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Ultraviolet AI exposure of human skin results in Langerhans cell depletion and reduction of epidermal antigen-presenting cell function: partial protection by a broad-spectrum sunscreen

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Summary

Background Ultraviolet (UV) B-induced effects on the skin immune system have been extensively investigated, but little is known regarding the immunological changes induced by UVA exposure of human skin. Recent data assessing the protection afforded by sunscreens against photoimmunosuppression stress the need for broad-spectrum sunscreens with an adequate UVA protection.

Objectives The purpose of this study was first to determine the changes observed in epidermal Langerhans cells (ELC) density and epidermal antigen-presenting cell (APC) activity after exposure of human skin to UVAI (340–400 nm) radiation, and secondly to assess the immune protection afforded *in vivo* by a sunscreen formulation containing a long wavelength UVA filter with a low UVA protection factor (UVA-PF = 3).

Methods Epidermal cell (EC) suspensions were prepared from skin biopsies 3 days after exposure to a single dose of UVAI (either 30 or 60 J cm^{-2}).

Results Flow-cytometric analysis of EC suspensions revealed that exposure to 60 J cm^{-2} UVAI resulted in a decreased number of ELC without infiltration of CD36+ DR+ CD1a− antigen-presenting macrophages into the epidermis, and a significant reduction of HLA-DR expression on viable ELC. *In vivo* exposure to both 30 and 60 J cm^{-2} resulted in a decreased allogeneic CD4+ T-cell proliferation induced by UVAI-irradiated ECs. The sunscreen application partially prevented (57 ± 9%) the decrease in epidermal allogeneic APC activity induced by 60 J cm^{-2} UVAI.

Conclusions *In vivo* UVAI exposure of human skin results in a decreased number of ELC and in a downregulation of epidermal APC activity. This last effect is partially prevented by prior application of a sunscreen with a low UVAI-PF value. These results indicate that increasing the absorption of UV filters for long UVA wavelengths may lead to an improved immune protection.

Key words: photobiology, photoimmunology, photoprotection

The solar ultraviolet (UV) radiation spectrum that reaches the earth is composed of intermediate wavelength UVB (290–320 nm) and long wavelength UVA (320–400 nm) radiation, which are arbitrarily divided into UVAII (320–340 nm) and UVAI (340–400 nm). UVB alters antigen-presenting cell (APC) function directly by affecting epidermal Langerhans cells (ELC) or indirectly by inducing keratinocytes to release immunomodulatory cytokines.[1,2] Hence, UVB exposure of human skin results in a dose-responsive decrease in immunization rates and promotes tolerance to epicutaneous antigens.[3] This UVB-induced immune unresponsiveness may play an important part in skin carcinogenesis by reducing host resistance to tumour growth.[4] The molecular mechanisms underlying the effects of UVA differ from UVB and lead to particular photobiological effects.[5] Indeed, UVA irradiation contributes to photoageing, triggers polymorphic light eruption and is thought to be less carcinogenic than

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UVB.[5] The amount of UVA radiation that reaches human skin is much greater than that of UVB, and exposure may increase due to tanning parlours, phototherapy and the use of sunscreens with high sun protection factor (SPF) and incomplete UVA protection, that may result in longer exposure times to sunlight. Little is known regarding the immunological changes induced by UVA exposure of human skin. In contrast with UVB, UVA depletes ELC[6] but does not reduce immunization rates.[7] However, the immunological effects of UVA seem to be wavelength dependent, as the exposure of human skin to UVAII (320–340 nm) results in decreased immunization capacity and increased induction of tolerance.[8] The purpose of this study was first to determine the changes observed in LC density of ELC and epidermal APC activity after exposure of human skin to UVAI radiation, and secondly to assess the immune protection afforded *in vivo* by a sunscreen formulation containing a long wavelength UVA filter.

