

Broad-Spectrum Sunscreens Provide Better Protection from the Suppression of the Elicitation Phase of Delayed-Type Hypersensitivity Response in Humans

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It is well established that ultraviolet radiation has immunomodulatory effects that may be involved in skin cancer. Recent studies have shown that ultraviolet A radiation (320–400 nm) as well as ultraviolet B (290–320 nm) is immunosuppressive. This means sunscreens that mainly absorb ultraviolet B (protection against erythema) may be less effective in preventing ultraviolet radiation-induced immunosuppression than broad-spectrum products. We have studied the effects of ultraviolet A exposure on the human delayed-type hypersensitivity response and compared the efficacy of sunscreens having different levels of ultraviolet A protection under both solar-simulated radiation and outdoor real-life solar exposure conditions. Delayed-type hypersensitivity was assessed using recall antigens. In a first study, two groups of volunteers were exposed to ultraviolet A (either full spectrum ultraviolet A or ultraviolet A1) without prior application of sunscreen and they were shown to exhibit significantly reduced delayed-type hypersensitivity responses. In order to compare the efficacy of sunscreens in preventing photoimmunosuppression, three groups of subjects received 10 cumulative exposures to solar-simulated radiation; one group was exposed unprotected and the other two were exposed after being applied either a ultraviolet B or a broad-spectrum sunscreen, each with the same sun protection factor 9, but with different ultraviolet A protection factors 9 and 2. Then, an outdoor study was conducted in which delayed-type hypersensitivity was assessed before and after six

daily exposures. Two different groups of subjects were treated with one of two sunscreens having the same sun protection factor 25 but different ultraviolet A-protection factors. In unprotected volunteers, responses to delayed-type hypersensitivity tests were significantly reduced irrespective of ultraviolet exposure conditions (full spectrum ultraviolet A, ultraviolet A1, solar-simulated radiation). The ultraviolet B sunscreen failed to protect from solar-simulated radiation-induced immunosuppression. In contrast, the broad-spectrum sunscreen having the same sun protection factor but providing high protection in the ultraviolet A range significantly reduced local ultraviolet-induced immunosuppression and prevented the distal effects. In the outdoor study, as compared with delayed-type hypersensitivity responses obtained before sun exposure, no alteration of immune response was detected when the skin was protected by broad-spectrum sunscreen sun protection factor 25 and ultraviolet A-protection factor 14. Conversely, a broad-spectrum sunscreen sun protection factor 25 ultraviolet A-protection factor 6 failed to protect against the sun-impaired response. The above studies clearly demonstrate the role of ultraviolet A in the induction of photoimmunosuppression together with the need for sunscreen products providing efficient photoprotection throughout the entire ultraviolet spectrum. **Key words:** human/photoimmunosuppression/sunscreens/ultraviolet A. *J Invest Dermatol* 117:1186–1192, 2001

Ultraviolet (UV) radiation has been shown to induce immunosuppression in humans (Hersey *et al*, 1983; Cooper *et al*, 1985; Kelly *et al*, 2000). Many studies have reported the effects of UVB (290–320 nm) radiation on immune responses such as contact hypersensitivity (CHS) or delayed-type hypersensitivity (DTH)

reactions to haptens, but relatively few studies have addressed the effects of UVA in humans (Hersey *et al*, 1983, 1987; LeVeé *et al*, 1997; Skov *et al*, 1997; Damian *et al*, 1999). Some authors have found that UVA had significant suppressive effects on CHS responses (Hersey *et al*, 1983; LeVeé *et al*, 1997), whereas others did not (Skov *et al*, 1997). Hersey *et al* (1987) reported that UVA induced suppression of DTH response to recall antigens (Multitest Pasteur/Mérieux). Damian *et al* (1999) showed that CHS to nickel was suppressed after one to three exposures to low UVA doses. Whereas commercially available sunscreens protected the skin from inflammatory responses (erythema and edema), some studies have shown that they provided little or no protection against UV-induced immune suppression (Ho *et al*, 1992; van Praag

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Abbreviations: SSR, solar-simulated radiation; UVA-PF, ultraviolet A protection factor.

