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UVA II Exposure of Human Skin Results in Decreased Immunization Capacity, Increased Induction of Tolerance and a Unique Pattern of Epidermal Antigen-Presenting Cell Alteration

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ABSTRACT

The risks incurred from increased exposure to UVA II (320–340 nm) (*i.e.* during sunscreen use and extended outdoor exposure, tanning parlors) are not well understood. Therefore, we explored the effects of UVA II on skin immune responses in humans. After a single local exposure (4 minimum erythemal dose [MED]) using a xenon arc lamp filtered with a narrow bandpass filter (335 ± 5 nm full width at half maximum), individuals were contact-sensitized with dinitrochlorobenzene (DNCB) through a UVA II exposure site or through normal skin. UVA II induced a marked decrease in the magnitude of skin immune responses ($P < 0.0001$). The UVA II group had only 29% successful sensitizations, as compared to 83% in the control group. The percentage of individuals who remained tolerant to DNCB after two sensitizations was 23.6% for the UVA II-exposed group, as compared to 3.8% in the controls ($P = 0.006$). UVA II also uniquely altered the type of antigen-presenting cells present in the epidermis. Human leukocyte antigen (HLA)-DR$^+$ cells in control epidermal cell suspensions (C-EC) comprised a single, homogeneous population of Langerhans cells (LC) with the phenotype: CD1a^{hi} DR^{mid} CD11b$^-$ CD36$^-$ (1.5 ± 0.3% of EC). UVA II irradiation reduced the number of such LC to 0.6 ± 0.2% of EC. Although cells expressing the macrophage phenotype: CD1a$^-$ DR^{hi} CD11b$^+$ CD36$^+$ were increased in UVA II skin, relative to C-EC, these comprised only 10.1 ± 6.1% of the DR$^+$ cells, which is less than that after UVB exposure. Also distinct from UVB, a third population was found in UVA II-EC, which exhibited a novel phenotype: CD1a$^+$ DR$^+$ CD36$^+$ CD11b$^+$; these comprised 11.1 ± 6.9% of the DR$^+$ UVA II-EC.

In conclusion, despite the above differences in infiltrating DR$^+$ cells, both UVB and UVA II reduce the skin's ability to support contact sensitization, induce active suppression (tolerance) and induce a reduction in LC.

INTRODUCTION

In addition to the well-publicized increases in UVB due to ozone depletion, the potential for greater exposure to UVA is also increasing. The major deleterious effects of UVB wavelengths are well established. Clinically, these include the discomforts of sunburn, accelerated aging and wrinkling of chronically exposed skin, and the well-established relationships between UVB exposure and skin cancer. Immunologically, UVB exposure creates an immunosuppressed state in the host that increases susceptibility to skin cancers (1–4) and microbes (5–7), which appears due to alterations in presentation of the antigen to antigen-specific T lymphocytes (2,8). These phenomena are nicely modeled by the contract sensitivity model of inducing antigen-specific delayed-type hypersensitivity, in which unresponsiveness and tolerance to antigens initially presented through UVB-exposed skin of both mice (9,10) and humans (11,12) is demonstrated to be due to T cells that block subsequent attempts to immunize through UV-irradiated skin (tolerance) (8,13,14). Mechanistically UVB depletes epidermal Langerhans cells (LC)† from the epidermis (15,16); however, the wavelength dependence of LC alteration and the wavelength dependence of contact sensitivity suppression are not the

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†*Abbreviations:* APC, allophycocyanin; DNCB, dinitrochlorobenzene; DPCP, diphenylcyclopropenone; EC, epidermal cells; EMA, ethidium monoazide; FITC, fluorescein isothiocyanate; HLA, human leukocyte antigen; LC, Langerhans cells; MED, minimal erythemal dose; PBS, phosphate-buffered saline; UVA I, 340–400 nm radiation; UVA II, 320–340 nm radiation.

same: UVA I (340–400 nm) depletes LC in mice and humans (16–18) but does not induce distant immunosuppression, in contrast to UVB (19–21), and does not create a distant (systemic) immunological susceptibility to UV skin cancer (22). Whereas UVB induces epidermal infiltration of neutrophils (23) and immunosuppressive macrophages (24–28), UVA I is unable to induce leukocyte infiltration (29) and allows a rapid recovery of LC function (30). Other biological effects of long-wave UVA (UVA I) appear to be distinct in many ways from those of UVB (31). These effects include: 1000-fold less biological activity, less carcinogenic potential (32) and differential effects on lipid mediators (33–35), cytokine induction (36,27), DNA (38) and proteases (39,40).

