treatments on murine Thy-1$^+$ dendritic epidermal cells (dEC) [19, 20]. We were interested in evaluating the effect of repetitive treatments with psoralens and UVA radiation on the dEC, as most previous studies have assessed only the acute effects of UVB radiation and 8-MOP plus UVA radiation. To do so, we determined the relative sensitivity of ATPase, Ia, and Thy-1 on dEC to each agent and whether changes in the morphology of LC occur after all treatments by measuring the dendricity of the cells with an image analyzer.

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Abbreviations:
8-MOP: 8-methoxypsoralen
ATPase: adenosine triphosphatase
dEC: dendritic epidermal cells
DI: dendricity index
IPUVA: isopsoralen plus UVA radiation
LC: Langerhans cells
PBS: phosphate-buffered saline
PUVA: psoralen plus UVA radiation
UV: ultraviolet

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MATERIALS AND METHODS

Animals Specific pathogen-free female mice of the inbred strain C3H/HeNCr (MTV^-) were obtained from the NCI-Frederick Cancer Research Facility animal production area (Frederick, MD). The mice were age-matched and were 9–12 wk old at the beginning of the experiments. The mice received NIH-31 open formula mouse chow and sterile water ad libitum.

Radiation Sources and Setup The UVA radiation was provided by a Dermalight 2001 using an optical H1 filter (Dermalight Systems, Studio City, CA). The lamp was rotated on its original stand to a horizontal position and mice were placed 22 cm below in a plastic cage separated into individual chambers. The cage was covered with a wire mesh. The spectral irradiance was measured under the mesh by an IL-700A research radiometer (International Light, Inc., Newburyport, MA) using a calibrated UVA detector. The irradiance value was 5.63 mW/cm^2. Additional measurement was made by an Optronics 742 Spectroradiometer (Optronic Laboratories Inc., Orlando, FL) and was similar to that found by IL-700A within 25%. The spectral output of the lamp was 99.5% between 320 and 400 nm with the peak emission occurring at 366 nm.

The UVB radiation was provided by a bank of 6 FS-40 sunlamps (Westinghouse, Bloomfield, NJ). Mice were housed on shelves 20 cm below the lamps and the cages were rotated systematically along the shelf before each UV exposure to ensure uniform irradiation over the course of the experiment. Sixty percent of the output of the lamps is within the UVB range (280–320 nm) with the peak emission at 313 nm. Mice were irradiated through wire cage tops. The irradiance value as measured by IL-700A radiometer with a calibrated UVB detector was 0.45 mW/cm^2.

Drugs The monofunctional psoralen used was angelicin (isopsoralen), obtained as a crystalline powder from HRI Associates, Inc. (Berkeley, CA). The drug was dissolved in 70% ethanol (w/v) to form a solution of 0.215%. 8-Methoxy-psoralen (Oxsoralen lotion 1%) was obtained from Elder Pharmaceuticals, Inc. (Bryan, OH) and diluted in 70% ethanol to form a 0.25% solution. Both drugs were kept shielded from light. Equimolar doses of the two drugs were used for the treatments.

Treatment Protocols Mice were divided into 10 experimental groups whose designations and protocols are established in Table I. In brief, treatments were given three times/wk over a 4-wk period. After the dorsal fur was removed with electric clippers, those mice that were to receive drug treatments were given 250 μg 8-MOP or 215 μg angelicin, in a 100-μl volume that was applied to the back. Forty-five to 60 min after the topical application (to allow maximal absorption of the drugs) the mice were irradiated with either 1 or 2.5 J/cm^2 UVA. The mice in the UVB group received 0.81 J/cm^2 at each treatment. Twenty-four h after the 1st, 3rd, 9th, and 12th treatments, mice from each group were killed by cervical dislocation, and their dorsal skin removed for preparation of epidermal sheets.

Table I. Experimental Groups

Groups	Drugs	Radiation
Control	none	none
8-MOP	8-methoxypsoralen[a]	none
Angelicin	angelicin[b]	none
UVA_1	none	1 J/cm^2 UVA
UVA_2	none	2.5 J/cm^2 UVA
UVB	none	0.81 J/cm^2 UVB
$PUVA_1$	8-methoxypsoralen[a]	1 J/cm^2 UVA
$IPUVA_1$	angelicin[b]	1 J/cm^2 UVA
$PUVA_2$	8-methoxypsoralen[a]	2.5 J/cm^2 UVA
$IPUVA_2$	angelicin[b]	2.5 J/cm^2 UVA

[a] 100 μl of 0.25% (w/v) in 70% ethanol.
[b] 100 μl of 0.215% (w/v) in 70% ethanol.

