Low-Dose UVA and UVB have Different Time Courses for Suppression of Contact Hypersensitivity to a Recall Antigen in Humans

Diona L. Damian, Ross St C. Barnetson, and Gary M. Halliday

Department of Medicine (Dermatology), University of Sydney at Royal Prince Alfred Hospital, Sydney, Australia

This study investigates the relative effects of low-dose solar-simulated ultraviolet, ultraviolet A, and ultraviolet B radiation on the elicitation of contact hypersensitivity to nickel in nickel-allergic volunteers. A xenon arc lamp with changeable filters was used to irradiate groups of volunteers daily, on separate areas of their lower backs, with both solar-simulated ultraviolet (ultraviolet B, ultraviolet AII + ultraviolet AI) and ultraviolet A (same ultraviolet AII content but twice the ultraviolet AI as the solar-simulated ultraviolet spectrum) for 1 and 2 d; 3, 4, and 5 d; and from 1 to 4 wk. A fourth group was irradiated for 1–5 d with the ultraviolet B component of solar-simulated ultraviolet. Following the final irradiation in each group, nickel-containing patches were applied to both ultraviolet-treated sites and adjacent, unirradiated control sites. Erythema caused by nickel contact hypersensitivity at each site was quantitated 72 h later with a reflectance erythema meter. By comparing the nickel reactions of irradiated and unirradiated skin, ultraviolet immunosuppression was assessed with the different spectra and durations of ultraviolet exposure. We found significant immunosuppression with daily doses of ultraviolet B and ultraviolet A equivalent to approximately 6 min of summer sun exposure, and that ultraviolet A and ultraviolet B exerted their maximal immunosuppressive effects at different times. Solar-simulated ultraviolet-induced immunosuppression was significant after one exposure, near-maximal after two exposures and remained elevated thereafter. Ultraviolet B-induced immunosuppression was lower than that induced by solar-simulated ultraviolet, but followed a similar time-course. In contrast, ultraviolet A-induced immunosuppression was transient, peaking after three exposures. Immune responses returned towards normal with subsequent ultraviolet A exposure, suggesting that an adaptive mechanism may prevent immunosuppression by continued ultraviolet A irradiation. ***Key words: immunosuppression/nickel allergy/sunlight/ultraviolet radiation. J Invest Dermatol 112:939–944, 1999***

Whereas the suppressive effects of ultraviolet (UV) radiation on human cutaneous immunity have been recognized for many years (Hersey *et al*, 1983; Cooper *et al*, 1985; Murphy *et al*, 1993), the relative immunosuppressive contribution of UVB (290–320 nm) and UVA (320–400 nm) is as yet unknown. Most previous work has concentrated on UVB-induced immunosuppression and relatively few studies in humans have directly examined the immune effects of UVA. Even fewer have directly compared UVA and UVB in the same model. UVA is known to have a number of deleterious effects on the skin, including genetic mutation (Burren *et al*, 1998; de Laat *et al*, 1997), reduced Langerhans cell activity (Aberer *et al*, 1981) isomerization of *trans*-urocanic acid (Webber *et al*, 1997) and generation of oxygen free radicals (Black, 1987), but the exact role of UVA in immunosuppression is still controversial.

Manuscript received October 26, 1998; revised January 27, 1999; accepted for publication March 7, 1999.

Reprint requests to: Associate Professor Gary Halliday, Department of Medicine (Dermatology), University of Sydney, Sydney NSW 2006 Australia.

Abbreviations: CHS, contact hypersensitivity; EI, erythema index; SSUV, solar-simulated UV.

Some groups have found that UVA has significant suppressive effects on primary contact hypersensitivity (CHS) (Hersey *et al*, 1983; LeVee *et al*, 1997) but others have not (Skov *et al*, 1997). Whereas UVA-induced suppression of delayed-type hypersensitivity (DTH) responses to recall antigens has been reported (Hersey *et al*, 1987; Moyal *et al*, 1997), Sjovall and Christensen (1986) found that elicitation of CHS to nickel was suppressed by UVB but not UVA.