The effects of the UVA II (320–340 nm) wavelengths are less well studied and somewhat controversial (38). However, the frequency and intensity of exposure is increasing due to a number of factors. Many people are increasing their use of sunscreen products. This may result in an increase in their exposure to UVA, from 1) incomplete UVA protection by sunscreens designed to protect against UVB and from 2) longer exposure times allowed by the sunscreen before visible sunburn is apparent. Others increase their exposure to UVA by frequenting tanning parlors where UVA II radiation is a major component of the output of the lamps used. Many sun-sensitive people do both, in search of a "safe" means of obtaining a cosmetically attractive "healthy tan." Furthermore, stratospheric ozone performs a filtering function on solar UVA II, albeit not to the same degree as to UVB and UVC, and ozone depletion may increase its irradiance (41).

The purpose of this study was to determine whether UVA II differs from UVB in its ability to suppress epicutaneous immunization with dinitrochlorobenzene (DNCB) through exposed skin of humans, to determine whether UVA II is able to induce tolerance to subsequent DNCB immunizations on normal skin, and to determine whether UVA II is able to induce the influx of $CD1a^-$ $CD36^+$ DR^+ epidermal macrophages such as those that infiltrate epidermis following UVB irradiation of human skin.

MATERIALS AND METHODS

Volunteers. Paid volunteers were recruited, were screened for skin type and health status, gave signed informed consent and were treated using Institutional Review Board approved procedures forms and protocols. Subjects were randomly assigned to control and to UVA-irradiated groups. The selected volunteers were limited to skin types I, II or III, were free from chronic disease and were not currently using medication.

UVA II light source. Ultraviolet A II radiation was supplied by a 5000 W xenon arc lamp (Optical Radiation Corporation, Azusa, CA) mounted in a modified Kratos lamp housing fitted with an IR-absorbing H_2O filter (Spectral Energy Corporation, Westwood, NJ), a UV-reflecting dichroic mirror (~270–520 nm) and a narrow bandpass filter (335 nm, 10 ± 2 nm full width, half maximum) (Andover Corporation, Salem, NH). Lamp output was monitored before and after each exposure, using an International Light IL-435 phototherapy radiometer fitted with a UVA filter on an SED 1240 detector (International Light, Inc., Newburyport, MA). Spectral output of the narrow bandpass filters used was checked using a Kratos 250 mm grating monochromator (Spectral Energy Corporation, Westwood, NJ), using an IL-770 radiometer fitted with an SED 400 detector, QNDS (broad spectrum) filter and W diffuser.

Ultraviolet B was provided by a bank of FS-20 sunlamps with output as previously described (12,25,42).

The UVA II source stability was monitored with an IL-435 phototherapy radiometer fitted with an SED 240 detector fitted with a UVA filter and wide angle window. The UVB source was monitored with an IL-435 phototherapy radiometer fitted with an SED 240 detector fitted with a UVB filter and wide angle window.

Irradiation. The minimal erythemal dose (MED) for UVA II was determined for each volunteer to enable delivery of biologically equivalent doses of UVR. The MED were determined by giving each subject a sequence of four exposures of increasing intensities, 10, 20, 30 and 40 J/cm^2. Four MED of UVA II were subsequently administered to an area of 2.5 cm by 5 cm on the left buttock of each volunteer in the irradiated group.

Sensitization. On the third day after irradiation, a 12 mm Finn chamber containing 48 μL (30 μg) of 0.0625% DNCB in acetone was placed on the irradiation site (left buttock). A similar chamber containing 48 μL (22.5 μg) of 0.0469% diphenylcyclopropenone (DPCP) in acetone was placed on the unirradiated right buttock as a control for unimpaired immune response and as a control for distant suppression. An equal number of unirradiated control volunteers was also sensitized on similar sites. The chambers remained in place on the skin for 48 h(121).

Challenge. Two to three weeks after sensitization, the skin fold thickness of the challenge sites was measured with an engineers' spring-loaded micrometer (Mitutoyo Manufacturing, Tokyo) and Finn chambers containing reduced quantities of the sensitizing chemicals were applied to the inner upper arms of each volunteer and left in place for 6 h, as modified from the method of Friedmann *et al.* (43). On the right arm, one chamber contained vehicle control and four contained increasing concentrations of DNCB (3.125 μg, 6.126 μg, 8.8 μg and 12.5 μg). An additional four chambers containing the control chemical DPCP (0.390 μg, 0.781 μg, 1.56 μg and 3.125 μg) were placed on the left arm (12).

Two days later, the intensity of the hypersensitivity response was determined by again measuring the skin fold thickness of the challenge sites. The difference between the before challenge and after challenge measurements was expressed as increase in skin thickness in mm. A visual evaluation of the response was also recorded using the National Allergic Contact Dermatitis Group (NACDG) scale (12).