Dendritic Epidermal Cell Staining Methods The remaining hair was removed from the dorsal skin with a razor blade, and the connective tissue was removed with a scalpel. The epidermis was separated from the dermis after a 2-h incubation at 37°C in buffered EDTA [21].

Adenosine Triphosphatase Staining for Langerhans Cells The epidermal sheets were fixed overnight at 4°C in 0.05 M cacodylate–2% paraformaldehyde solution. After fixation, the samples were washed three times with saline and incubated at 37°C for 45 min in a solution containing ATP powder, Tris-Mal, $MgSo_4$, and $Pb(NO_3)_2$. The samples were then washed again in saline and immersed for 60 min in ammonium sulfide solution. The stained specimens were mounted in glycerol phosphate-buffered saline (PBS) on glass slides.

Immunofluorescence Staining for Langerhans Cells and Thy-1$^+$ Dendritic Epidermal Cells Epidermal sheets were fixed in acetone for 30 min and then refrigerated overnight at 4°C in a 1:30 dilution of monoclonal antibody in PBS. The antibodies used were mouse anti-mouse Ia^k (specificity 2) for LC and rat anti-mouse Thy 1.2 for Thy-1. Both antibodies were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA). The specimens were washed in PBS and incubated in a 1:100 dilution of a second antibody in PBS. Fluorescein-conjugated $F(ab')_2$ fragment goat anti-mouse IgG and rhodamine-conjugated IgG fraction of goat anti-rat (heavy- and light-chain specific) antibody were used as the second reagents, respectively (Cooper Biomedical, Inc., Malvern, PA). The specimens were mounted on glass slides in a 9:1 solution of glycerol-PBS in 0.1 M *n*-propylgalate.

Counting Dendritic Epidermal Cells The number of ATPase$^+$, Ia^{k+}, and Thy-1$^+$ epidermal cells were determined by the same observer using a Nikon Optiphot microscope (Nikon Inc., Garden City, NY). Between 10 and 20 fields from each experimental group were counted at ×400 magnification using a WHK 10 × 20 L optical grid (Olympus Optical Co., Tokyo, Japan). The significance of differences between the mean number of the cells in the various groups was determined using the Student's two-tailed *t*-test or the one-way analysis of variance (ANOVA) test. The data are reported as percentages of the number of cells in the control, untreated mice.

Image Analysis We used the ATPase-stained specimens obtained after the 12th treatment to evaluate the effect of the various treatments on the dendricity of the LC. Quantitative measurements of the area and perimeter of the LC were performed using the IBAS image analyzer (Zeiss Inc., Thornwood, NY). The samples were viewed with a Zeiss Universal photomicroscope, using a 40× objective and projected onto a video screen. The video image of the cells was calibrated with a stage micrometer (Nikon). Each area examined on the video screen covered a field corresponding to an area of 0.17 mm^2 on the slide specimen. Five fields from each experimental group were examined depending on the available surface area. The major problems encountered in attempting to quantitate the morphologic features of dendritic cells in whole epidermal mounts was the limited depth of focus of the specimen, especially in a high magnification. By virtue of the image analyzer's electronic enhancement capabilities, an average of images displayed on two focal planes could be obtained. All electronic enhancements and editings were cross-checked with the original image and refocused on a separate video monitor to minimize over or underestimation errors caused by electronic enhancement. The circularity of each LC was calculated according to the following formula:

$$\text{Cell circularity} = \frac{4\ \text{Pi} \times \text{cell image area}}{(\text{perimeter of LC})^2}$$

The dendricity index (DI) was determined as one-cell circularity [22]. Thus, a perfectly round cell with no dendrites would have a DI of 0, whereas a highly dendritic cell would have a DI close to 1. The significance of differences between the DI of the different experimental groups was calculated using the Student's two-tailed *t*-test.

Histological Examination Skin samples from the various experimental groups were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Samples were taken after the 1st, 3rd, 9th, and 12th treatments from PUVA-treated groups and from the IPUVA-treated groups after the 12th treatment.