Subjects and methods

Subjects

The study was undertaken from January to June 1999 after approval of the French Ethics Committee. The age of male volunteers ($n = 24$) ranged between 21 and 48 years (mean ± SD 27 ± 7 years) (15 phototype II and nine phototype III). Females were excluded because of the reduced cutaneous immune responsiveness that may occur in menstruating women.[9]

Ultraviolet A irradiation conditions

The radiation source used throughout this study was a 3000-W Jetsun 55 UVA lamp (Cosmed, Bagnolet, France). The emission spectrum (330–450 nm, maximum at 380 nm) (Fig. 1) was determined with a spectroradiometer MACAM 3010 (MACAM Photometric Limited, Livingston, U.K.) and contained 0·7% UVAII radiation. Two sites on the buttocks of each volunteer ($n = 7$) were irradiated with single doses of 30 and 60 J cm^{-2}, respectively. Seven subjects were irradiated on one site with a single dose of 30 ($n = 3$) or 60 J cm^{-2} ($n = 4$). According to the UV climatology proposed by Sabziparvar *et al.*,[10] a dose of 60 J cm^{-2} UVA is within the range of that received in Europe by a sunbather exposed for some hours during a normal July afternoon. To evaluate the photoprotection afforded by the sunscreen, 10 subjects were exposed to a dose of UVAI corresponding to 60 J cm^{-2}. Irradiance was 490 mW cm^{-2} at a distance of 17 cm. Keratotome biopsies were performed on non-irradiated and UV-irradiated sites 3 days after UV exposure.

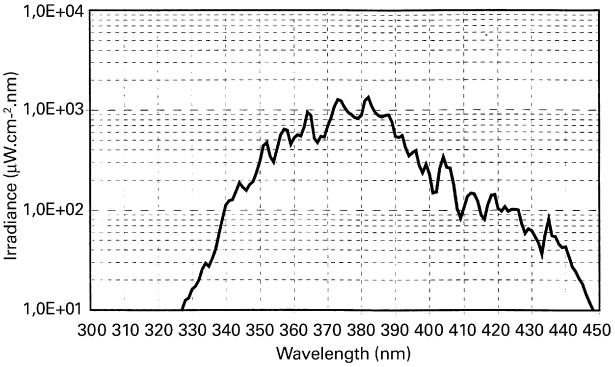

Figure 1. Emission spectrum of Jetsun 55 UVA lamp.

Sunscreen

The sunscreen cream tested (L'Oreal, Clichy, France) was an oil-in-water emulsion containing 7% octocrylene (UVB filter) and 3% butyl methoxydibenzoylmethane (long wavelength UVA filter) (Fig. 2). With such a composition, the absorption spectrum of the sunscreen cream covered the entire UV range. The sunscreen and the vehicle (Veh) preparations (2 mg cm^{-2}) were applied on two different sites on the buttocks 20 min before UV exposure ($n = 10$). The UVAI-PF was determined for each volunteer ($n = 9$) by using the persistent pigment darkening method[11] after irradiation with the UVAI light source.

Antibodies

The panel of monoclonal antibodies (mAbs) employed was: fluorescein isothiocyanate (FITC)-conjugated

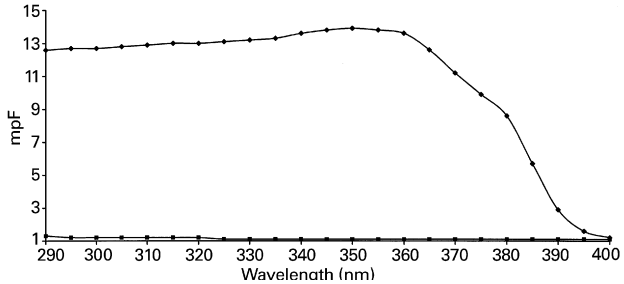

Figure 2. Ultraviolet absorption spectrum of vehicle (■) and sunscreen (◆). Monochromatic protection factors (mPF) were obtained by spectrophotometric measurements of the formulation applied as a thin layer (2 mg cm^{-2}) on Transpore tape.

anti-CD36 (Immunotech, Marseille, France), phycoerythrin (PE)-conjugated antihuman leucocyte antigen (HLA)-DR (Sigma, St Louis, MO, U.S.A.) and FITC anti-CD1a (CLB, Amsterdam, the Netherlands). Streptavidin Tricolor (SA-TC) (200 μg mL^{-1}) (Caltag, Burlingame, CA, U.S.A.) was used as a marker of non-viable cells.[12] Appropriate isotype controls included FITC mouse IgG1 (Caltag), PE mouse IgG2a (Caltag) and FITC mouse IgG2b (Caltag).