Table I. The eight treatment groups used

Study no.	Group no.	No. of subjects ^a	Treatment	Cumulative dose
I	1	8 F, 4 M	No UV	
	2	6 F, 5 M	12 exposures Full spectrum UVA	352 J per cm ²
	3	9 F, 2 M	12 exposures UVA 1	352 J per cm ²
II	4	10 F, 5 M	10 exposures SSR	14.5 MED (SD = 2.3) 75 J per cm ² UVA (SD = 3.4)
	5	12 F	10 exposures SSR + sunscreen A	58 MED (SD = 4.5) 15 J per cm ² UVA (SD = 99.7)
	6	6 F, 5 M	10 exposures SSR + sunscreen B	58 MED (SD = 3.3) 315 J per cm ² UVA (SD = 63.6)
	7	8 F, 8 M	6 d of exposure Sunlight + sunscreen C	50.5 standardized MED 340 J per cm ² UVA
III	8	8 F, 8 M	6 d of exposure Sunlight + sunscreen D	50.5 standardized MED 340 J per cm ² UVA

^aF, female; M, male.

et al, 1991). Others reported data showing that sunscreens could completely prevent these effects (Wolf *et al*, 1993; Roberts and Beasley, 1997).

It is not established whether the sun protection factor (SPF) of a sunscreen can predict its ability to protect against photo-induced immune suppression (Young and Walker, 1999). It is possible that the degree of protection provided by a sunscreen not only depends on the SPF but also on the absorption spectrum and in particular, the absorbing potency it affords in the UVA range. Some studies seem to indicate this trend (Bestak *et al*, 1995; Damian *et al*, 1997; Serre *et al*, 1997; Fourtanier *et al*, 2000).

Here, we report studies in human volunteers to assess the role of UVA in eliciting immune suppressive effects. We also investigated whether broad-spectrum sunscreens were more effective than sunscreens absorbing UVB only. We assessed the effects of either UVA or solar-simulated radiation (SSR) or real sunlight on the elicitation phase of the DTH response to recall antigens (Multitest Pasteur/Mérieux).

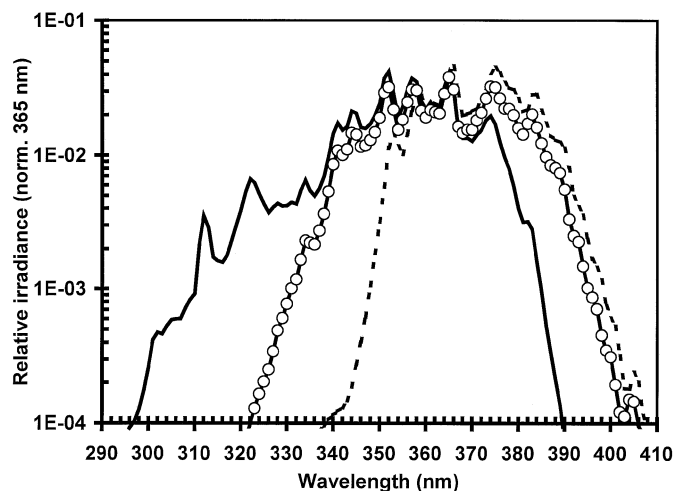
MATERIALS AND METHODS

Subjects Female and male Caucasian volunteers were recruited after study approval by an ethics committee. Study inclusion criteria included Fitzpatrick skin type II or III (Fitzpatrick, 1988), aged between 18 and 40 y with general good health. Exclusion criteria included disease conditions or medications causing immune suppression or the risk of photosensitization. None of the volunteers had experienced sun exposure for at least 4 wk prior to the study. Seventy-five subjects were recruited for the indoor studies. Three of them dropped out for personal reasons without any relation to the treatment. For the outdoor study, 32 subjects were included and all completed the test. In each experiment the volunteers were divided into groups of 11–16 volunteers with no sex randomization, excepted for the outdoor study in which each group included eight males and eight females (**Table I**).

In a first experiment (study I), the effects of full spectrum UVA (group 2) and UVA1 (group 3) were compared. Volunteers unexposed to UVA (group 1) served as controls to measure the reaction variability to Multitest antigens in the absence of UVA exposure.

In a second experiment (study II), we compared the ability of two SPF 9 sunscreens to prevent immunosuppression induced by SSR exposure (groups 5 and 6). A control group was exposed to SSR without applying sunscreen prior to exposure (group 4). In a third experiment (study III), two groups of 16 volunteers applied SPF 25 sunscreens before being exposed to outdoor real sun exposure (groups 7 and 8).

In each study, the volunteers were assembled after an initial Multitest on the back. They were then randomized into the different groups according to their initial reaction to the Multitest in order to obtain a similar average response between the groups excepted for study I group 3 which was added after the initial randomization.