RESULTS

Phototoxicity The mice in the $PUVA_1$ and $PUVA_2$ groups exhibited a gross phototoxic reaction in the irradiated site shortly after the second treatment. The phototoxic reaction was especially severe in the $PUVA_2$ group, and so we decreased the dose of UVA in this group to 2 J/cm² after the fourth treatment. A severe gross phototoxic reaction occurred also with UVB radiation. The phototoxic effects were seen also on histologic examination. Acute, severe dermatitis was present with inflammatory cells consisting of neutrophils, macrophages, and lymphocytes. In contrast, no phototoxic reactions were noted grossly or histologically in any of the mice in the $IPUVA_1$ or $IPUVA_2$ groups even after completion of the study. In addition, no phototoxicity was observed in any of the mice that received UVA alone.

Effect of Angelicin and 8-MOP on Cutaneous Immune Cells Treatments with angelicin or 8-MOP alone for 4 wk had no significant effect on the number of ATPase⁺, Ia⁺, or Thy-1⁺ epidermal cells, compared with that in the untreated mice (Table II).

Effect of UVA Radiation Alone After a single exposure to UVA_1 or UVA_2 the number of ATPase⁺ cells was reduced by more than 50% ($p < 0.001$). Surprisingly, the same single exposure caused no significant depletion of Ia⁺ cells (Fig 1). Repeated treatments resulted in nearly completed disappearance of the ATPase⁺ cells from the epidermis, but in both the UVA_1 (Fig 1*A*) and UVA_2 mice (Fig 1*B*), there was a slight, but not significant, increase in the number of Ia⁺ cells during the first nine exposures. Although at the end of study the number of Ia⁺ cells was significantly lower than that in the control group ($p = 0.003$ for UVA_1 and $p = 0.002$ for UVA_2), the percentage of control was still much higher than for the ATPase⁺ cells. These results suggest that ATPase is more sensitive than the Ia marker on dEC to relatively low doses of UVA radiation.

The lower dose of UVA (Fig 1*A*) caused a significant reduction in the number of Thy-1⁺ dEC after three exposures ($p < 0.001$) but after other cumulative exposures, the number of Thy-1⁺ dEC was not significantly reduced from that of the controls. The higher doses of UVA radiation, however, significantly decreased the number of Thy-1⁺ dEC at all time points examined (Fig. 1*B*), even after a single exposure ($p = 0.002$). By the completion of the study the number of Thy-1⁺ dEC decreased by nearly 50% ($p < 0.001$). These findings demonstrate that UVA radiation depletes either the number or the marker on Thy-1⁺ dEC in mouse skin.

Effects of Psoralens Plus Ultraviolet A Radiation on Langerhans Cells Although UVA_1 and UVA_2 decreased the number of ATPase⁺ epidermal cells after the first and third treatments, IPUVA had no effect on the number of ATPase⁺ cells after the same number of treatments (Fig 2*A*, *B*). In contrast, the first and third $PUVA_1$ and $PUVA_2$ treatments resulted in marked depletion of the ATPase⁺ cells ($p < 0.001$) (Fig. 3*A*, *B*). Nevertheless, after 4 wk of treat-

Table II. Effect of Monofunctional and Bifunctional Psoralens Without UVA Radiation on the Number of ATPase⁺, Ia⁺, and Thy-1⁺ Dendritic Epidermal Cells

	Controls[a]	8-MOP[b]	Controls[a]	Angelicin[c]
ATPase⁺ cells	806 ± 45	731 ± 91	908 ± 79	967 ± 70
Ia⁺ cells	920 ± 29	893 ± 33	787 ± 36	775 ± 35
Thy-1⁺ cells	609 ± 25	567 ± 23	595 ± 27	577 ± 35

[a]No statistically significant differences from treated groups, calculated by Student's *t* test. Figures represent number ± standard error of the mean from 10 to 20 microscopic fields.
[b]Mice were treated topically with 250 μg of 8-MOP three times/wk for 4 wk.
[c]Mice were treated with 215 μg of angelicin three times/wk for 4 wk.