In all of these human studies and in most murine studies, there has been a tendency to use large and often erythemal doses of UVA in order to achieve "biologic equivalence" with UVB. Erythemal equivalence, however, does not imply immunosuppressive equivalence. It has been shown by our group and others that dark-skinned subjects are just as susceptible as pale-skinned subjects to immunosuppression by a given UV dose, whereas the erythemal susceptibility of the two groups will be markedly different (Vermeer *et al*, 1991; Damian *et al*, 1997).

There is also some evidence to suggest that high- and low-dose UVA might exert opposite effects on cutaneous immunity. Recent work by Reeve *et al* (1998) in hairless mice found that a single high-dose UVA exposure reversed the immunosuppressive effect of UVB radiation immediately preceding or following it, and that UVA was more immune protective in higher doses. This contrasts with the results of Bestak and Halliday (1996) who found significant immunosuppression in C3H/HeJ mice when UVA was given for

4 wk at a low daily dose. Thus the relative immunosuppressive effect of chronic vs. acute UV exposure is also unclear.

Allergy to nickel (in earrings, watchbands, and other costume jewellery) is common in the general population and we have previously shown that elicitation of nickel CHS is readily suppressed by suberythemal solar-simulated UV (SSUV); this represents immune suppression rather than suppression of nonspecific inflammation, as UV exposure had no effect on patch test responses to the nonspecific irritant, sodium lauryl sulfate (Damian *et al*, 1997). We also found that UV suppression of nickel CHS was only prevented by sunscreens containing UVA as well as UVB filters, thus suggesting indirectly that acute, low-dose UVA may be immunosuppressive. Using this method to measure the relative effects of low-dose UVB, UVA, and SSUV, we found that all were immunosuppressive, but that their time-courses of immunosuppression were different. We also report that acute UVA exposure may be more immunosuppressive than chronic exposure.

MATERIALS AND METHODS

Subjects Sixty healthy, nickel-allergic volunteers (59 female, one male) of Fitzpatrick's skin types I–V (Fitzpatrick, 1988) were recruited from the local university (students and staff), hospital staff, and general population. A further 12 volunteers (six men and six women), who were not necessarily allergic to nickel, were recruited for minimal erythema dose (MED) testing with the UVB-only source in order to determine its erythemal equivalence with respect to the SSUV source.

None of the volunteers had experienced sun exposure of their backs for at least 4 wk preceding the study. Ethical approval was obtained for these experiments from both the Sydney University and Royal Prince Alfred Hospital Ethics Committees, and all volunteers provided written informed consent prior to entry into the study.

Nickel patch testing Nickel allergy was initially confirmed by patch testing with 9 mm Finn chambers (Epitest, Tuusula, Finland). Each volunteer was tested with at least three concentrations of $NiS0_4.6\ H_20$ in a petrolatum base (Trolab Hermal, Reinbek, Germany) from 0.03125% to 2%, and a placebo patch, containing only petrolatum, was also included in the test array. For each volunteer, the three nickel concentrations were chosen according to the reported severity of their allergy, in order to minimize the risk of unduly large reactions. For example, subjects reporting only minimal symptoms after prolonged contact with costume jewellery were tested with 2%, 0.5%, and 0.125% nickel, whereas those reporting severe blistering after only brief contact with jewellery were tested with 0.5%, 0.125%, and 0.03125%. The nickel-containing patches were taped to the mid-back, removed after 48 h and the response assessed 24 h later. A positive nickel reaction consisted of erythema with induration at the site of the patch test, as per the International Contact Dermatitis Research Group definition (Adams, 1981). By determining the minimum nickel concentration likely to elicit a mild but confluent nickel reaction (this may have been one of the three-test doses, or may have been a concentration above, below or intermediate between the three-test doses), the occurrence of larger or vesicular reactions was minimized during subsequent patch testing.

Nickel-induced erythema was measured with a reflectance spectrometer (Diastron, Hampshire, U.K.), with subjects resting in the prone position. The nickel-induced erythema index (EI) at each test site was calculated as the difference between the mean of four erythema readings at the nickel test site and the mean of four readings taken from skin adjacent to each test site.