Epidermal cell (EC) isolation. For evaluation of UVA II effects on epidermal antigen-presenting cell population density and surface antigen profile, volunteers were irradiated with 4 MED UVA II as described above. Three days following irradiation, UVA II-irradiated skin and unirradiated control skin samples were obtained from each volunteer by keratome biopsy and EC suspensions were prepared as previously described (42). Briefly, the epidermis was separated from the dermis by overnight incubation with Dispase® (Collaborative Research, Waltham, MA) and EC suspensions obtained by incubation for 10 min in 0.125% trypsin in phosphate-buffered saline (PBS). The action of the trypsin was stopped by transfer of the epidermis to PBS containing 20% fetal calf serum plus 0.05% DNase to remove DNA released from damaged cells. The tissues were gently agitated to disperse the EC and cell clumps were removed by filtration through 112 mesh NYTEX® nylon screen (42).

Staining to identify EC phenotypes. Aliquots of EC suspensions were preincubated for 30 min at 4°C with mouse IgG1, mouse IgG2 (Sigma) and normal human AB serum, at 20 μL each per 10^6 cells. The cells were then stained with specific antibodies directly conjugated to the fluorochromes (or to biotin in the case where the streptavidin allophycocyanin [APC] secondary fluorochrome was used) at 2 μg/10^6 cells for 45 min at 4°C, washed three times and the cells incubated with APC for 30 min. After one wash, ethidium monoazide (EMA) was added to each sample (to identify nonviable cells), incubated under fluorescent lamps for 10 min (23,44), washed again and fixed in formaldehyde for flow cytometric analysis. Epidermal cell phenotypes were identified by four-color flow cytometry (42).

Viable EC (EMA negative) were selected based on low EMA intensity (lack of uptake of EMA by viable cells). The specific antibodies used for obtaining EC subpopulations were: PE anti-human leukocyte antigen (HLA)-DR (Ig2a isotype) and PE anti-CD11b (IgG1 isotype) both from Becton Dickenson, San Jose, CA. The PE anti-CD1a (T6 RD1) from Coulter, Hialeah, FL; fluorescein isothio-

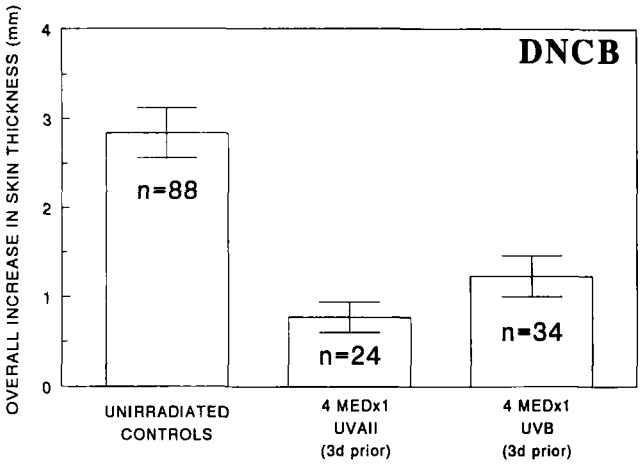

Figure 1. Four MED UVA II (335 ± 5 nm) reduces the mean response to DNCB sensitization in humans to a level equal to that produced by a 4 MED UVB exposure. A single exposure of either UVA II or UVB given 3 days prior to sensitization significantly reduces the response to DNCB sensitization as measured by the mean increase in skin thickness (mm) at challenge (y axis). Unirradiated controls, mean = 0.284 ± 0.28 mm, n = 88; UVA II-irradiated, mean = 0.77 ± 0.17 mm, n = 24; and UVB-irradiated, mean = 1.23 ± 0.23 mm, n = 34. For UVA II-irradiated subjects *vs* control subjects, $P = 0.000014$; for UVA II-irradiated *vs* UVB-irradiated are not significantly different, $P = 0.144$; for UVB-irradiated *vs* control, $P = 0.000113$.

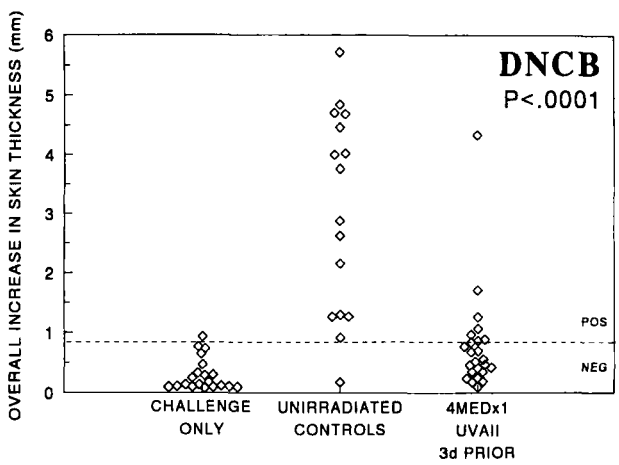

Figure 2. Scattergram reveals relatively homogenous reduction of DNCB response in human subjects sensitized on UVA II-exposed skin. A single exposure of UVA II (335 ± 5 nm) given 3 days prior to sensitization significantly reduces the response to DNCB sensitization as measured by the mean increase in skin thickness (mm) at a challenge site (y axis). Challenge only, mean = 0.301 ± 0.06 mm, n = 20; unirradiated controls, mean = 3.05 ± 0.043 mm, n = 16; UVA II-irradiated, mean = 0.775 ± 017 mm, n = 24. The response of the UVA II-irradiated subjects differed significantly from that of the unirradiated control subjects, $P < 0.0001$.