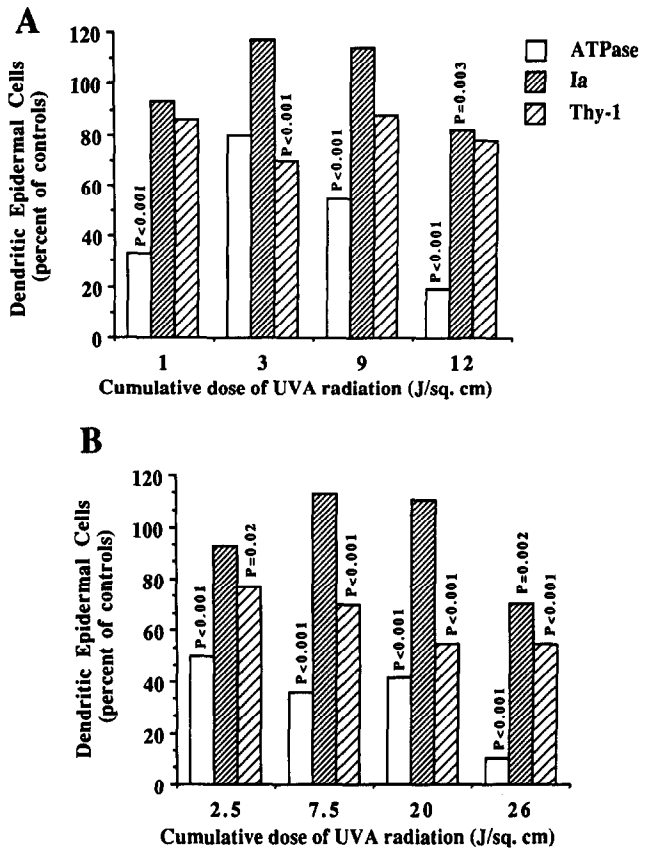


Figure 1. Number of ATPase⁺, Ia⁺, and Thy-1⁺ dendritic epidermal cells (percentage of controls) during repeated exposures to 1 J/cm² UVA *(A)* and 2.5 J/cm² UVA *(B)*.

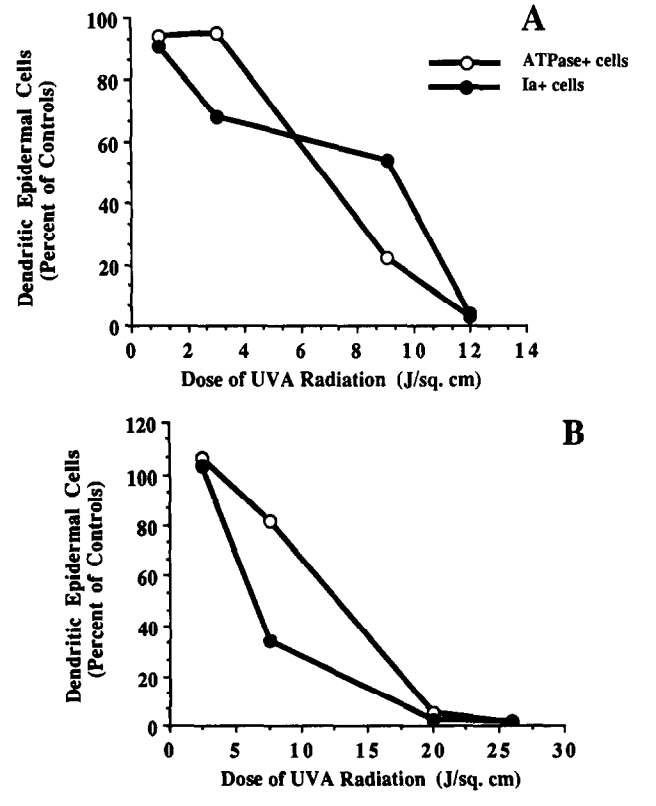


Figure 2. Effect of monofunctional psoralen followed by 1 J/cm² UVA *(A)* and 2.5 J/cm² UVA *(B)* on the number of ATPase⁺ and Ia⁺ epidermal cells, expressed as percentage of controls.