UV source SSUV irradiation was provided by a 1000 W ozone-free xenon arc lamp with a collimated 7.5 cm^2 beam (Oriel, Stratford, CT). The lamp emission was filtered by two 280–400 nm dichroic mirrors (to attenuate the visible and infrared component) and an atmospheric attenuation filter (Oriel) to remove UVC (<290 nm) and modify the UVB spectrum so that it more closely approximated sunlight. This was the solar-simulated spectrum (**Fig 1**). By substituting a UVB and UVC blocking filter (Oriel) for the atmospheric attenuation filter, a primarily UVA-only spectrum was produced. In order to generate a UVB-only spectrum (with only minimal residual UVA), two 260–320 nm dichroic mirrors (Oriel) were installed in place of the 280–400 nm dichroics used for the solar-simulated spectrum. This UVB-only spectrum was also filtered with the atmospheric attenuation filter.

Spectral irradiance of the lamp, with its different combinations of filters, was measured at the Commonwealth Scientific and Industrial Research Organization (Sydney, Australia). The integrated irradiance of the solar-simulated spectrum at the skin surface was 5.1 mW per cm^2 UVB (290–320 nm), 9.7 mW per cm^2 UVAII (320–340 nm) and 26.6 mW per cm^2 UVAI (340–400 nm). The daily dose of SSUV comprised 143 mJ per cm^2 UVB, 271 mJ per cm^2 UVAII and 745 mJ per cm^2 UVAI.

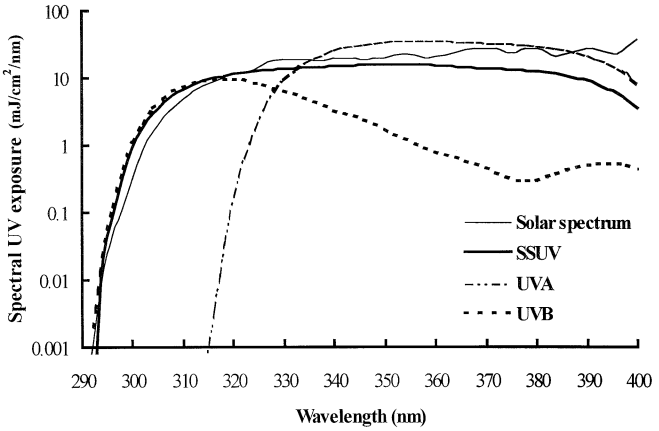

Figure 1. The SSUV spectrum of the Oriel Solar Simulator approximates solar UV (absolute daily spectral exposure doses). The solar spectrum here represents 6 min exposure to the standard sun UV spectrum as defined in the European Cosmetic Toiletry and Perfumery Association Sun Protection Factor Test Method (1994). This spectrum is a rounded average of the solar spectra of Sayre *et al* (1990) and Diffey and Robson (1989) (cloudless sky at low altitude, at latitudes 35°N and 40°N, respectively). The SSUV, UVB, and UVA spectra represent the subjects' absolute levels of daily UV exposure.

The UVA spectrum had an irradiance of 5.9 μW per cm^2 of UVB (> 315 nm), 3.3 mW per cm^2 UVAII and 24.4 mW per cm^2 of UVAI; the daily UV dose given with this "UVA-only spectrum" was 233 mJ per cm^2 UVAII (i.e., approximately equal to that within the solar-simulated spectrum) and 1709 mJ per cm^2 UVAI. There was also a small amount of UVB present in the "UVA-only" spectrum (daily UVB exposure was 0.42 mJ per cm^2 UVB; 0.41 mJ per cm^2 of this UVB radiation was > 315 nm).

The "UVB-only" spectrum had an irradiance of 5.1 mW per cm^2 UVB, 4.4 mW per cm^2 UVAII and 1.9 mW per cm^2 UVAI. The daily dose of UVB from this source was 144 mJ per cm^2, with a small amount of residual UVA (123 mJ per cm^2 UVAII and 53 mJ per cm^2 UVAI).

The irradiance of the lamp output was monitored at least daily with an IL1350 broadband radiometer using SED 038 (UVA) and SED 240 (UVB) detectors (International Light, Newburyport, MA). All subjects in each group were irradiated with the same daily doses of UV regardless of skin color or MED.