cyanate (FITC) anti-Cd11b (AMAC, Westbrook, ME), FITC anti-CD1a (OKT6), FITC anti-CD36 (OKM5) (both, Ortho, Raritan, NJ), and biotinylated CD11c (Sigma, St. Louis, MO) were all of IgG1 isotype. Biotinylated anti-HLA-DR, with isotype IgG2a, was from Becton Dickenson. The HLA-DR$^+$ cells were selected from among the viable cells based upon intensity of APC staining. Displaying the HLA-DR$^+$ population of cells on histograms whose axes displayed the intensity of staining for a pair of the other antigens allowed identification of cell populations that were single positive, double positive or triple positive for the antigens being analyzed.

RESULTS

Effects of UVA II radiation on human sensitization to DNCB

Like UVB, a single 4 MED dose of UVA II, given 3 days prior to sensitization with DNCB, strongly suppresses the contact sensitivity response in humans, as measured by the mean increase in skin fold thickness (edema) in mm upon DNCB challenge (Fig. 1). The unirradiated control subjects were pooled from concurrent and historical controls for comparison to both the UVB and UVA II groups. The increase in mean thickness was 2.84 ± 0.28 mm, n = 88, for the unirradiated control group. The mean thickness increase for the UVA II-irradiated subjects was 0.775 ± 0.17 mm, n = 24, a significant reduction ($P = 0.000014$) upon comparison to the unirradiated controls. As previously described (12), the mean increase in skin fold thickness for the UVB-irradiated subjects, 1.23 ± 0.23 mm, n = 34, was also significantly different from the controls, $P = 0.00013$. The difference between the UVA II-irradiated group and the UVB group was not significant, $P = 0.14$. Thus, at 4 MED, UVA II is as effective as UVB in inducing suppression of the contact hypersensitivity response to DNCB.

Because heterogeneity of the human population has raised the possibility of low and high susceptibility subsets that may correlate with skin cancer susceptibility (11), we also examined the data set for the presence of distinct groups, using a scatter plot (Fig. 2). The data for the unsensitized challenge group are also presented for comparison to the concurrently randomized positive control group (DNCB through normal skin) and the UVA II group. Whereas the mean irritant response to DNCB (challenge only) was 0.301 ± 0.006 mm, n = 20, the positive control group demonstrated a mean response of 3.053 ± 4.25 mm, with 93.8% positive responses. By contrast, there were only 29.2% positive responses in the UVA II-irradiated, mean = 0.775 ± 0.17 mm, n = 24, $P = 0.02$. Although 2 of the 24 subjects exhibited a fully successful sensitization through UVA II-exposed skin, it is not clear whether this percentage reflects a distinct subgroup cluster.

Effect of UVA II on an immunization through a site distant to the UVA II exposure

Each volunteer was sensitized on a matching site on the opposite (right) buttock with a second, noncross-reacting sensitizer (DPCP) to test for distant (systemic) effects of the UV radiation exposures. As for DNCB, the response to DPCP sensitization was measured by the mean increase in skin thickness (mm) at challenge sites (Fig. 3). The DPCP is less efficient at sensitizing than is DNCB, sensitizing fewer than 75% of the unirradiated volunteers in the groups being presented, yet the DPCP response was frequently stronger than the DNCB response, often resulting in blisters at the challenge sites, which made measurement difficult. The challenge doses of DPCP were adjusted downward in some of the groups during the study, in an attempt to alleviate the blistering problem. Only the volunteers receiving the original dosages are included here, which explains the smaller numbers of volunteers shown. For the challenge only