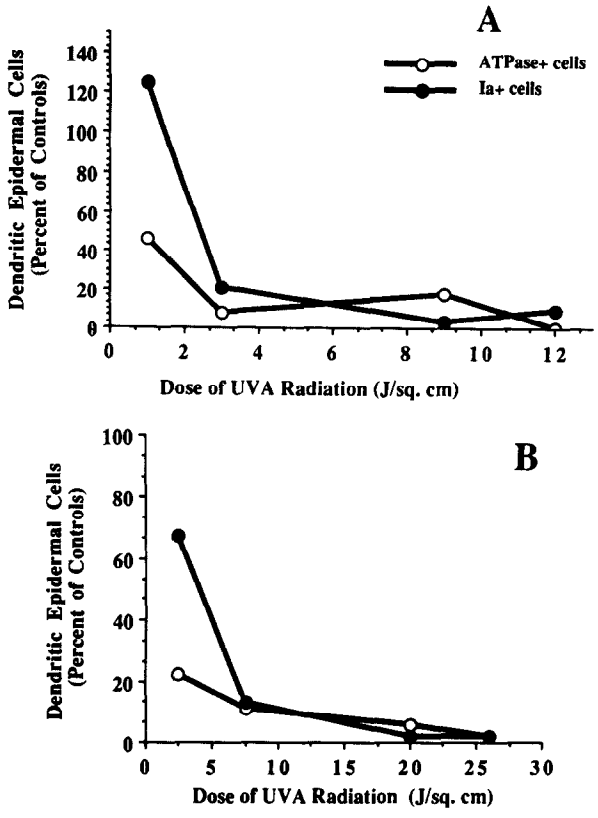


Figure 3. Effect of 8-MOP followed by 1 J/cm² UVA *(A)* and 2.5 J/cm² UVA *(B)* on the number of ATPase⁺ and Ia⁺ epidermal cells.

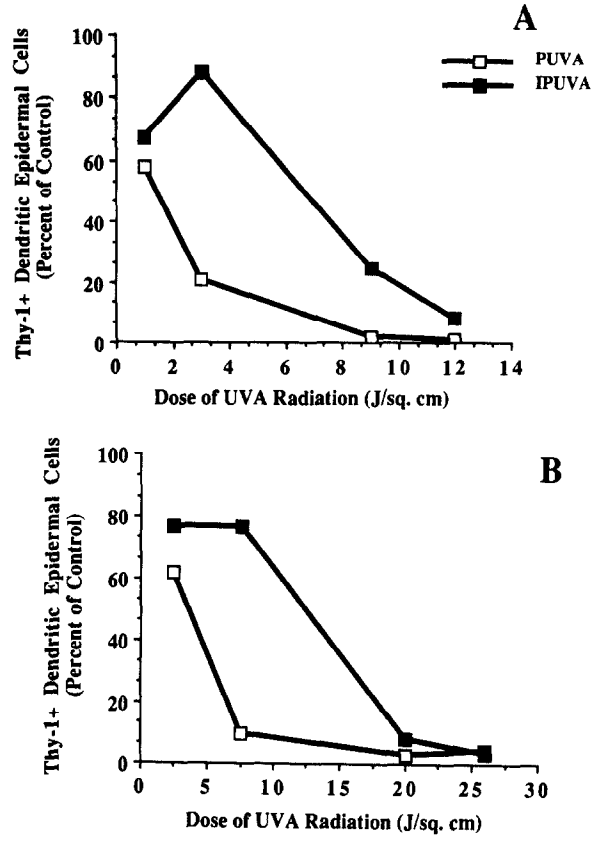


Figure 4. Effect of repeated PUVA and IPUVA treatments on the number of Thy-1⁺ dendritic epidermal cells. *A:* Each exposure to UVA = 1 J/cm². *B:* Each exposure to UVA = 2.5 J/cm². After the fourth exposure each exposure to UVA = 2 J/cm².

ment, all four treatment groups, $IPUVA_1$, $IPUVA_2$, $PUVA_1$, and $PUVA_2$, showed complete disappearance of ATPase⁺ cells from the epidermis. From these observations it appears that a cumulative dose of approximately 12 J/cm² of UVA radiation (given as 1 J/cm² per exposure) after equimolar applications of either the monofunctional or bifunctional psoralen is sufficient to eliminate ATPase from murine LC. Doses of UVA radiation lower than 7.5 J/cm², however, had almost no effect on ATPase⁺ LC in the mice treated with IPUVA.

The same general trend occurred with Ia⁺ cells, although the third treatment with $IPUVA_1$ and $IPUVA_2$ did significantly reduce the number of Ia⁺ cells ($p < 0.001$) (Fig 2). At the end of the experiment, the Ia⁺ cells in the IPUVA groups were reduced to between 1.6% and 3.9% of the number in untreated mice ($p < 0.001$); a similar depletion occurred in the PUVA-treated mice.