Epidermal cell suspensions and flow-cytometric analysis

Keratotome specimens were obtained under aseptic conditions and placed in Dispase (50 U mL^{-1}) (Collaborative Research Inc., Boston, MA, U.S.A.) overnight at 4 °C. The epidermis was removed and teased into a cell suspension after brief trypsinization as described previously.[13] Flow cytometry was performed using a FACScan (Becton Dickinson, Mountain View, CA, U.S.A.) equipped with an air-cooled argon laser. Forward light scatter and 90° light scatter were used for gating out debris. Light scatter, FITC (FL1), PE (FL2) and SA-TC (FL3) fluorescence were collected on 4-decade logarithmic scales. Data for 30,000 events were stored in a Listmode archive file. Listmode data were analysed using Cellquest software (Becton Dickinson).

Isolation of purified resting antigen-presenting cell-depleted CD4+ T cells

Allogeneic peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation and then washed three times with phosphate-buffered saline and 1% fetal calf serum. Twenty million PBMC were incubated with 1 mL of an antibody cocktail: anti-HLA-DR (Caltag; 1 : 20), anti-CD8 (Caltag; 1 : 50), anti-CD14 (Caltag; 1 : 50) and anti-CD20 (Caltag; 1 : 50). Positively stained cells were removed from the PBMC preparation by incubation with magnetic beads coated with goat antimouse IgG (Dynabeads M-450, Dynal, Oslo, Norway) at a ratio of three beads per cell, as previously described.[13] The purity of the resulting APC-depleted, resting CD4+ lymphocytes was verified by fluorescence-activated cell sorter staining to be $>95\%$ (data not shown).

Functional assessment of alloantigen-presenting capacity of epidermal cells

Eighty thousand epidermal cells (EC) were mixed with 50,000 allogeneic purified CD4+ T lymphocytes in round-bottomed microtitre wells for 7 days at 37 °C in 5% CO_2−95% air. T-cell proliferation was measured over the last 18 h of culture by adding 1 μCi of [^{3}H]thymidine ([^{3}H]TdR) (ICN Biomedicals, Orsay, France) to each well. Cells were then harvested on a cell harvester and the [^{3}H]TdR incorporation was measured on a Packard scintillation counter. CD4+ T cells were unresponsive to concanavalin A (10 mmol L^{-1}) (Sigma).

Statistical analysis

The Wilcoxon signed ranks test was used to compare cell numbers and fluorescence intensities. The Spearman rank-order correlation coefficient was used to compare UVAI-PF values with percentages of protection against immunosuppression.

Results

Ultraviolet AI irradiation and Langerhans cells

Keratotome biopsies were performed 3 days after UVAI irradiation because UVB-induced epidermal CD1a− CD36+ DR+ macrophages reach a maximum number 72 h after a high UVB exposure.[14] Unirradiated EC (C-EC), 30 J cm^{-2} UVA-irradiated EC (UV30-EC) and 60 J cm^{-2} UVA-irradiated EC (UV60-EC) contained a unique and homogeneous population of CD1a+ DR+ ELC that was negative for the CD36 monocyte/macrophage marker. Furthermore, no CD36+ DR+ cells were detected within non-irradiated and UVA-exposed epidermis ($n = 8$). The percentage of CD1a+ DR+ cells among UV60-EC ($0{\cdot}69 \pm 0{\cdot}14\%$) ($n = 8$) was significantly reduced compared with that of C-EC ($1{\cdot}27 \pm 0{\cdot}25\%$) ($n = 8$) ($P < 0{\cdot}03$), while the number of ELC after 30 J cm^{-2} exposure remained unchanged ($0{\cdot}95 \pm 0{\cdot}14$) ($n = 8$) ($P > 0{\cdot}1$). Similarly, the percentage of viable CD1a+ DR+ SA-TC− cells among UV60-EC ($0{\cdot}42 \pm 0{\cdot}17\%$) ($n = 8$) was reduced compared with that of viable ELC among C-EC ($0{\cdot}86 \pm 0{\cdot}25\%$) ($n = 8$) ($P < 0{\cdot}05$), whereas there was no difference after exposure to 30 J cm^{-2} ($0{\cdot}5 \pm 0{\cdot}17$) ($n = 8$) ($P > 0{\cdot}1$). A representative cytogram is shown in Figure 3 and the means of grouped data from multiple subjects are summarized in Figure 4.