**Figure 1. Emission spectra of the Uvasun5000 metal halide lamp.**

The UVA 1 (340–400 nm) spectrum (---) was obtained with one Schott WG 360/2 mm thick and two Schott UG5/3 mm thick filters. The full UVA (320–400 nm) spectrum (O—O) was obtained with one Schott WG 335/3 mm thick and two Schott UG5/3 mm thick filters. The SSR (290–390 nm) spectrum (—) was obtained with a short cut-off glass filter/8 mm thick and a Schott UG11/3 mm thick filter.

UV sources and dosimetry

UVA—Study I Two UVA spectra were obtained from a metal halide lamp (UVASUN 5000 Mutzhas, Munich, Germany) by using different filters: (i) a Schott WG335/3 mm thick filter (Clichy, France) and two Schott UG5/3 mm thick filters that delivered a 320–400 nm radiation spectrum (UVA), or (ii) a Schott WG360/2 mm thick and two Schott UG5/3 mm thick filters delivering a 340–400 nm spectrum (UVA1). The spectra are shown in **Fig 1**.

SSR—Study II The source used to compare two SPF 9 sunscreens (see Results and **Table II**) was also the metal halide lamp (UVASUN 5000 Mutzhas, Munich, Germany) but equipped with filters to give a spectrum between 290 and 390 nm: the filters were a Schott UG11/3 mm thick filter (Clichy, France), and a Mutzhas custom made short cut-off glass filter/8 mm thick (Munich, Germany).

Indoor dosimetry The spectral power distribution for the laboratory UV radiation sources was measured with a calibrated spectroradiometer Macam 3010 (Macam, Livingston, U.K.). The output was monitored with a Centra OSRAM (Berlin, Germany) radiometer equipped with UVB and UVA sensors.

Table II. UV filters in sunscreen products A, B, C, D and protection factors determined in humans

Study no.	Products	UV filters combination	SPF	UVA-PF
II	A	9% OC + 2% PBSA + 0.7% TDSA + 2% BMDM	9 (1.3)	9 (1.3)
	B	9% OC + 1% PBSA	9.3 (1.6)	2.1 (1.3)
III	C	10% OC + 0.5% TDSA + 2.5% BMDM + 1.5% DT + 4%TiO ₂	25 ^a	13.7 (2.8)
	D	4-MBC + OT + BMDM + TiO ₂	25 ^a	5.9 (1.4)

^aSPF labeled on the packaging. Mean (SD). Number of volunteers =10–12; OC, Octocrylene or Uvinul® N539; PBSA: phenylbenzimidazole sulfonic acid or Eusolex R232; TDSA, terephthalylidene dicamphor sulfonic acid or Mexoryl® SX; BMDM, butyl methoxydibenzoylmethane or Parsol® 1789; DT, drometrizole trisiloxane or Mexoryl® XL; 4-MBC, 4-methyl benzylidene camphor or Eusolex R6300; OT, octyl triazone or Uvinul RT150; TiO₂: titanium dioxide.

Natural sunlight Two sunscreens SPF25 were compared in a study performed in outdoor under actual sun in Turkey (latitude 38°N) during June 1999. The solar UV exposure of volunteers was monitored with an 2-channel PMA self-recording radiometer (Solar-Light Co, Philadelphia, PA), equipped with a UV erythema (280–380 nm) sensitive cell (Uve) and a UVA (320–400 nm) sensitive cell. During exposure, the Uve irradiance was recorded every 10 min on a horizontal plane and expressed as erythema effective (ee) intensity in terms of standard minimal erythema dose (MED) per hour (standard MED = 21 mJ per cm²) (Solar-Light Inc., 1993). At the same time, UVA irradiance was recorded as mW per cm². The cumulative UV doses received by all volunteers were calculated as standard MED (Uve) and J per cm² (UVA).

Sunscreens For the indoor study II, two prototype sunscreen preparations (A and B) were formulated in the same oil-in-water vehicle. The products were designed to have the same SPF but different UVA-PF. Their characteristics are shown in **Table II**.

SPF was determined according to the European Cosmetic Toiletry and Perfumery Association (COLIPA) recommendations (1994) using the metal halide lamp (290–390 nm), which was used for the immunoprotection study (study I). UVA-PF was determined on 10 subjects using an *in vivo* method based on persistent pigment darkening dose (Moyal *et al*, 2000), a method adopted by the Japanese Cosmetic Industry Association (JCIA) in, 1996. For the immunoprotection study, products A and B were applied at 2 mg per cm² 15 min prior to each exposure on both exposed body sites (see section on *UV exposures*).