An interesting observation from Figures 2 and 3 is that the decreases in the number of Ia⁺ and ATPase⁺ cells in the PUVA and the IPUVA groups differed within the same general trend. In the PUVA-treated groups the number of ATPase⁺ cells decreased more than the Ia⁺ cells until a cumulative dose of 7.5 J/cm² was reached, whereas in the IPUVA-treated groups the number of Ia⁺ cells decreased more than the number of ATPase⁺ cells at the lower cumulative doses. At the cumulative dose ≥12 J/cm² these differences disappeared, as there were almost no detectable ATPase⁺ or Ia⁺ cells remaining in the epidermis.

Effects of Psoralens Plus Ultraviolet A Radiation on Thy-1⁺ Dendritic Epidermal Cells Like their effect on LC markers, the end result of IPUVA and PUVA treatments was total disappearance of the Thy-1⁺ dEC from the epidermis ($p < 0.001$ for all experimental groups) (Fig 4). The effect of PUVA, however, was observed earlier in the course of treatment than that of IPUVA. As shown in Figure 4, cumulative doses of 3 or 7.5 J/cm² of UVA caused marked depletion of Thy-1⁺ dEC when preceded by 8-MOP treatment (79% and 90%, respectively), whereas only modest decreases occurred after these doses of UVA when preceded by angelicin treatment (12% and 23%, respectively). This observation shows that the relative effects of psoralens on the Thy-1 marker can differ depending on the structure and binding capacity of the psoralen, at least at cumulative doses of UVA radiation below 7.5 J/cm².

Effect of Ultraviolet B Radiation on Langerhans Cells and Thy-1⁺ Dendritic Epidermal Cells The administration of a single dose of 0.81 J/cm² of UVB radiation greatly reduced the number of ATPase⁺, Ia⁺, and Thy-1⁺ epidermal cells in mouse skin (Fig 5). As was the case with UVA radiation alone and PUVA treatments, ATPase was more sensitive to the effects of UVB irradiation than the Ia marker. The data in Figure 5 show a slight parallel increase in the number of ATPase⁺ and Ia⁺ cells after the third UVB treatment (cumulative dose, 2.4 J/cm²). These numbers,

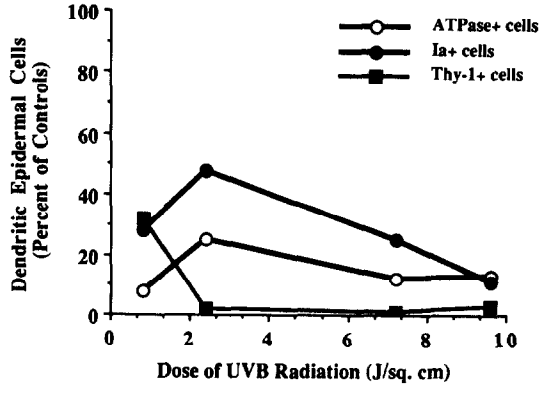


Figure 5. Effect of repeated exposure to UVB radiation on the number of ATPase⁺, Ia⁺, and Thy-1⁺ dendritic epidermal cells. Each exposure is 0.81 J/cm².

however, decreased again with continued irradiation, and at a cumulative dose of 9.6 J/cm², there was almost complete depletion of both ATPase⁺ and Ia⁺ cells. It should be noted from the data shown in Figure 5 that Thy-1⁺ dEC were completely eliminated by UVB radiation, whereas Ia⁺ and ATPase⁺ were not. After three treatments, few or no Thy-1⁺ dEC could be detected in the mouse epidermis.

Effect of Psoralens and Ultraviolet Radiation on the Morphology of Langerhans Cells Image analysis was performed on a total of 423 LC in skin taken from each experimental group at the end of the treatments. Figure 6 graphs the mean DI of the cells from each group. The ATPase⁺ cells in the control group and in the 8-MOP- or angelicin-treated mice, which appeared dendritic (Fig 7*a*), had a high DI. All other ATPase⁺ cells appeared more round or oval with almost no dendrites (Fig 7*b*); they had a lower DI. There was no significant difference between the mean DI of LC of the control, 8-MOP, or angelicin groups. All other groups showed a significant difference ($p < 0.001$) between their mean DI and that of cells from the control group.

