Quantitative and Morphologic Changes of Langerhans' Cells after Ultraviolet A Irradiation of Human Epidermis

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Three different surface markers (OKT6, HLA-DR, and adenosinetriphosphatase) were compared to identify Langerhans' cells, and the changes in number and morphology of these cells were studied at different intervals after irradiation of human skin by a 2.5-fold minimal erythema dose of ultraviolet A. Morphologic alteration and decreased surface-marker reactivity became evident on day 2 and were most pronounced on day 3 or day 4 (injury phase). The recovery phase started between day 4 and 1 week and was complete by 3 weeks. HLA-DR$^+$/OKT6$^-$ (DR$^+$T6$^-$) cells were present at all time intervals. The ratio of these cells to the sum of DR$^+$T6$^-$ and DR$^+$T6$^+$ cells was 0.3% before irradiation, reached a peak of 65.4% at day 4, and decreased to 0.6% by 3 weeks. *Key words: Adenosinetriphosphatase; HLA-DR; OKT6; Normal skin.* (Received August 10, 1986.)

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Our group previously found no significant differences among the three epidermal Langerhans' cell surface markers—OKT6, HLA-DR, and adenosinetriphosphatase (ATPase)—to identify Langerhans' cells (LC) in normal epidermis (1). However, in diseased states, the epidermis may be infiltrated by cells sharing with LC HLA-DR or ATPase (or both) surface markers (2). In diseased skin, the reliability of HLA-DR and ATPase to label LC is questionable. On the contrary, OKT6 is nonreactive with bone marrow cells and normal peripheral mononuclear cells and may be a particularly valuable marker for LC in diseased skin (2).

The effect of ultraviolet A radiation on LC has been relatively unexplored. Examining 1-μm epoxy-embedded sections by light microscopy, Gilchrest et al. (3) found that a 2.5-fold minimal erythema dose of ultraviolet A (MED$_A$) radiation reduced the percentage of LC at day 2 to approximately one-fifth of control value. However, vertically sectioned specimens are inferior to epidermal sheets for enumeration of LC (4), and they did not continue the examination beyond 2 days. Using ATPase stain on epidermal sheets, Aberer et al. (5) found morphologic and histochemical alterations of LC, which were most pronounced at 24 h after irradiation of more than 100 J/cm^2 of ultraviolet A; however, they did not examine the sheets after 24 h. Koulu et al. (6) noted no effect on LC on day 7 after giving up to 100 J/cm^2. The spectral distribution of their source of ultraviolet light A was such that the majority of ultraviolet light A given was >340 nm. In our study, we used double-labeling techniques for HLA-DR and OKT6 markers as well as separate ATPase staining on epidermal sheets to evaluate depletion and repopulation of LC at different intervals up to 3 weeks after irradiation with 2.5-fold MED$_A$.

METHODS AND MATERIALS

Subjects

Four healthy Caucasians with skin type I or II (two men and two women), 25 to 35 years of age, were enrolled in the study after giving informed consent. None of the subjects had a history of skin disease or photosensitivity.
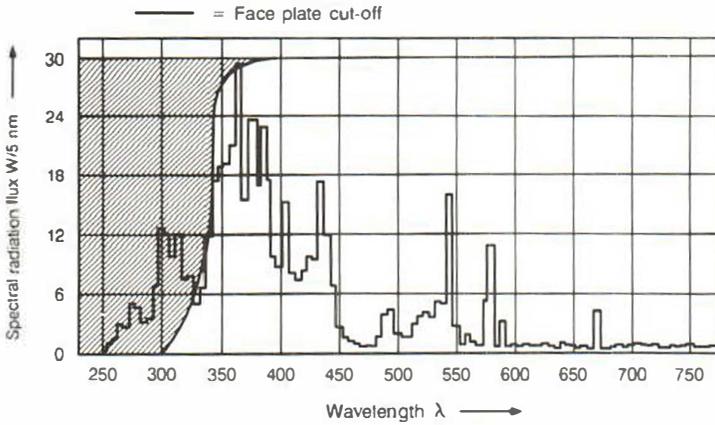
Fig. 1. Spectral emission of ultra-violet lamp used in the study.

Ultraviolet light source

A halide radiation lamp (National Biological Corp. Osran Ultramed 400 watts) was mounted in an irradiation unit (National Biological Corp. Hid 400 System) with a 3-mm thick window glass shield interposed between the source and the target. The wavelength spectrum of this glass-filtered ultraviolet radiation was between 300 and 800 nm, with a maximal output at 365 nm (spectral distribution curve provided by the manufacturer, Fig. 1). A 6.25-mm thick window glass shield was inserted for the additional elimination of ultraviolet B radiation. At a distance of 30 cm from the treatment area, the irradiance of ultraviolet B was 0.0004 mW/cm^2 as measured by an IL 433 radiometer. At the same distance, the irradiance of ultraviolet A was 12 mW/cm^2 as measured by an IL 441 UVA radiometer.