UV irradiation Using the SSUV source, the MED of the skin of the lateral mid-back was determined for each subject in the SSUV and UVA studies as the lowest UV dose at which erythema was observed. In the UVB-only study, the MED was determined using the UVB-only source.

Separate groups of volunteers were used for the different experiments with their different irradiation protocols. In each group, the skin of one or both sides of the mid-back was irradiated within a 4 × 6 cm template, which comprised up to six irradiated sites. The position of the various unirradiated and irradiated patches within the template was randomly varied in each subject so as to counteract any potential anatomic influence on the intensity of nickel CHS responses.

In the first group (irradiation protocol 1), subjects were irradiated with both SSUV and UVA, daily for 5, 4, and 3 d to different areas of their backs. There were thus six irradiated sites: three receiving SSUV and three receiving UVA for 3, 4, and 5 d. The sites irradiated for 5 d received UV daily from Monday to Friday in 1 wk. The sites irradiated for 4 d received UV on Monday, Wednesday, Thursday, and Friday of that same week, whereas the sites irradiated for 3 d received UV on Tuesday, Thursday, and Friday. In all cases, nickel patches were placed on each of the irradiated segments on Friday, immediately after the final UV exposure. Patches were simultaneously placed on adjacent, unirradiated skin in each subject to serve as immunologically intact controls. The patches were left in place for 48 h, and the resulting nickel CHS measured with the erythema meter 24 h later, on the following Monday.

The second group of volunteers (irradiation protocol 2) were also irradiated with SSUV and UVA at different sites, but for 1 d (Thursday)

Table I. Subject characteristics

	3, 4, 5 d SSUV, UVA (protocol 1)	1, 2 d SSUV, UVA (protocol 2)	1–5 d UVB (protocol 3)	1–4 wk SSUV, UVA (protocol 4)
No. eligible subjects	16	16	16	5
No. excluded	1	3	1	1
Mean age (y)	26	34	32	29
Range	18–37	18–62	20–53	23–41
Mean MED (mJ per cm^2 UVB)	269	224	306	167
Range	128–717	108–307	108–358	128–538
No. subjects of skin type[a]				
I	0	1	0	1
II	6	5	3	1
III	6	8	8	3
IV	3	2	5	0
V	1	0	0	0
Mean EI of unirradiated control site	80.1	68.1	65.3	61.6

[a]Fitzpatrick's classification (1988).

and 2 d (Wednesday and Thursday) only. Nickel patches were simultaneously applied to the different sites on Friday (24 h after the final irradiation) and measured the following Monday.

A third group of volunteers (irradiation protocol 3) was irradiated daily with UVB only, for 5, 4, 3, 2, and 1 d to different sites. The timing of this irradiation protocol was identical to that described in protocols 1 and 2 combined, with nickel challenge always being performed on Friday and the CHS response assessed the following Monday.

The fourth group of six subjects (irradiation protocol 4) was irradiated with SSUV and UVA at different sites for 1, 2, 3, and 4 wk. Each week, the various sites were irradiated on 4 d (Monday, Wednesday, Thursday, and Friday). The sites irradiated for 4 wk received this weekly irradiation protocol from week 1, for the 4 consecutive weeks of the experiment. The sites receiving 3 wk UV began their UV exposure in week 2, and the sites receiving 2 wk' UV began their exposure in week 3. The sites receiving only 1 wk UV did not begin their exposure until week 4. Hence the various sites, with UV exposures over 1–4 wk, all had their final UV exposures on the same day (Friday of week 4), with nickel patch testing done immediately thereafter.

Analysis of data The magnitude of immunosuppression at each test site was determined by comparing the nickel-induced erythema (EI) of the irradiated test sites with nickel-induced erythema at the unirradiated control site:

Immunosuppression (ΔEI) =
EI (unirradiated control) – EI (irradiated test site)

Statistical comparisons were made by comparing nickel-induced erythema at control sites with nickel-induced erythema at test sites in each subject via paired two-tailed Student's t-tests. Results were considered significant if $p < 0.05$, and are presented as mean ± SEM. Correlations, using linear regression analysis, were also considered significant if $p < 0.05$.