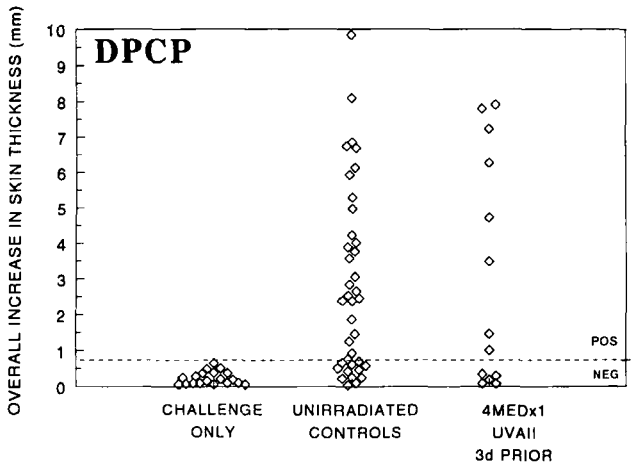

Figure 3. *Scattergram of DPCP responses in human subjects sensitized distant from UVA II-exposed skin reveals distant immunosuppression.* The DPCP sensitization on the buttock opposite to the DNCB sensitization was measured by the mean increase in skin thickness (mm) at a challenge site (shown on the y axis). Concurrently randomized challenge only negative controls, mean = 0.241 ± 0.04 mm, n = 19; unirradiated positive controls, mean = 2.54 ± 0.36 mm, n = 36; UVA II-irradiated subjects, mean = 3.15 ± 0.89 mm, n = 13.

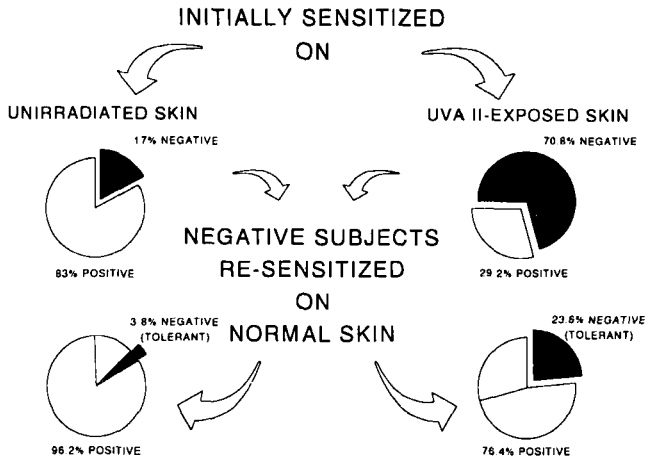

Figure 4. DNCB sensitization through a UVA II-exposure site causes decreased responsiveness and increased tolerance in 24% humans tested. The DNCB sensitization on UVA II-exposed skin resulted in 70.8% of individuals being unresponsive to challenge after the initial sensitization. These were then resensitized on normal skin. Of these individuals, 23.8% were again unresponsive to DNCB sensitization. By contrast, among individuals not receiving UVA II exposure yet unresponsive to initial DNCB sensitization (17%), only 3.8% were still negative after resensitization on normal skin.

group, the mean skin fold thickness increase was 0.19 ± 0.06 mm, n = 20; for the unirradiated controls, the mean skin fold thickness increase was 2.88 ± 0.41 mm, n = 38. The mean skin fold thickness increase was 3.15 ± 0.89 mm along the UVA II-irradiated volunteers (n = 13).

Toleragenic effects of UVA II

To determine whether sensitization on UVA II-exposed skin induces tolerance to subsequent exposure to DNCB, individuals who did not respond to challenge after the initial sensitization (70.8%) were resensitized on normal skin 2 weeks to 5 months later. Of these individuals, 23.8% were again unresponsive to DNCB sensitization, whereas, among unirradiated control individuals unresponsive to DNCB sensitization (17%), only 3.8% were still negative after resensitization on normal skin. This difference is significant, P = 0.0006.

Effects of UVA II radiation on HLA DR$^+$ epidermal cell subsets

Ethidium monoazide-negative viable cells were selected, and from them cells staining intensely with APC-HLA-DR were gated and selected for further analysis (Fig. 5a,e). Both the unirradiated EC (1) and the UVA II-irradiated EC (b) contained distinct populations of HLA-DR$^+$ cells. The EC stained with the isotype control contained only a negligible amount of background staining within the comparable areas (not shown).

The HLA-DR$^+$ cells in unirradiated control epidermis were homogeneously CD1a^{hi} CD11c^{lo} (Fig. 5b), CD36$^-$ (Fig. 5c) and CD11b$^-$ (Fig. 5d). By contrast, UVA II-irradiated epidermis contained a heterogeneous population of DR$^+$ cells. One population was represented by reduced LC (CD1a^{hi}, CD11c^{lo}, CD36$^-$ CD11b$^-$ cells in Fig. 5f circle F; 5g box G; 5h box H). A second population could be identified within an HLA-DR$^+$ CD11c^{hi} non-LC population that was CD1a$^-$ (Fig. 5f box I with 14.5% cells) relative to 0.0% cells that are DR$^+$ CD11c^{hi} CD1a$^-$ in control skin (Fig. 5b box L). In contrast to LC, which were CD36$^-$ CD11b$^-$, these HLA-DR$^+$ CD11c^{hi} cells in UVA II-irradiated EC expressed macrophage markers and were thus CD36$^+$ (Fig. 5g circle J) and CD11b$^+$ (Fig. 5h circle K).