DISCUSSION

Although several studies showed that PUVA treatment and UVB irradiation altered the number and morphology of epidermal LC [23,24], it was unclear whether these effects were related to phototoxicity and whether the ATPase and the Ia markers of the cells were equally affected. Previous studies examined the effect of PUVA treatment on ATPase⁺ and Ia⁺ cells in the epidermis [14,24]; however, in none of the studies were both markers examined simultaneously. Moreover, the effect of the various treatment modalities on dEC was evaluated at various time points after cessation of the treatment and not during the treatment. This is of special interest to understand the role of dEC in the evolution of skin cancers during repeated exposure to UV radiation or long-term photochemotherapy.

In the present study we demonstrated that a monofunctional psoralen plus UVA radiation has different effects on dEC than does a bifunctional psoralen plus UVA. First, a single treatment with IPUVA had no effect on the number of ATPase⁺ and Ia⁺ epidermal cells, whereas a single PUVA treatment caused marked decreases in all markers. Multiple treatments with PUVA and IPUVA had almost the same depleting effects on these markers. These effects occurred despite a complete lack of overt phototoxicity in the IPUVA-treated skin. Second, the effect of PUVA on Thy-1⁺ cells was more pronounced than that of IPUVA. Third, ATPase seemed more sensitive to PUVA treatment than the Ia marker, whereas no such differential sensitivity was observed after IPUVA treatment. The differential sensitivity of the two markers to PUVA, UVB, and UVA alone suggests that at least at low doses, the LC are not destroyed, but rather only the surface markers are affected. Koulu et al [15] similarly noted that the ATPase in human skin was more sensitive to moderate UVB doses than the immunologic markers Ia and T6. Furthermore, Aberer et al [13] demonstrated that the disappearance of both Ia antigen and ATPase activity of the LC did not necessarily reflect the complete absence of the cell population. They demonstrated by electron microscopy that substantial numbers of morphologically unaltered LC were present while their surface markers were no longer identifiable. Hanau et al [25] demonstrated that acute low-dose UVB radiation destroyed the ATPase on LC but did not deplete the LC population. They were able to show, however, that the LC contained abnormal LC granules or were deficient in these granules.

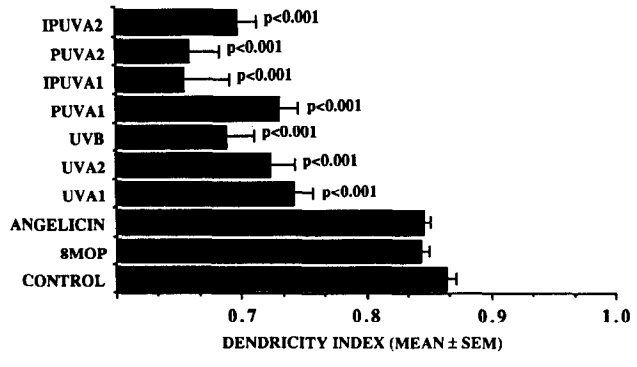
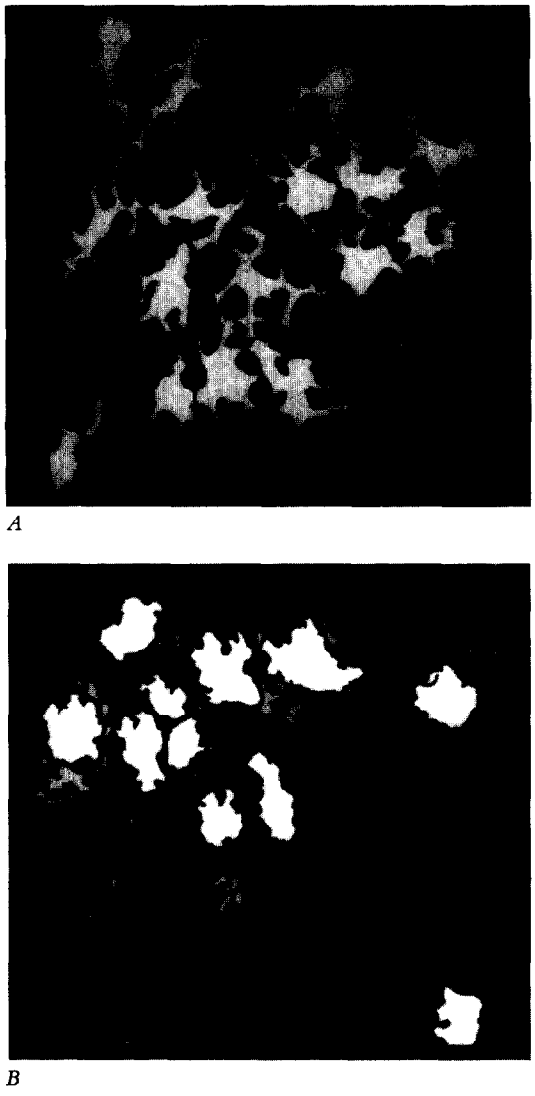