RESULTS

Nickel-allergic subjects None of the volunteers suffered significant adverse effects from the nickel patch testing, and all but one completed the study. This subject did not complete the study for unrelated reasons. Six subjects were excluded from the results because of insufficient nickel reaction at the unirradiated control site (i.e., lack of confluent induration), despite an acceptable reaction to the initial patch test. Characteristics of the eligible subjects in each group are shown in **Table I**.

The UVB source was erythemally equivalent to the solar-simulated UV source Twelve subjects of mean age 31 y (range, 20–58 y) were MED tested with both the solar-simulated source and the UVB-only source. Their mean SSUV MED was 288 ± 46 mJ per cm^2 UVB (with 2.1 J per cm^2 accompanying UVA), whereas their mean MED with the UVB source was 290 ± 30 mJ per cm^2 UVB (with 354 mJ per cm^2 UVA). Correlation of the volunteers' SSUV MED with their UVB MED gave an r-value of 0.95 ($t = 9.9$; $p < 0.001$).

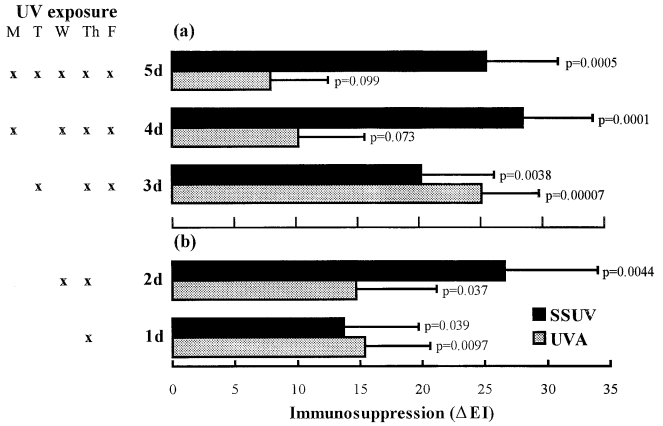

Figure 2. Immunosuppression of nickel CHS reached maximal levels with 2 d of SSUV and 3 d of UVA exposure. Immunosuppression is shown here as the mean reduction in nickel-induced erythema with each irradiation protocol (ΔEI). These data represent the results from two separate groups of 16 volunteers: one group (*a*) received 3, 4, and 5 d exposure to SSUV and UVA to different areas of their backs (i.e., six irradiated sites as per protocol 1), and the second group (*b*) received 1 and 2 d exposure (protocol 2). After the final irradiation on Friday, nickel patches were applied to all irradiated sites, and also to adjacent, unirradiated sites serving as controls (mean ± SEM; two-tailed Student's t-test, comparison of unirradiated control sites with irradiated sites).

The acute immunosuppressive effects of SSUV and UVA are maximal with 2 and 3 d UV, respectively In the group exposed to 3, 4, and 5 d of SSUV and UVA (protocol 1), significant mean immunosuppression (ΔEI) of 19.9 ± 5.8 was observed with 3 d of SSUV exposure ($n = 16$; $p < 0.01$)(**Fig 2*a***). With 4 d of SSUV exposure, immunosuppression peaked at ΔEI of 28 ± 5.6 (equivalent to ≈34% immunosuppression; $p < 0.001$) and with 5 d of SSUV, mean ΔEI was 25.1 ± 5.8 ($p < 0.001$). In contrast, UVA-induced immunosuppression was maximal with 3 d of exposure (mean ΔEI 24.7 ± 4.6; $p < 0.0001$) and then declined with 4 and 5 d of exposure to 10.1 ± 5.3 and 7.9 ± 4.6, respectively (both NS; $p > 0.05$).

In the group of volunteers irradiated with protocol 2, there was significant immunosuppression (ΔEI) of 13.7 ± 6 and 26.6 ± 7.4 with 1 and 2 d SSUV, respectively ($n = 16$; $p < 0.05$; $p < 0.01$) (**Fig 2*b***). With 2 d of UVA exposure, ΔEI was 14.7 ± 6.4 ($p < 0.05$) and with 1 d mean ΔEI was 15.4 ± 5.2 ($p < 0.01$). Thus SSUV and UVA caused immunosuppression over different time-courses. Immunosuppression due to UVA peaked after 3 d and then became refractory to further UVA doses, whereas immunosuppression due to SSUV reached near-maximal levels after two exposures and remained high after five exposures.