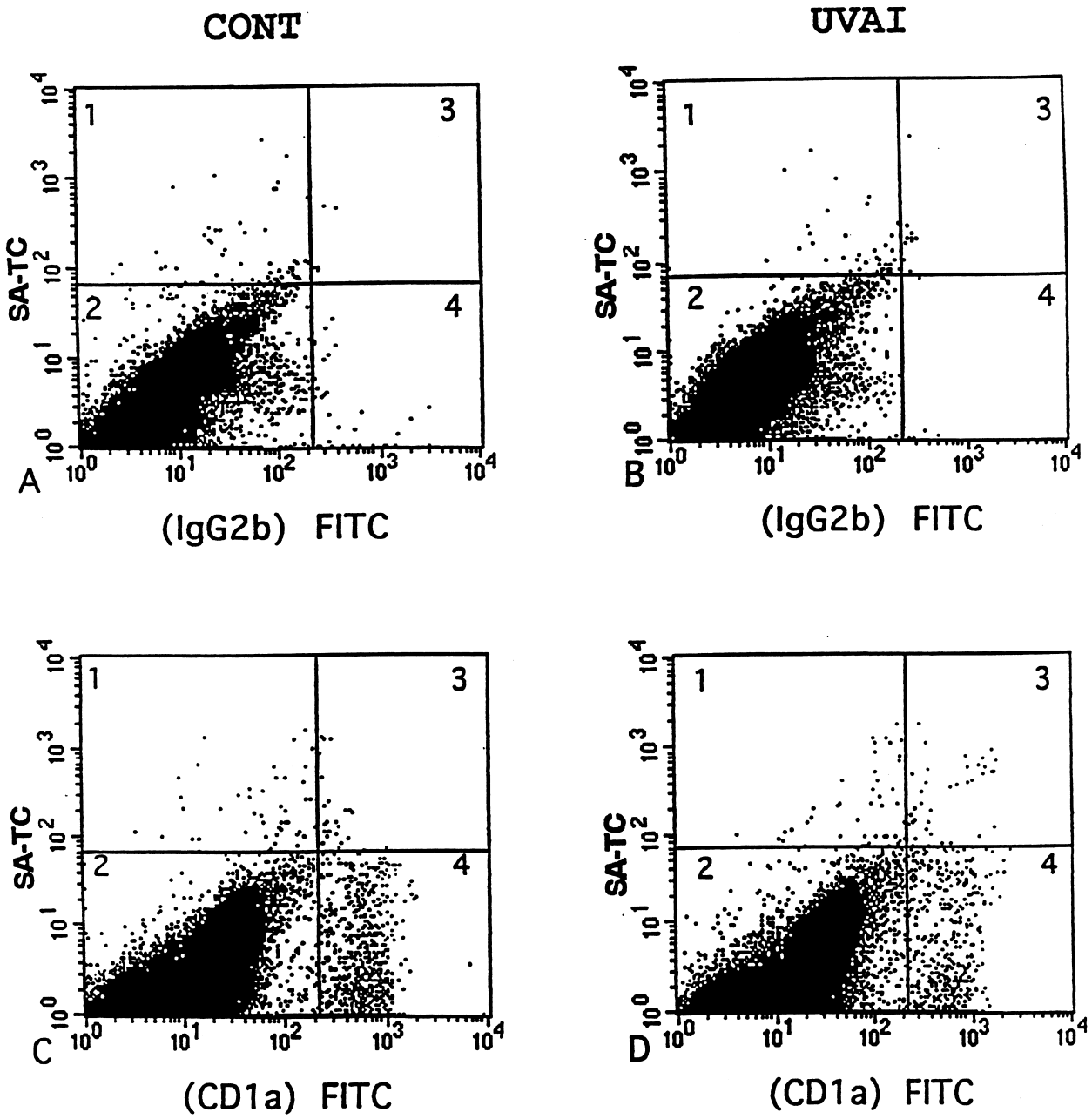

Figure 3. Flow-cytometric analysis of normal and ultraviolet AI (340–400 nm) (UVAI)-irradiated epidermal cells (ECs). EC suspensions were obtained from non-irradiated (CONT) and irradiated cutaneous sites 72 h after UVAI exposure at 60 J cm^{-2}. Specific staining was relative to mouse IgG2b; non-viable ECs were stained with Streptavidin Tricolor (SA-TC). After UVAI exposure, the CD1a+ SA-TC– Langerhans cell subset (1·4%) is reduced compared with that of non-irradiated epidermis (2·25%) (D vs. C, quadrant 4). FITC, fluorescein isothiocyanate.