For the outdoor study III, two commercially available sunscreen products (C and D) were selected. Their characteristics are shown in **Table II**. Products C and D had the same SPF 25 and their UVA-PF were determined using the persistent pigment darkening method as described above. Volunteers were exposed to sunlight after sunscreen was applied over the whole body except on the areas covered by swim wear and the opaque armband (see section on *UV exposures*), before each exposure period. The amount of applied product was weighted and calculations based on body surface indicated that it averaged out at approximately 0.8 mg per cm² per application.

The UV radiation transmission spectra (T) of the products were obtained using a modified Diffey and Robson (1989) method. In this method, the UV radiation transmitted through a roughened quartz plate, with and without sunscreen applied, was measured spectroradiometrically. The monochromatic protection factors (mPF) were calculated ($mPF = 1/T$) and represented as a function of wavelengths (**Figs 2 and 3**).

UV exposures Under laboratory conditions two exposure areas (30 cm × 20 cm) were delineated, one on the back and the other one on the abdomen in order to expose a large part of the body and feasible with the surface of the solar simulator beam. Both sites were exposed to the same UV regimen.

UVA study I In group 2 (full-spectrum UVA) and group 3 (UVA1) 12 daily exposures were performed (Monday to Friday in the first 2 wk and Monday and Tuesday in the third week) on both delineated areas. In both groups, to avoid erythema the UVA exposure dose was progressively increased from 20 J per cm² to 48 J per cm² (increment of 10% every 2 d for 6 d followed by an increase of 12% each day for the last 6 d). The cumulative UVA dose received was 352 J per cm². The daily UVA doses administered were realistic, considering that 20 J per cm² of UVA is equivalent to about 1 h sunlight on the French Riviera

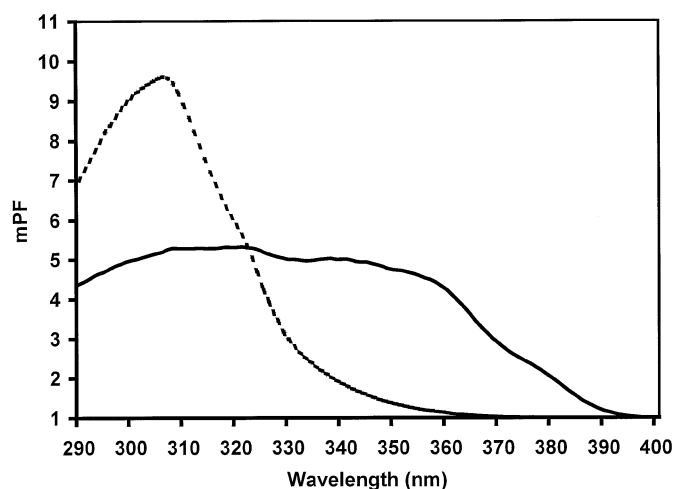


Figure 2. Monochromatic protection factors of sunscreens A and B. The mPF spectra of products A (—) and B (---) were generated by spectroradiometric measurements between 290 and 400 nm according to a modified Diffey method. These spectra clearly show that protective efficacy in the UVA range for product A is much higher than for product B.

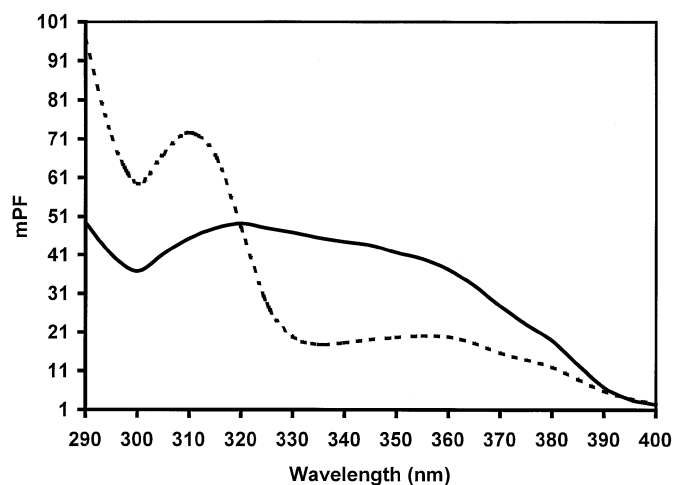


Figure 3. Monochromatic protection factors of sunscreens C and D. The mPF spectra of products C (—) and D (---) were generated by spectroradiometric measurements between 290 and 400 nm according to a modified Diffey method. Two broad-spectrum sunscreens having similar SPF can differ significantly in UVA absorption potency as shown on these spectra.