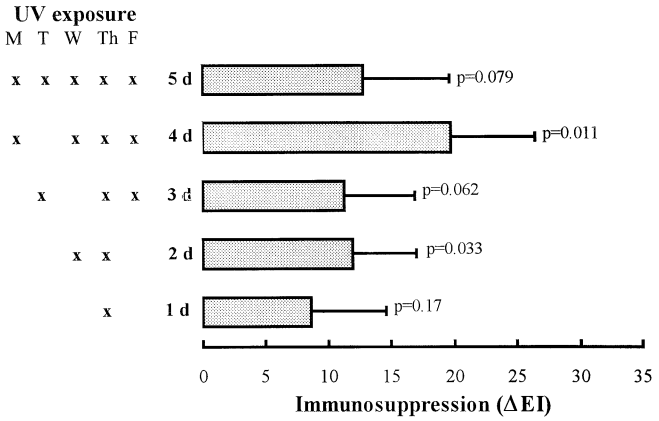

Figure 3. UVB-induced suppression of nickel CHS has a time-course parallel to that of SSUV. Immunosuppression is shown as the mean reduction in nickel-induced erythema (ΔEI) at sites exposed to 1, 2, 3, 4, and 5 d of UVB (protocol 3), patch tested on Friday and then measured 72 h later (Monday). At all time points, immunosuppression due to SSUV was greater than immunosuppression due to UVB (n = 16; mean ± SEM; two-tailed Student's t-test).

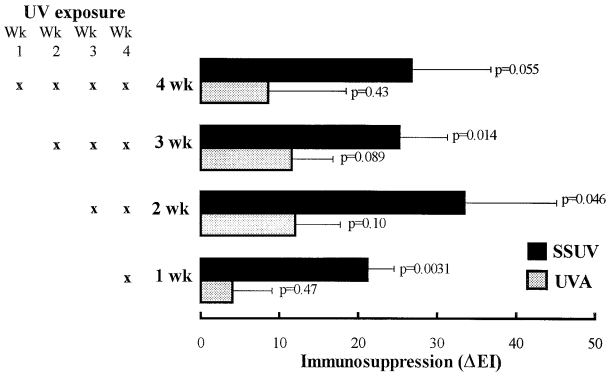

Figure 4. Chronic irradiation for 1–4 wk with SSUV but not UVA induces immunosuppression. Five volunteers were exposed to SSUV and UVA four times per week (Mon, Wed, Thu, Fri) for 1–4 wk as per protocol 4. After the final UV exposure in the fourth week, nickel patches were placed on the various irradiated areas and also on adjacent, unirradiated areas. Nickel-induced erythema at each site was read 72 h later, and immunosuppression is shown as the mean reduction in nickel-induced erythema (ΔEI) with each UV protocol (n = 5; mean ± SEM; two-tailed Student's t-test).

UVB is less immunosuppressive than SSUV, but has a similar time-course of immunosuppression At all time-points, SSUV was more immunosuppressive than UVB alone, but had an approximately parallel time-course of immunosuppression (**Fig 3**). Mean ΔEI with 1–5 d of UVB was 8.6 ± 6, 11.9 ± 5.1, 11.3 ± 5.6, 19.7 ± 6.7, and 12.8 ± 6.8, respectively, with immunosuppression reaching significance with 2 d of UVB exposure and maintaining similar levels with up to 5 d of exposure.

Chronic SSUV but not UVA induces immunosuppression In the five subjects exposed for 1–4 wk (protocol 4), significant SSUV-induced immunosuppression was observed after 1, 2, and 3 wk of exposure (mean ΔEI 21.2 ± 3.3, 33.4 ± 11.7, and 25.2 ± 6.1, respectively; $p < 0.01$, $p < 0.05$, and $p < 0.05$), and relatively high but nonsignificant immunosuppression with 4 wk (mean ΔEI 26.8 ± 10; NS, $p > 0.05$). After 1 wk (i.e., 4 d) of UVA exposure, significant immunosuppression was not observed. UVA also failed to induce immunosuppression at sites exposed for 2, 3, and 4 wk (**Fig 4**).