Experimental protocol

The MED was determined by exposing the skin of the right buttock to 20% increments of ultraviolet A doses starting from 25 J/cm^2 (3). Erythema was evaluated visually at 24 and 48 h. Scores were $+$=minimally perceptible with indistinct borders; $1+$=pink-red and macular; $2+$=fiery red-purple and macular; and $3+$=edematous. Subsequently, an area of 8 cm^2 of the opposite buttock was exposed to a 2.5-fold MED dose of ultraviolet A (average MED_A=50 J/cm^2).

Six-millimeter punch biopsy specimens from the first two volunteers were obtained from the left buttock immediately before exposure to ultraviolet A (0 hours) and at 1 day, 2 days, 3 days, 4 days, 1 week, 2 weeks, and 3 weeks. Biopsy specimens were not taken from the other two volunteers at 1 and 4 days. The samples were immediately snap-frozen in liquid nitrogen and stored in a freezer less than 1 week before processing.

Table I. *Reduction of OKT6, HLA-DR, and adenosinetriphosphatase positive cells[a] at different time intervals after irradiation in four volunteers*

Interval between exposure and biopsy	OKT6-positive cells/mm^2	HLA-DR-positive cells/mm^2	Percentage of DR$^+$T6$^-$ cells[b]	ATP-ase positive cells/mm^2
0	749 ± 197	751 ± 200	0.3	746 ± 175
1 day[c]	641 ± 157	658 ± 160	2.6	632 ± 159
2 days	219 ± 117	269 ± 73	18.6	198 ± 100
3 days	46 ± 59	83 ± 74	44.6	84 ± 66
4 days[c]	28 ± 11	81 ± 12	65.4	27 ± 7
1 week	221 ± 88	297 ± 72	25.6	146 ± 24
2 weeks	366 ± 102	390 ± 85	6.2	316 ± 120
3 weeks	650 ± 215	660 ± 198	0.6	483 ± 145

[a] Mean ± standard deviation.

[b] Percentage is number of DR$^+$T6$^-$ cells to sum of DR$^+$T6$^-$ and DR$^+$T6$^+$ cells times 100.

[c] Performed on first two volunteers only.
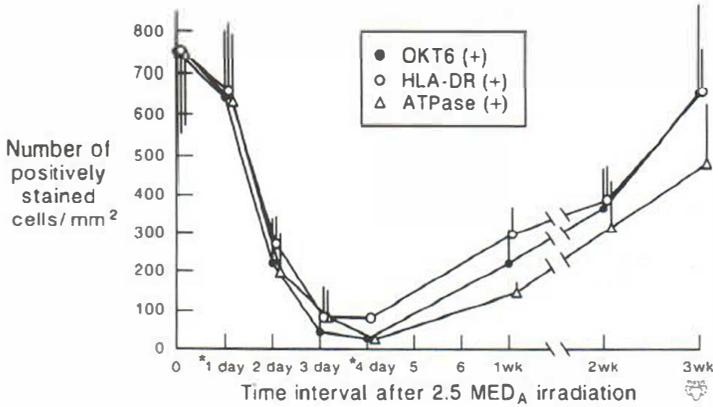
Fig. 2. Number of OKT6, HLA-DR, and ATPase-positive cells at different time intervals after 2.5-fold minimal erythema dose of ultraviolet A radiation. $n=4$; M ± SD shown. Asterisk refers to study performed on two volunteers only.

Staining technique

Epidermal sheets were obtained by incubating specimens in buffered edetate (EDTA) solution, as previously described (7, 8). Each 6-mm epidermal sheet was bisected. One half of the epidermal sheet was used for parallel double stains; the other half for modified ATPase stain. The technique was performed as follows. Epidermal sheets were overlaid with mouse anti-human HLA-DR serum (Becton Dickinson) at a dilution of 1 : 51 at 37°C for 1 h. After two 10-min rinses in Veronal buffer, the sheets were overlaid with rhodamine-conjugate goat anti-mouse immunoglobulin (Cappel Laboratories) at a dilution of 1 : 26 at 37°C for 1 h. For controls, normal mouse serum or mouse anti-human antimyoglobulin antibody were substituted for mouse anti-human HLA-DR serum. Specimens were rinsed twice for 10 min in Veronal buffer and overlaid with fluorescein isothiocyanate (FITC)-labelled mouse anti-human OKT6 serum (Ortho Pharmaceutical Corp.) at a dilution of 1 : 22 at 37°C for 1 h. For controls, FITC-labelled anti-mouse serum was substituted for FITC-labelled mouse anti-human OKT6 serum. Frozen sections were stained by the same procedure as above, except for a shorter incubation time (30 min) and a shorter rinse time (10 min). Finally, the specimen was immersed in Veronal buffer for 30 min and mounted with Veronal buffer glycerin and 1% *p*-phenylenediamine. LC were counted and photographed under an epi-fluorescent microscope (Leitz, Otholux 11, 200 Watts light pressure mercury vapor illumination) using appropriate excitation and barrier filters.