Table III. Effect of UVA exposure on DTH (study I): Total score expressed in mm (mean \pm SEM) and DTH response variation (%)

Test site	Group 1 control nonexposed	% variation	Group 2 exposed full spectrum UVA	% variation	Group 3 exposed UVA 1	% variation
Pre-UV	10.8 \pm 2.9		8.1 \pm 2.1		17.3 \pm 2.4	
Post-UV non-exposed site	12 \pm 3.4	+ 11%	3.1 \pm 1.7 ^a	- 61.7%	6.5 \pm 0.9 ^a	- 62.4%
Post-UV exposed site	9.8 \pm 3.3	- 9.3%	2.7 \pm 1.3 ^a	- 66.7%	7.3 \pm 1.0 ^a	- 57.8%

^aSignificantly different from pre-UV ($p < 0.05$).

at noon in summer. The initial 20 J per cm² are lower than an average UVA MED, which is approximately 30 J per cm² for Fitzpatrick skin type II or III (Fitzpatrick, 1988).

Laboratory sunscreen study II Using the SSR source, the MED was determined for each volunteer in groups 4, 5, and 6 on the upper part of the back the day before the first exposure.

In group 4 (SSR irradiated control), 10 daily exposures over 2 wk were carried out on both delineated areas. The SSR dose was progressively increased by 10% from 0.8 individual MED to 2 individual MED. The cumulative SSR dose was on average 14.5 MED (SD = 2.3). The UVA dose included was on average 75 J per cm² (SD = 13.4). This progressive approach was chosen to minimize erythema. If a perceptible redness was observed 24 h after exposure, the dose was not increased.

In groups 5 (SSR + product A) and 6 (SSR + product B) SSR doses on both delineated areas were increased at the same rate as in group 4, but the doses were multiplied by half the SPF of sunscreens (**Table II**). We chose to expose the skin to SSR doses that did not exceed the protective efficacy of the sunscreens against erythema. So if 24 h after an exposure a perceptible redness was observed, the dose was not increased for the next exposure. The cumulative SSR dose delivered was on average 58 individual MED (SD = 4.5 for group 5 and SD = 3.3 for group 6) with an average UVA component of 315 J per cm² (SD = 99.7 for group 5 and SD = 63.6 for group 6).

Outdoor sunscreen study III Groups 7 and 8 were each exposed to the same daily solar UV radiation dose for 6 d, including an exposure both each morning and each afternoon.

The duration of daily exposure ranged from 3 h (1st day) to 5 h (6th day), thus the UV radiation dose was progressively increased from 6 to 10 standardized erythematous doses (MED = 21 mJ per cm²) per day (Solar-Light Inc., 1993). All volunteers received the same cumulative dose of 50.5 standardized MED with a UVA component of 340 J per cm². The entire body was exposed except areas covered by the standardized swim wear (bikini for females, slip for male) and one area (35 cm²) on one arm, which was protected by an UV opaque armband. This area enabled the distal effects of sun exposure to be assessed.

Immune response to recall antigens: elicitation of DTH reaction The effects of UV radiation exposure on the elicitation of DTH reaction were measured using Multitest Kits Pasteur/Mérieux (Lyon, France). This Multitest kit includes seven antigens (tetanus toxoid, diphtheria toxoid, *Streptococcus*, tuberculin, *Candida albicans*, *Trichophyton mentagrophytes*, and *Proteus mirabilis*). As the negative control substance, there was a 70% sterile glycerin solution, the antigen vehicle.

Measuring the immune response to recall antigens that most people encounter during childhood immunizations, offers a unique advantage as no active immunization of the test volunteer is required.

For the indoor studies, all Multitests were carried out on the back. An initial test was done on the right or left side of the upper back the week before UV exposure. Two subsequent tests were done 24 h after the last UV radiation exposure: one on a nonexposed site for the evaluation of distal immunosuppression (the opposite side of the first Multitest) and the other on the exposed site for the assessment of both local and distal immunosuppression.

For the outdoor study, Multitests were carried out on the back (exposed site) and on one arm (unexposed site) 1 wk before the first exposure and 72 h after the last sun exposure. All DTH test responses were measured 48 h after applying the Multitest.

The diameter (mm) of each positive reaction, identified as erythema accompanied by local induration, was measured in two directions and averaged. The mean diameters of each positive reaction for each subject were added to obtain the total score.