Ultraviolet AI exposure and HLA-DR expression on viable epidermal Langerhans cells

Tricolor-labelled streptavidin coupled to Cy-5 (SA-TC) is a reliable marker for non-viable cells[12,15] and non-viable EC are responsible for the non-specific background to isotype controls binding,[12] indicating that the concomitant use of a marker of viability is necessary to determine precisely the mean fluorescence intensity (MFI) of specific mAbs on a cell subset. Thus, SA-TC negative viable ELC were electronically selected and gated for further analysis. The MFI of specific mAbs on viable EC (SA-TC–) was determined by subtracting the non-specific background (isotype controls) from the

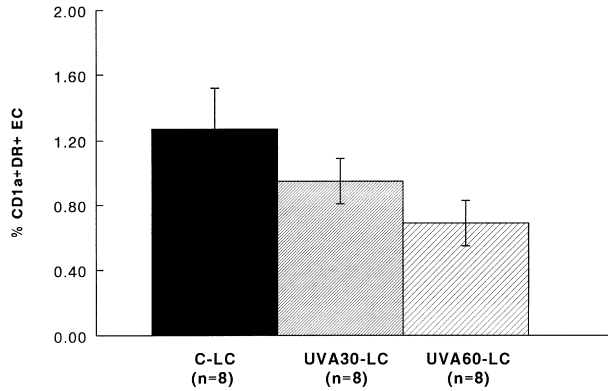

Figure 4. Decreased number of CD1a+ DR+ Langerhans cells (LC) in the epidermis 3 days after ultraviolet AI (340–400 nm) (UVAI) exposure at either 30 (UVA30-LC) or 60 J cm^2 (UVA60-LC). The mean percentage of epidermal LC was significantly reduced from 1·25 ± 0·25% (C-LC) to 0·7 ± 0·15% (UVA60-LC) after a single UVAI dose (60 J cm^{-2}) ($P < 0·03$; $n = 8$). The decreased number of LC after 30 J cm^{-2} (UVA30-LC) (0·95 ± 0·15%) was not statistically different from that of C-LC ($P > 0·1$; $n = 8$).

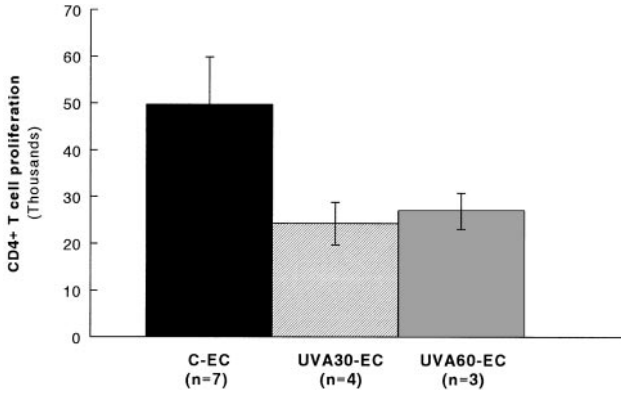

Figure 5. Decreased allogeneic CD4+ T-cell proliferation induced by ultraviolet AI (340–400 nm) (UVAI) exposure. UV-irradiated epidermal cells (EC) harvested 3 days after UVAI exposure at 30 J cm^{-2} (UVA30-EC) or 60 J cm^{-2} (UVA60-EC) and non-irradiated EC (C-EC) were used to stimulate allogeneic CD4+ T cells. T-cell proliferation was assessed by the amount of [^{3}H] uptake after 7 days culture. Results represent the mean ± SEM c.p.m. (× 10^3). Each experiment was performed in triplicate.

fluorescence intensity of SA-TC– cells stained with specific mAbs.[12,16] UVAI exposure at 60 J cm^{-2} resulted in a decreased MFI of HLA-DR (1268 ± 164) ($n = 6$) on CD1a+ SA-TC– ELC compared with that of non-irradiated skin (1802 ± 310) ($n = 6$) ($P < 0·03$), whereas MFI of HLA-DR on viable ELC was unaffected by a 30-J cm^{-2} UVAI exposure (1385 ± 286) ($n = 6$) ($P > 0·2$). The dose-dependent decrease of HLA-DR expression on viable ELC seems to be specific as MFI of CD1a on CD1a+ DR+ SA-TC– ELC was not modified by UVAI exposure (data not shown).