Elicitation of nickel CHS in unirradiated skin is not impaired by irradiation of adjacent skin In 21 of the 48 subjects in protocols 1–3, the concentration of nickel used in the final patch test (i.e., following UV exposure) was the same as that eliciting a minimal but confluent reaction in the initial patch test (i.e., without prior UV exposure). This group of 21 subjects comprised five from protocol 1, nine from protocol 2, and seven from protocol 3. In the remainder of subjects, the nickel concentration used for final patch testing was different from those causing confluent responses in the initial patch test array.

When the initial patch tests (i.e., prior to commencement of the UV exposures) were compared with the unirradiated control patch tests (i.e., after the UV exposures), the intensity of nickel CHS at the unirradiated sites was not significantly affected by exposure of adjacent skin to the various UV protocols Thus, these UV protocols caused local but not systemic immunosuppression.

DISCUSSION

In order to compare more directly the immunosuppressive effects of low-dose SSUV and its UVA and UVB components, we used various filters to modify the same xenon arc lamp. Although the doses of UVA and UVB delivered separately were reasonably well matched with the doses delivered within the SSUV irradiation, none of the filters provided a clean, "vertical" cut-off between wavebands. The filter used to generate the UVA spectrum not only blocked UVB transmission, but also attenuated the shorter-wavelength UVAII. Longer daily irradiation with the UVA source was therefore required to maintain equivalent amounts of UVAII in the UVA and SSUV spectra. Whereas this achieved almost identical total daily UVAII exposure in the two protocols, it also meant that the UVA sites received twice the UVAI of the SSUV sites.

Our UVB and SSUV spectra were erythemally equivalent and well-matched in the waveband 290–310 nm, although the UVB spectrum contained 6% less 310–320 nm radiation than the SSUV spectrum. The dichroic mirrors used to produce the UVB spectrum removed most but not all of the UVA: the "UVB" spectrum contained some UVA, predominantly UVAII, but this amount was less than 10% of that in the UVA spectrum and 17% of that in the SSUV spectrum.

Using suberythemal UV doses, equivalent to approximately 6 min of midday summer sun exposure (Diffey and Robson, 1989; Sayre *et al*, 1990) and 0.6 of the volunteers' mean solar-simulated MED, we found that elicitation of nickel CHS was significantly suppressed by even single exposures to SSUV and UVA, and by two exposures to UVB, but that the different wavebands exerted their maximal immunosuppressive effects at different time points and to different extents.

SSUV-induced immunosuppression reached significance after even one exposure, increased to near-maximal levels after two exposures and remained high at all time points up to 4 wk. In contrast, UVA-induced immunosuppression was significant after one and two exposures, but peaked with three exposures and then rapidly diminished. With 4 d–4 wk of UVA exposure, significant immunosuppression was not observed. Our UVA spectrum contained no wavelengths less than 315 nm, and the small amount of UVB present (315–320 nm) was less than 0.3% of that in the SSUV and UVB spectra. The acute immunosuppressive effects of our UVA source therefore cannot be attributed to "contaminating" UVB.

The different time-courses of SSUV and UVA-induced immunosuppression suggest different mechanisms of action. Whereas both UVA and UVB are known to have genetic effects, they appear to regulate differentially the expression of tumor suppressor and apoptosis inhibiting genes (Wang *et al*, 1998). Although UVA and UVB reduce the number and function of Langerhans cells in human skin (Aberer *et al*, 1981), UVAI was found by Baadsgaard *et al* (1989) to cause only a transient decrease in antigen-presenting cell capacity: despite significant reduction immediately after exposure to high-dose UVAI, Langerhans cell activity had fully recovered 3 d later. LeVee *et al* (1997) found that exposure to UVAII caused an influx of novel $CD1^+$ DR^+ $CD36^+$ $CD11b^+$ Langerhans cells, and suggested that this may represent a migrant subset repopulating

the epidermis after an initial reduction in density. This novel subset was not identified after irradiation with UVB. Rapid recovery of antigen-presenting activity 3 d after high-dose UVA may explain the findings of Skov *et al* (1997) that primary sensitization of human volunteers to diphenylcyclopropenone was not reduced 3 d after exposure to a single, erythemal dose of UVAI. Further, early recovery of immune function is consistent with our observation of significant immunosuppression after 3 d but not after 4 d of low-dose UVA.