Figure 6. Mean dendricity indices of Langerhans cells in the various experimental groups after 12 treatments. p values represent differences from the untreated control.



A

B

Figure 7. *A:* Photomicrograph of an image analysis of normal Langerhans cells. Note the dendritic morphology of the cells. *B:* Effect of PUVA treatment on morphology of Langerhans cells as detected by image analysis.

The mechanisms by which UV irradiation or psoralens plus UVA affect the markers of LC are still unclear. It is possible that monofunctional and bifunctional psoralens have different affinities for these markers. Recently, it has been demonstrated that psoralens not only bind to DNA, but also to other structures in the cytoplasm and cell membrane [26]. Laskin et al [27] showed that psoralens bound to mammalian cells and activated by a low dose of UVA radiation have direct biologic effects on the cell surface membrane. The same biological effect, inhibition of epidermal growth factor binding, was also achieved by high doses of UVB radiation.

Although the effect of UV radiation on LC has been a point of interest for some time, its effects on Thy-1⁺ dEC is a relatively new area of investigation. In 1986, Aberer and associates [28] demon-

strated that low-dose UVB radiation (0.04 or 0.08 J/cm²) had no effect on the number of Thy-1⁺ dEC, whereas high doses of UVB (0.28 or 0.4 J/cm²) and PUVA caused an almost complete disappearance of these cells from murine skin. Okamoto and Kripke [29] also found that low-dose UVB radiation (0.096 J/cm²) had no effect on Thy-1⁺ dEC. Aberer et al found no effect of UVA alone on Thy-1⁺ dEC after a cumulative dose of 3.6 J/cm². We used higher doses of UVA and achieved significant depletion of Thy-1⁺ dEC. We also observed that complete elimination of Thy-1⁺ dEC from the skin is more readily achieved by UVB radiation than complete elimination of ATPase⁺ or Ia⁺ cells. Similarly, Orita [30] found that Thy-1⁺ dEC were depleted more than Ia⁺ cells by UVB radiation. Thus, chronic exposure to UV radiation with or without psoralens is associated with changes in the ratio of Thy-1⁺ and Ia⁺ dendritic cells in the epidermis and eventually leads to elimination of detectable Thy-1⁺ dEC from the epidermis. It is possible that these changes contribute to the development of skin cancers by perturbing cutaneous immune surveillance.

We and others have noticed that during UV radiation the morphology of the LC is changed. The main change, which is seen almost exclusively in ATPase-stained preparations, is loss of dendrites. In addition, the cell becomes round or oval and sometimes huge. Our data demonstrate that UVA, UVB, PUVA, or IPUVA all cause similar morphologic changes in LC. Because this alteration was scarcely detected by Ia staining, we hypothesize that ATPase on the dendrites of the LC is more sensitive to UV radiation than ATPase on the cell body and is destroyed on these structures first. Recently, Azizi et al [22] demonstrated similar morphologic changes in the LC of human epidermis overlying basal cell carcinomas. Whether rounding of the LC was caused by UV radiation or by the tumor itself was unclear. Our results, however, show that UV irradiation of mouse skin can decrease the dendricity of LC, as determined by ATPase staining.

In conclusion, our results show that despite the lack of phototoxicity, a monofunctional psoralen plus UVA radiation has the same ultimate effects as a bifunctional psoralen plus UVA on cutaneous immune cells. This conclusion should be kept in mind when monofunctional psoralens are considered for use as therapeutic agents in the treatment of dermatologic diseases. Second, differences between the number of ATPase⁺ and Ia⁺ cells after irradiation indicate that the absence of markers may not always reflect the absence of cells. Third, the fact that UVA alone affects cutaneous immune cells raises a question about the potential of artificial tanning devices to alter immune function. Further studies are needed to assess the significance of these changes in cutaneous immune cells on the immunologic function and integrity of the skin.

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