Modified ATPase stain

One half of the epidermal sheet was also used for ATPase stain. Other steps follow those previously described in detail (9, 10), except for reduced concentration of ATP (31.25 mg/dl) and $Pb(NO_3)_2$ (1 ml for each 10 ml of ATP).

Counting method and statistical method

Each individual specimen stained with OKT6, HLA-DR, and ATPase was measured with a calibrated ocular grid (net micrometer disc 1 mm^2/400 squares, 1490 A, American Optics). The cells that were OKT6 positive and HLA-DR positive were counted in 10 fields at a magnification of ×500. ATPase-positive cells were also counted in 10 fields at a magnification of ×400. Tukey's method was employed for statistical evaluation of data.

RESULTS

Tissue from the first two volunteers had morphologic alterations and changes in surface-marker reactivity which became evident by day 2 and were most pronounced by day 3 or 4 after irradiation. Therefore, biopsy specimens at days 1 and 4 for the other two volunteers were not taken.

There were no significant differences ($p > 0.05$) among the three surface markers used to enumerate LC at different time intervals after 2.5-fold MED_A irradiation, except at 1 week ($p < 0.05$) (Table I and Fig. 2). HLA-DR phenotype seemed to be reexpressed earlier than OKT6 and ATPase.

A control experiment in which mouse serum or mouse anti-human myoglobulin were sub-
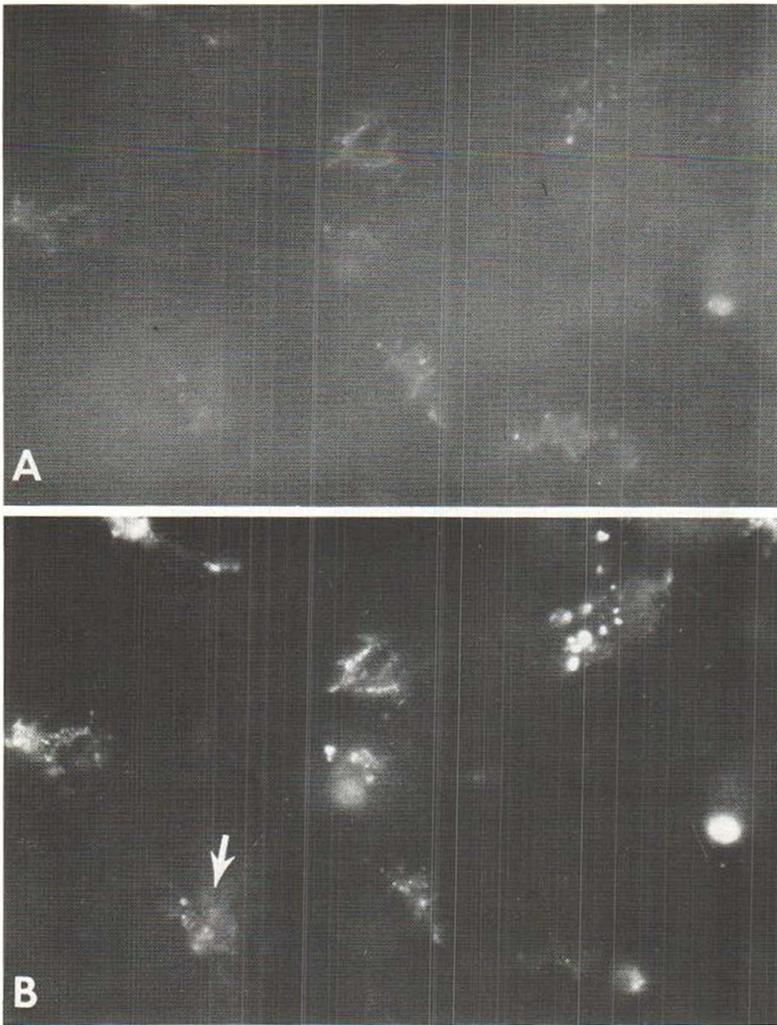
Fig. 3. Two days after 2.5-fold minimal erythema dose of ultraviolet A radiation. The dendritic processes were either grossly attenuated or absent. *A*, FITC-labelled OKT6-positive cells. (×12.5.) *B*. Rhodamine-labelled HLA-DR-positive cells. One DR^+T6^- cell is seen (*arrow*). (×11).

stituted for anti-HLA-DR serum and FITC-labelled anti-mouse serum for anti-OKT6 did not have positive staining.