Ultraviolet AI exposure and epidermal alloantigen-presenting cell activity

Incubation of CD4+ T cells with UVA30-EC vs. C-EC revealed a decrease in the allogeneic T-cell proliferation in response to UVA30-EC relative to C-EC in each of the four subjects tested. T-cell proliferation induced by UVA30-EC (24209 ± 4553 c.p.m.) ($n = 4$) was reduced compared with that of C-EC (46891 ± 11990 c.p.m.) ($n = 4$). The percentage of decrease in c.p.m. of UVA30-EC relative to C-EC in each subject was 50%, 30%, 64% and 28%, respectively (mean ± SEM = 42·7 ± 8·5%) (Fig. 5). Similarly, T-cell proliferation induced by UVA60-EC (26887 ± 3880 c.p.m.) ($n = 3$) was reduced compared with that of C-EC (41597 ± 9083 c.p.m.) in each of the three subjects tested. The percentage decrease in c.p.m. of UVA60-EC relative to C-EC in each subject was 44%, 36% and 60%, respectively (mean ± SEM = 46·8 ± 7%) (Fig. 5).

Sunscreen application and protection from ultraviolet AI-induced reduction of epidermal alloantigen-presenting cell activity

Ten volunteers (mean ± SD age 28 ± 1·5 years, six phototype II and four phototype III) were irradiated with a single dose of UVAI corresponding to 60 J cm^{-2}. The epidermal APC activity was assessed 3 days later. T-cell proliferation induced by EC obtained from UV-irradiated and veh-treated sites (25001 ± 5095 c.p.m.) was significantly reduced (42 ± 4% of decrease) ($n = 10$), compared with that of EC obtained from vehicle-treated and non-irradiated sites (C-EC) (41548 ± 6849 c.p.m.) ($n = 10$) ($P = 0·005$; Wilcoxon test). The sunscreen formulation did not completely protect from UV-induced immunosuppression as the APC activity of sunscreen treated and UV-irradiated EC (33890 ± 6184 c.p.m.) was still reduced (18·6 ± 5% of decrease), compared with that of C-EC ($P = 0·02$) (Fig. 6). The percentage protection from immunosuppression by the sunscreen (PPIS) was determined by the following formula: [1 − (S-SS/S-Veh)] × 100, where S-SS represents the percentage suppression of alloreactivity in sunscreen-treated, UV-irradiated volunteers, and S-Veh is the percentage suppression in veh-treated and UV-irradiated volunteers.[17] The protection afforded by the sunscreen filters was partial (PPIS = 57 ± 9%, $n = 10$) but the mean UVAI-PF of the tested sunscreen as determined with our radiation source was relatively low (3 ± 0·2, $n = 9$). However, PPIS values were well

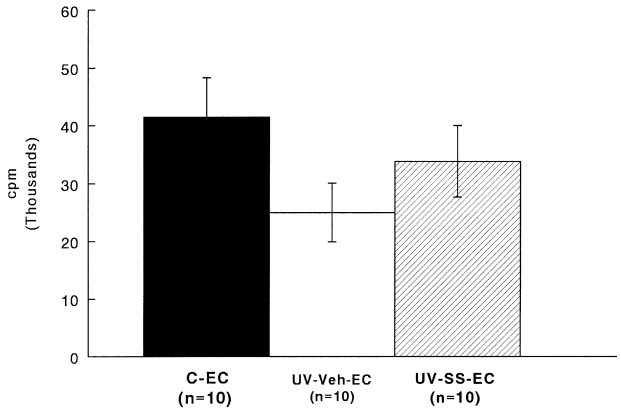

Figure 6. Partial protection afforded by a long wavelength ultraviolet (UV) A filter against the UVAI (340–400 nm) -induced downregulation of epidermal antigen-presenting cell activity. For each volunteer ($n = 10$), epidermal cells (EC) were harvested from non-irradiated and vehicle-treated cutaneous sites (C-EC) and from UVAI-exposed sites (60 J cm^{-2}) pretreated with either the vehicle (UV-Veh-EC) or the sunscreen (UV-SS-EC). EC were used to stimulate allogeneic CD4+ T-cell proliferation. Results are expressed as mean ± SEM c.p.m. ($\times 10^3$) of triplicate samples after 7 days culture.

correlated with those of UVAI-PF ($r_s = 0{\cdot}79$) (Spearman test).