Data pertaining to the above cell populations (illustrated in Fig. 5) were pooled from multiple subjects (Table 1). The HLA-DR$^+$ portion of the viable EC was reduced by the irradiation from 1.5% of EC in the controls to 0.8% of EC in the UVA II-treated group (P = 0.005). Among the HLA-DR$^+$ cells, the CD1a$^+$ cells were reduced by UVA II from 90.1% of DR$^+$ cells in controls to 75.0% of the lowered DR$^+$ cells within UVA II-EC. The CD1a$^+$ LC expressing CD36 represented 0.8% of LC in control skin but rose to 11.1% of DR$^+$ cells in UVA II-expressed skin. Within the expanded CD1a$^-$ population, CD36$^+$ monocytic/macrophagic cells rose from <0.0% to 10.1%, bringing the total CD36$^+$ population to 18 ± 3% of DR$^+$ UVA II-EC (not shown). Likewise, CD11b$^+$ monocytic/macrophagic cells increased from 3.2% in DR$^+$ cells in controls to 23.7% of DR$^+$ cells in the UVA II-treated group. The identities of the various subsets, as well as their quantitative relationships, are summarized in Fig. 6 as means of grouped data from multiple subjects. Each shared shading designation denotes a specific subset and whether it expresses the indicated marker (Fig. 6).

DISCUSSION

Ultraviolet A II has many biological activities in common with UVB. Ultraviolet A II is more biologically active than UVA I and highly relevant doses may be acquired during outdoor exposure as well as during tanning parlor exposure. Particularly high UVA II doses may occur when outdoor exposure times are increased due to the use of UVB-absorb-