By fluorescent microscopy, the morphologic appearance of LC significantly changed at day 2 (Fig. 3); this change became most profound at day 3 or 4. The dendritic processes were either grossly attenuated or absent during this period. The recovery phase started by 1 week and was complete by 3 weeks. At this time, most OKT6, HLA-DR, ATPase-positive cells were dendritic in shape. The morphologic change of positively stained cells was relatively parallel to the change in number.

$HLA-DR^+/OKT6^-$ $(DR^+/T6^-)$ cells were present at all time intervals, but their numbers reached a peak at day 4 and diminished to almost 0% at 3 weeks.

DISCUSSION

Statistically significant differences could not be demonstrated among surface markers (OKT6, HLA-DR, and adenosinetriphosphatase) to label LC on human skin after treatment

with 2.5-fold MED_A. The exception occurred only at 1 week. However, significant variation in the density of LC (11) among our volunteers may have rendered the statistical evaluation insignificant.

While the total number of cells diminished significantly after treatment, there was a dramatic increase in the ratio of DR^+T6^- cells to the sum of DR^+T6^+ cells and DR^+T6^- cells in the epidermis after radiation, with a peak of 65% at day 4 and a steady decrease to 0.6% at week 3. The origin of the DR^+T6^- cells is not clear. Cooper et al. (12) found 0.1% DR^+T6^- cells in epidermal cell suspension immediately after ultraviolet light treatment and a striking increase to 5.7% by day 3. Cooper et al. (12) suggested that DR^+T6^- cells are bone-marrow-derived or of dermal-derived monocyte-macrophage lineage because of the presence of OKM5 marker, a strong ability to phagocytize melanin, and the lack of appearance of DR^+T6^- cells when epidermal cells were placed in culture after ultraviolet light radiation. Because of the presence of cells that are not likely to be of Langerhans' cell origin in the skin treated with ultraviolet light, we suggest that OKT6 is a more specific surface marker to identify LC in the diseased skin.

The depletion of LC after ultraviolet light treatment is dose-related (5, 13). The effect of ultraviolet light on Langerhans' cell density was difficult to compare using variably designed treatment schedules. By giving 2.5-fold MED_A to the volunteers' buttocks, Gilchrest et al. (3) demonstrated that the progressive reduction in LC induced by ultraviolet A was approximately one-fifth of control values at 48 h. Our study showed less reduction, nearly one-third. This discrepancy may be due to different methods of assessing LC. Gilchrest et al. (3) identified the cells on the vertical section by their relatively clear ample cytoplasm with occasional dendrites, distinctive convoluted nucleus, and prominent nucleolus. We assessed LC by labelling them with different surface markers on the epidermal sheet.

Obata & Tagami (13) found incomplete recovery of cell number but a restoration of regular distribution of cells that were HLA-DR positive after 14 days for both ultraviolet B (200 mJ/cm^2 of 300 nm) and psoralen-ultraviolet A (1 J/cm^2 of 360 nm) treatment groups. However, Toews et al. (14) found that the number and morphology of LC returned to normal about 2 weeks after radiation with ultraviolet B (FS-20 sunlamp 10 mJ/cm^2 for 4 consecutive days). These differences suggest that the recovery rate is also dose-related. Friedmann et al. (15) found that the LC returned to normal by 3 weeks after cessation of psoralen-ultraviolet A therapy. We are not able to find equivalent studies regarding 2.5-fold MED_A effect on the reappearance of LC. In our study, the injury phase persisted at least 96 hours and the recovery phase began between day 4 and 1 week and was completed by 3 weeks.

The negative results reported by Koulu et al. (6) could have been due to differing spectral emission of ultraviolet light A; our light source likely had ultraviolet light A <340 nm. Also, they may have not noted an effect on LC at 7 days because the recovery phase may have occurred.

We have shown that the density and morphology of human LC can be dramatically altered by exposure to erythrogenic doses of ultraviolet A and recovery seems to occur over a period of 3 weeks. These studies are important to our understanding of the function and alteration of the LC by natural and therapeutic sources of ultraviolet radiation. It may be that the near and far ends of the ultraviolet light A spectrum can affect LC differently. This aspect will need to be reviewed carefully.

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