Statistical analysis Statistical comparisons were made by comparing the DTH response after UV radiation exposure with DTH response before UV radiation exposure in each subject via paired two-tailed Student's *t* tests. Variance analysis between groups was performed to compare results on the difference of the total scores (pre-UV-post-UV). Results were considered significant if $p < 0.05$ (software SPSS).

RESULTS

SPF and UVA-PF determinations The results are shown in **Table II**. It can be seen that the SPF of the products were both 9 for indoor SSR study II but that product A had a much higher UVA-PF (UVA-PF = 9) than product B (UVA-PF = 2.1). SPF testing was not done for the sunscreens used in outdoor study III but each had a labeled SPF of 25. Product C, however was shown to have a much higher UVA-PF (UVA-PF = 13.7) than product D (UVA-PF = 5.9).

Elicitation of local DTH response was decreased by repeated UVA and SSR exposures

Study I and study II group 4 No significant variation between the response to the three Multitests was observed in control group 1, which was not exposed to UV (**Table III**). In group 2 (full spectrum UVA) and group 3 (UVA 1) a significant ($p < 0.05$) and equivalent decrease in the DTH responses was observed either on the exposed or nonexposed sites when compared with the test done before UVA exposure. Percent immune suppression ranged between 57% and 67% (**Table III**). Under full spectrum UVA exposure conditions we observed a slight erythema on the volunteers from the fourth exposure up to the last exposure. Under UVA 1 exposure conditions, we did not observe any erythema, only a moderate tan was noticed.

In group 4, exposed to repeated low erythematous SSR doses without sunscreen protection, there was a significant decrease (approximately 60%) ($p < 0.05$) in the response to antigens after exposure as compared with the initial response. This decrease was not significantly different on exposed (71%) and nonexposed (59%) skin sites (**Table IV**). The immunosuppressive effects were equivalent to the one observed after repeated UVA or UVA 1 exposures (57–67%).

A moderate erythema developed in three volunteers after the third exposure and in five volunteers after the sixth exposure when the daily SSR dose was 1.6 MED. The erythema was observed for 3 d before the onset of tan. The other volunteers only developed a just perceptible erythema.

When the initial Multitests (before exposure) were compared with unirradiated controls after UV radiation exposure, the intensity of DTH response at unirradiated sites was significantly ($p < 0.05$) reduced by exposure of adjacent skin to the two UVA protocols and SSR (**Tables III and IV**). Thus, all UV protocols caused distal as well as local immunosuppression.

Indoor sunscreen study II A significant decrease ($p < 0.05$) in the response was observed in group 6 (SSR + sunscreen SPF 9 UVA-PF 2) on both exposed (65%) and nonexposed (52%) skin sites. There was no significant difference ($p > 0.05$) in the immunosuppression rate measured between groups 4 (exposed unprotected)

Table IV. Immunoprotection afforded by sunscreens (indoor study II). Total scores expressed in mm (mean \pm SEM) and DTH response variation (%)

Test site	Group 4 control exposed without sunscreen	% variation	Group 5 exposed with sunscreen A	% variation	Group 6 exposed with sunscreen B	% variation
Pre-UV	10.7 \pm 1.5		13.1 \pm 2.9		13.3 \pm 3.3	
Post-UV non-exposed site	4.4 \pm 0.9 ^a	– 58.9%	11.5 \pm 2.4 ^b	– 12.2%	6.3 \pm 1.9 ^a	– 52.6%
Post-UV exposed site	3.1 \pm 0.6 ^a	– 71%	9.2 \pm 1.9 ^{ab}	– 29.8%	4.6 \pm 2.2 ^a	– 65.4%

^aSignificantly different from pre-UV ($p < 0.05$).^bSignificantly different from group 4 and group 6 for the same site ($p < 0.01$).**Table V. Immunoprotection afforded by sunscreens (outdoor study III). Total score expressed in mm (mean \pm SEM) and DTH response variation (%)**

Test site	Group 7 exposed with sunscreen C	% variation	Group 8 exposed with sunscreen D	% variation
Pre-UV back site	20.8 \pm 2.3		21.2 \pm 2.4	
Post-UV exposed back site	19 \pm 2.2	– 8.6%	17.1 \pm 1.8 ^a	– 19.3%
Pre-UV arm site	14.7 \pm 2.1		18.2 \pm 2.8	
Post-UV non-exposed arm site	14 \pm 1.5	– 4.8%	12.1 \pm 1.8 ^b	– 33.5%

^aDifferent from pre-UV ($p < 0.1$).^bSignificantly different from pre-UV ($p < 0.05$).

and 6 (exposed protected with SPF 9 UVA-PF 2) on exposed and nonexposed skin sites (**Table IV**).