Discussion

In vivo exposure of human skin to high UVB doses results in ELC depletion, and infiltration of antigen-presenting CD36+ DR+ CD1a− monocytes/macrophages into the epidermis.[14,18] These macrophages derive from transcapillary migration and from *in situ* proliferation of a dermal precursor.[19] They differ from ELC in their responsiveness to FcIgG receptor-mediated signalling,[20] in high production of interleukin (IL)-10[21] and in their inability as APC to induce early upregulation of T-cell IL-2Rα.[22] UV-induced macrophages (UV-Mph) are the dominant APC type within UVB-exposed epidermis[14] and they preferentially activate naive (suppressor-inducer) CD4+ T lymphocytes,[23,24] which may be critical for inducing immune tolerance in mice[25,26] and in humans.[3] The UV wavelengths for the induction of CD1a− DR+ UV-Mph predominantly lie within the UVB band.[6] In contrast to UVB, a high UVAI exposure results in a rapid recovery of ELC alloreactivity without induction of autoreactivity.[27] Similarly, erythemogenic doses of UVA radiation do not reduce the immunization rates to epicutaneous allergens,[7] although UVB and UVAII irradiation as well as solar-simulated UV exposure of human skin result in decreased immunization capacity.[8,28,29] Flow cytometric analysis of UV-irradiated epidermal cell suspensions failed to detect any CD36+ DR+ cells indicating that both CD1a− CD36+ DR+ UV-Mph and putative repopulating CD1a+ CD36+ DR+ dendritic cells from the dermis[8] do not infiltrate the epidermis after UVAI exposure. Moreover, our results indicate that a moderate *in vivo* UVAI exposure is sufficient to decrease the epidermal alloantigen presentation 3 days later. The altered APC activity after UVAI exposure may result from both the decreased number of ELC and the reduced expression of major histocompatibility class (MHC) class II molecules on residual ELC, as it occurs after *in vivo* UVB exposure.[30] The downregulation of MHC class II molecules on UV-irradiated resident ELC may be due to IL-10 production[31] as UVAI irradiation of human keratinocytes *in vitro* induces the release of IL-10[32] and *in vivo* UVAI exposure of human skin results in a slight increase in epidermal IL-10 concentration.[33] Alternatively, the release of *cis*-urocanic acid after UVAI irradiation[33] may account for reduced HLA-DR expression on UV-irradiated resident ELC.[34] Expression of accessory molecules that are essential for antigen presentation to T cells may also be modulated by UVA irradiation,[35,36] although recent data demonstrate that, in contrast with UVB radiation *in vivo*, UVAI irradiation does not interfere with the activation-dependent upregulation of accessory molecules such as B7 on ELC.[37] Reactive oxygen species generated by UVA may also play a crucial role in the suppression of the antigen-presenting function of ELC.[38,39] Our results indicate that UVAI shares immunosuppressive activities with UVB and UVAII, and thus may contribute to UV-induced immunosuppression in humans.

Although conflicting results have been reported on the protective effect of sunscreens against UV-induced immunosuppression, recent human data suggest that broad-spectrum sunscreens are necessary to prevent the decreased cutaneous immunity that occurs after solar-simulated exposure.[29,40–42] Our findings indicate that a UVAI filter does not completely protect from the reduced epidermal APC activity induced by an acute UVAI exposure. However, one should note that the UVAI-PF of our sunscreen, as determined with our radiation source, was relatively low ($3 \pm 0{\cdot}2$, $n = 9$) and a 60-J cm^{-2} UVAI dose may thus be beyond the absorption capacity of the sunscreen. Nevertheless, PPIS values were well correlated to UVAI-PF, suggesting that, at least for UVA, higher protection factors for long wavelengths may lead to an improved immune

protection. This should prompt manufacturers to increase efficacy of sunscreen products by reinforcing protection against UVAI radiation, especially in those containing UVB filters with high SPF values.

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