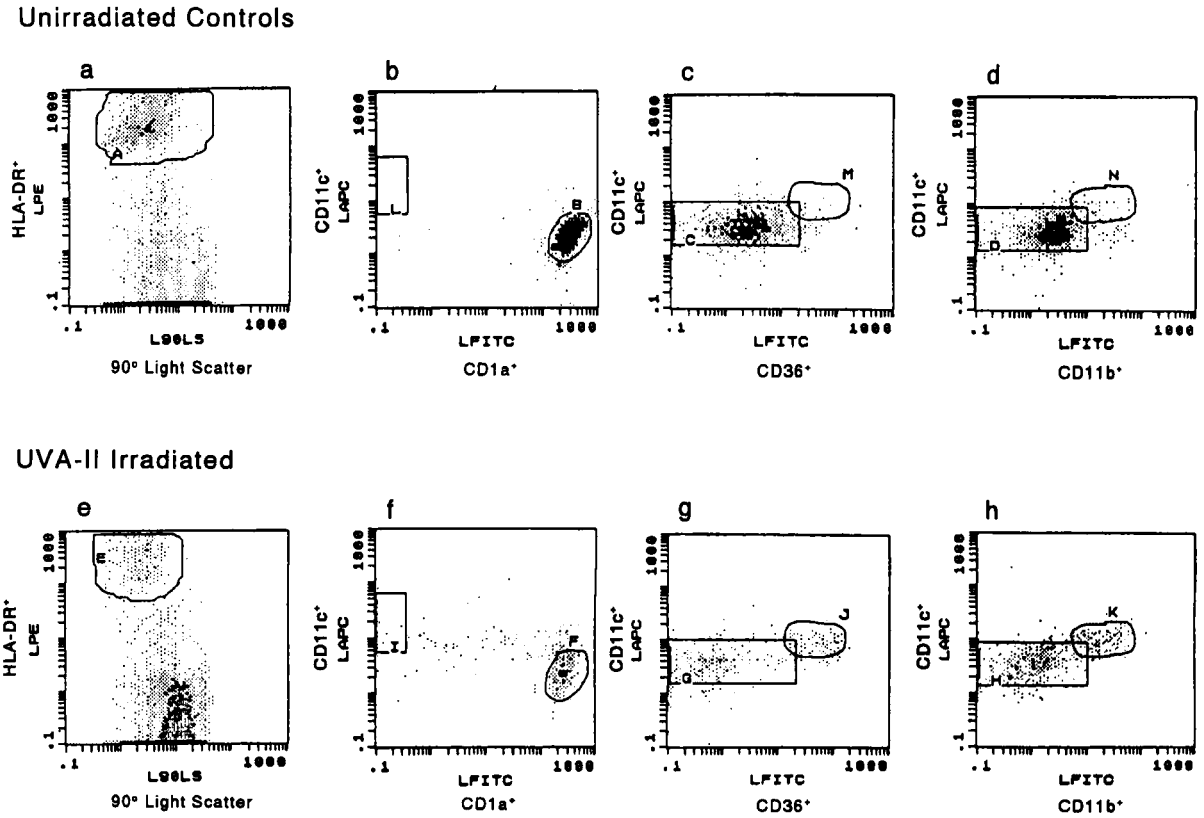

Figure 5. Ultraviolet A II irradiation induces a population of macrophage-like CD1a$^-$, CD36$^+$, CD11b$^+$ among the HLA-DR$^+$, CD11c$^+$ epidermal cells in human skin, similar to that induced by UVB. Four-color flow cytometric analysis shows contrasting patterns of cell phenotypes between UVA II-irradiated and unirradiated control skin. Frames a and e show selection of HLA-DR$^+$ cells in both groups. The HLA-DR$^+$ cells were then analyzed for CD11c, CD1a, CD36 and CD11b. Frame b (unirradiated control skin) shows a distinct population of CD1a$^+$ cells (gate B) but no CD1a$^-$ cells (gate L) among the CD11c$^+$ cells. In UVA II-irradiated skin (frame f), with the same selection, CD1a$^+$ cells are reduced in number (gate F) and there is a small, strongly CD1a$^-$, CD11c^{hi} population seen (gate I). There are insignificant numbers of CD36$^+$ cells (frame c, gate M) and CD11b$^+$ cells (frame d, gate N) in the unirradiated controls; however, the UVA II-irradiated cells clearly show the presence of both CD36 (frame g, gate J) and CD11b (frame h, gate K), both of which are CD11c^{hi}.

ing sunscreens. In addition, because UVA II intensity at the earth's surface is attenuated by stratospheric ozone, especially those wavelengths in the shorter, more energetic portion of this waveband, ozone depletion is predicted to result in increased biologic activity of UVA II wavelengths reaching the earth (45). The ability of UVA photons to alter the dose–response relationship of UVB on EC (46), and the active role of UVA II in photocarcinogenesis (47–50), in immunologic alteration (15,17,19,20,37,51,52) and in processes active in photoaging (18,33,39,40,53–55) makes it imperative to understand the actions of UVA II in human skin *in vivo*.

We report here that UVA II has immunologic effects on human skin, which exhibit a distinctive pattern that shares some features with UVB exposure effects and some features with UVA I exposure effects. The human skin immunologic response to UVA II is similar to UVB in that there is: (a) a reduced ability to support contact sensitization; (b) a capacity to induce active suppression of DNCB responsiveness (tolerance); (c) a reduction in the number of LC (phenotype:

Table 1. UVA II* depletes CD1a$^+$ DR$^+$ LC but concomitantly induces minor populations of CD1a$^-$ DR$^+$ cells expressing CD36 and/or CD11b

Waveband	% of EC HLA-DR$^+$	% of DR$^+$ CD1a$^+$	CD1a$^+$ CD36$^+$	CD1a$^-$ CD36$^+$	CD36$^+$ CD11b$^+$	CD11b$^+$
Control	1.5 ± 0.3†	90.1 ± 1.4‡	0.8 ± 0.50§	0.0 ± 0.0§	0.2 ± 0.2§	3.2 ± 1.1‡
UVA II	0.8 ± 0.1	75.0 ± 4.2	11.1 ± 6.9	10.1 ± 6.1	8.6 ± 1.9	23.7 ± 8.7

*All skin was tested 3 days after 4 MED UV irradiation.
†Percentage of total (viable) epidermal cells expressing the indicated phenotype (mean of n = 22, ±SEM).
‡Percentage of DR$^+$ cells expressing the indicated phenotype (mean of n = 10, ±SEM).
§Percentage of DR$^+$ cells expressing the indicated phenotype (mean of n = 4, ±SEM).

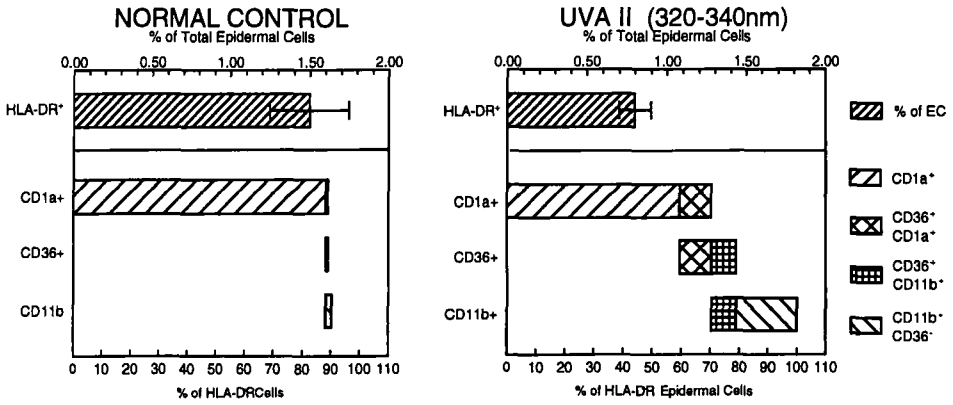

Figure 6. Proportional relationships of HLA-DR$^+$ subsets in epidermis from normal (a) and UVA-II (b)-exposed skin. Data expressed as % of total EC for HLA-DR-expressing cells (top panels) or as % of HLA-DR$^+$ EC for cells expressing CD1a, CD36 or CD11b. From triple color analysis, cell populations are shown as distinct bar shadings to indicate subsets of the following phenotypes: 1) CD1a$^+$ CD36$^-$ CD11b$^-$ DR$^+$; 2) CD1a$^+$ CD36$^+$ CD11b$^-$ DR$^+$; 3) CD1a$^-$ CD36$^+$ CD11b$^+$ DR$^+$; 4) CD1a$^-$ CD36$^-$ CD11b$^+$ DR$^+$.