In group 5 (SSR + broad-spectrum sunscreen SPF 9 UVA-PF 9) the DTH response was slightly decreased (– 29.8%, $p < 0.05$) on exposed sites and unchanged on nonexposed sites.

As the initial responses to Multitest were not different between groups ($p > 0.1$) we compared the three groups and showed that the difference in efficacy between the broad-spectrum product and the nonbroad-spectrum product was highly significant ($p < 0.01$) for both local and distal immune suppression (**Table IV**). We did not observe any sunburn in the study groups.

Outdoor sunscreen study III Both products were protective against erythema, but the tan developed was significantly higher on group 8 (protected by the SPF 25 UVA-PF 6 sunscreen) than on group 7 (protected by sunscreen SPF 25 UVA-PF 14).

The outdoor study demonstrated that, in comparison with DTH responses before solar exposure, no alteration of local and distal immune response was detected when the skin was protected by sunscreen SPF 25 UVA-PF 14 (group 7). Conversely, in Group 8 on which sunscreen SPF 25 UVA-PF 6 was applied, a moderate decrease ($p < 0.1$) in the immune response was observed on the irradiated skin of the back (– 19.2%) (local effect) and a significant decrease ($p < 0.05$) was measured on the nonirradiated skin of the arm (– 33%) (distal effect) (**Table V**).

DISCUSSION

Whereas it is generally agreed that UVB and SSR are immunosuppressive (Cooper *et al*, 1985; van Praag *et al*, 1991; Wolf *et al*, 1993), few data have been reported on the effects of UVA.

We have investigated the effects of UVA on the elicitation phase of the DTH response. It was important to know if UVA could affect this response and thereby possibly compromise the ability of vaccination to trigger a response after contact with the relevant antigen (Jeevan and Kripke, 1990). Needs for research on this issue have recently been listed in Selgrade *et al* (1997). By measuring the effects of different parts of the UV spectrum and the protection afforded by sunscreens on immune DTH response to recall antigens, we generated data relevant to the issue. In our studies volunteers were exposed to repeated, realistic UVA doses.

Furthermore, we compared the effects of full spectrum UVA (UVA) ν long-wave UVA (UVA 1) and SSR. We also studied the effects of UVA indirectly by comparing sunscreens having the same SPF but with a different UVA-PF.

We have shown that the elicitation of DTH response was significantly reduced, both locally and at a distance, by all UV radiation sources used. Furthermore, the exposure protocols we applied resulted in a similar degree of immune suppression irrespective of the source. Of course, the UV dose ranges tested are limited but preliminary experiments from our group (data not shown), with lower repeated doses of SSR (a total of 5 MED over 5 d) or with real sun exposure (1 MED per day for 12 d) resulted also in a decrease of DTH reaction (decrease of 20% and 34%, respectively). We never observed whatever the wavebands, the exposure regimen or the UV dose, an enhancement of the elicitation response to recall antigens. Sunscreen studies indirectly confirmed the suppressive effects of UVA by showing that products having a similar SPF with higher UVA protection provided significantly better prevention than those with a low UVA protection. It was evidenced both with SSR and natural sunlight. These findings confirm those of Damian *et al* (1997) in humans whereas our results without sunscreens confirm those of Ullrich (1999) who found that acute low SSR doses and full spectrum UVA suppressed the DTH elicitation response to *Candida albicans* in the mouse.

Few other investigators have studied the effects of UVA on the elicitation phase of CHS or DTH responses. Damian *et al* (1999) reported the effects of full spectrum UVA (320–400 nm) on the elicitation of CHS response to nickel in humans. In this study, a single low dose (4 J per cm²), was suppressive as were low-dose exposures (1.9 J per cm² per exposure) over 1–3 d. In contrast, there were no suppressive effects when a low-dose cumulative UVA exposure was applied over a longer period (4 d–4 wk). The authors proposed the development of an adaptive response with longer exposure periods.

Others (Hersey *et al*, 1983; LeVeé *et al*, 1997; Skov *et al*, 1997) have investigated the effects of UVA on the suppression of induction phase of CHS response to chemical haptens. These studies assessed the effects of UV radiation on cutaneous immune

function before sensitization, whereas studies on the elicitation phase assessed the effects after sensitization. It should be stated, however, that the immunologic and photobiologic relationships between these two phases are unknown as well as their respective role in tumor development.

LeVeé *et al* (1997) reported that a single exposure to 4 MED UVA 2 (centered at 335 nm) was highly effective to suppress CHS induction by dinitrochlorobenzene; however, in another experiment, 3 MED UVA 1 had no effect (Skov *et al*, 1997). In both studies, there was no evidence of systemic suppression when diphenylcyclopropanone was applied to a site distant from that exposed to UVA, although LeVeé *et al* (1997) showed that 4 MED of UVA 2 had toleragenic effects. Hersey *et al* (1983) found that 12 cumulative exposures to UVA from a solarium delivering some UVB (0.9–1.4% of total UV) suppressed the induction phase of CHS response to dinitrochlorobenzene.

A recent study on the induction phase of CHS response to dinitrofluorobenzene in hairless mouse by Reeve *et al* (1998) showed that a single UVA exposure, immediately after or before an acute dose of 3 MED of UVB or SSR, abrogated the immunosuppressive effects. The effects of pure UVA applied before or after UVB or SSR exposure, however, may not be relevant to real-life conditions except perhaps for people using a sun parlor just before solar exposure.

Donawho *et al* (1996) questioned whether UVB irradiation of C3H mice could inhibit the elicitation of DTH response as well as the rejection of melanoma cells. They demonstrated that elicitation of a DTH response to alloantigen was diminished in UV irradiated ears and that tumor rejection was impaired in melanoma immune mice challenged in a UV-irradiated site. Our results showing that UVA and SSR exposures suppress the elicitation of DTH to recall antigens in humans confirm these findings in animals and suggest that sunlight induces suppression of the efferent arm of immune reaction. The suppression of the efferent arm may promote the outgrowth of skin cancer.

Many investigators have studied the protective effects of sunscreens on UV radiation-induced suppression of CHS or DTH reactions in mice (Bestak *et al*, 1995; Roberts and Beasley, 1995; Gueniche and Fourtanier, 1997; Fourtanier *et al*, 2000) and some in humans (Damian *et al*, 1997; Serre *et al*, 1997). The degree of immune protection reported varies greatly.

Our study in humans confirms previous results (Fourtanier *et al*, 2000) in the hairless mouse model in which the level of immunoprotection afforded by two broad-spectrum sunscreens having the same SPF but with different UVA-PF was evaluated. Both sunscreens showed a preventive effect on UV-induced suppression of CHS to dinitrofluorobenzene but the product having the higher UVA-PF showed significantly greater protection.

An important conclusion of our study is that the SPF, an indicator of protection against sunburn, is not a proper indicator of the level of protection against the suppression of the elicitation phase of immune response induced by repeated UV exposures. It also strongly suggests that sunscreens with improved UVA protection have a higher immune protection factor (IPF). In the future it may be important to specify an IPF for sunscreen or at least to demonstrate that the IPF is not lower than the SPF; however, this would require the development of standardized methods based on the dose–response characteristics of immunosuppression in humans. Kelly *et al* (2000) have determined SSR dose–response for the suppression of the induction phase of CHS response in humans. Recently they have also studied the effects of a UVB sunscreen in this model and they concluded that the IPF of the sunscreen was about 50% of its SPF. The authors also suggested that UVA was important in immunosuppression (personal communication).

The induction phase of CHS response in humans cannot be used for the routine assessment of IPF as it necessitates sensitization that can only be induced once. This means that only one person per UV radiation dose can be studied subsequently requiring a large number of volunteers to be involved if UV radiation dose–response studies are to be carried out; however routine studies could be done on the

elicitation phase and it may be useful to make comparative studies on immunoprotection of these two phases in relation to the SPF. Our outdoor study, in which the volunteers self-applied the sunscreens confirmed previous reports that sunscreen users apply less than 2 mg per cm² in practice (Wulf *et al*, 1997; Azurdia *et al*, 1999). Overall, our calculations led to estimate an average applied amount of 0.8 mg per cm². Nevertheless the products performed well, although it was observed a significant difference in the immunoprotection provided by the two products. These data suggest to recommend applying a higher SPF than might be actually needed in order to ensure that most adequate protection is provided both against sunburn and the alteration of immune function.

In conclusion, the present studies provide direct and indirect evidence that UVA has a suppressive effect on cutaneous immunity when assessed by the elicitation phase of DTH response. This effect occurs on irradiated sites and on distal sites suggesting a possible systemic effect.

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