CD1a$^+$ DR$^+$ CD36$^-$ CD11b$^-$) and, (d) epidermal infiltration with cells of macrophagic phenotype (CD1a$^-$ DR$^+$ CD36$^+$ CD11b$^+$). Ultraviolet A II differs from UVB in that it induces the appearance of a minor population of LC with a unique phenotype: CD1a$^+$ DR$^+$ CD36$^+$ CD11b$^+$ and results in a less potent depleting effect on CD1a$^+$ DR$^+$ LC than does UVB.

It is not clear at this point whether the immunosuppressive effects of UVA II on contact sensitization induction in human subjects is due predominantly to the UVB-like effects or to the UVA-like effects. Ultraviolet A II is considered to contain both UVB and UVA I photons and the biologic activity of this waveband may be the result of a balanced or combinatorial effect of both types of photons, without an overwhelming dominance of one over the other (38,56). Thus, study of this waveband may actually reflect the balance of processes that occur in response to the more intense mix of UVA I and UVB photons that occur in natural sunlight.

The dose of UVA II used in the current study, 4 MED, resulted in a highly suppressed immune response to DNCB in 90% of the subjects tested (Fig. 2). This effect is at least as, and possibly more suppressive than an equally erythemogenic single dose of UVB (Fig. 1), after which only about 60% of subjects are highly suppressed using fluorescent sunlamps (12).

Altered immunization *in vivo* is likely related to the changes observed in antigen-presenting cell composition of the epidermis after UVA II exposure. Ultraviolet A II clearly reduced LC density to levels comparable to similar doses of UVA and UVB. Ultraviolet A I and UVB both reduce LC density (57,58), but UVA I results in only a very transient inhibition of LC functional activity, with extremely rapid recovery (30). Because LC that have newly repopulated the epidermis or in atopic dermatitis skin can doubly express CD1a and CD36 (59,60) and normal human LC in the dermis can doubly express CD1a and CD11b (42), it is possible that the novel population of CD1a$^+$ cells expressing CD36 and CD11b in UVA II skin are repopulating immigrants from the dermis or blood (Fig. 4) (Table 1) (Fig. 6, double diagonal cross hashes).

With regard to macrophage infiltration (Table 1) (Fig. 6, double vertical horizontal cross hashes and single wide diagonal hashes) the degree of infiltration is markedly less after UVA II exposure ([10.1% CD36$^+$ CD1a$^-$ cells among DR$^+$ UVA II EC] $\times$ [0.8% DR$^+$ cells among total UVA II EC] = 0.08% of all UVA II EC that are macrophagic cells) (Table 1) than after UVB exposure (3–15% of all UVB EC that are macrophagic cells) (24,61) and in the same range as after UVA I exposure ([33% CD36$^+$ among DR$^+$ UVA I EC] $\times$ [0.3% DR$^+$ among total UVA] $\approx$ 0.1% of all UVA I EC that are macrophagic cells) (16). Whether these epidermal changes are reflected in the dermis, as they are after UVB (61), remains to be determined but may be a critical element dictating the outcome of immunization through UVA II-exposed skin.

In summary UVA II, the shorter wavelength, more energetic component of the UVA waveband, is clearly immunosuppressive. Its unique features of LC depletion with induction of LC of novel phenotype and without a high degree of macrophagic induction is more reminiscent of UVA I than UVB. In distinction to UVA I, however, UVA II induced tolerance to the contact sensitization, whereas murine UVA I studies have indicated an inability of UVA I to induce tolerance, despite LC depletion (19,20). These findings indicate that conditions of increased UVA II exposure, such as during prolonged outdoor exposure under sunscreen, or tanning parlor exposure or exposure to stratospheric ozone-depleted solar radiation, will result in cutaneous immunosuppression to normally immunogenic agents introduced through the exposed skin.

We report here that a single erythemagenic exposure to UVA II can reduce the immunization rate and induce tolerance to a potent immunogen such as DNCB. The appearance of complex mixtures of cells with antigen-presenting cell potential in the UVA II-exposed epidermis is further evidence that UVA II, although distinct in certain aspects from UVB, shares critical immunosuppressive activities with UVB.

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