Comparison of the acute immunosuppressive time-courses of SSUV and UVB revealed an approximately parallel relationship. Both spectra produced high levels of immunosuppression after 2 d, although SSUV was more immunosuppressive than UVB at all time points. This is most likely to have been due to the UVA (and perhaps predominantly the UVAII) content of the SSUV spectrum. We observed that with 2, 4, and 5 d of exposure, the "UVB-only" and "UVA-only" protocols seemed to have roughly additive effects (i.e., addition of UVB-induced and UVA-induced immunosuppression after 2, 4, and 5 d of exposure approximated the level of immunosuppression observed after two, four, and five exposures to SSUV, respectively). With three exposures, however, UVA and SSUV seemed to have effects trending in opposite directions: UVA-induced immunosuppression was maximal, whereas SSUV-induced immunosuppression was slightly lower than with either two or four exposures. This implies that, at least at certain critical time-points, the immunosuppressive effects of UVA and UVB are not simply additive but may reflect interactions between the different wavebands.

It is unclear why the immunosuppressive time-course of SSUV (i.e., UVB + UVA) paralleled that of UVB, with a slight decrease in immunosuppression after three exposures, rather than following the increase observed with three UVA exposures. One possible explanation is that the immunosuppressive effects of UVA, and particularly UVAI, may be determined by the total, cumulative UVA dose, rather than the timing or fractionation of its delivery. In this study, the dose of UVAI contained in three exposures to the UVA spectrum (≈5.1 J per cm^2) was equivalent to the total dose of UVAI contained in ≈7 exposures to the SSUV spectrum (≈5.2 J per cm^2). This could account for the increased immunosuppression seen with 2 wk of SSUV (i.e., eight exposures). Once the cumulative dose of UVAI reaches a critical level, it might then induce the adaptive mechanism(s) preventing immunosuppression by continued irradiation.

Our results contrast with previous, murine studies which have shown enhanced elicitation of CHS after exposure to relatively low, acute doses of UVB (Grabbe *et al*, 1995) as well as higher, erythemal UVB exposures (Polla *et al*, 1986). This may reflect different UV effects in mice and humans; our findings confirm those of Sjovall and Christensen (1986), who reported suppression of nickel CHS by chronic exposure of human subjects to UVB but not UVA. The UVA in their study was delivered four times per week for 3 wk, causing a small but not significant decrease in clinical assessments of the nickel reactions (12%). Our 3 wk UVA protocol, which used UVA doses approximately nine times smaller, resulted in a nonsignificant decrease in EI of 24% in our small group of five subjects. We and others have found that measurement of nickel CHS with the reflectance erythema meter provides a more sensitive, reproducible, and dose–response assessment of small changes in CHS intensity than clinical assessment of the responses (Memon and Friedmann, 1996; Damian *et al*, 1997) so it may be that Sjovall and Christensen's (1986) results would have been more similar to ours had they used an erythema meter. Alternately, it may be that higher doses of long wavelength UVA are immunoprotective, as suggested by Reeve *et al* (1998).

A possible protective mechanism of UVAI is the induction of the anti-oxidant ferritin in the dermis and epidermis. Applegate *et al* (1998) found that *in vivo* ferritin levels in human skin are markedly increased by UVAI, and to a lesser extent by UVAII, but not SSUV. Reeve *et al* (1998) have suggested that UVAI might be protective by inducing a photoproduct which competitively inhibits *cis*-urocanic acid. Thus acute UVA exposure may initially be immunosuppressive but then induce protective mechanisms. In our study, daily exposures to the SSUV and UVA spectra comprised equivalent doses of UVAII, but different doses of UVAI: the UVAI content of our UVA spectrum was double that of the SSUV spectrum. This "excess" long wavelength radiation may have prevented immunosuppression by continued UV exposure, and accounted for the short duration of our UVA-induced immunosuppression.

In contrast to our results, Moyal *et al* (1997) found that high-dose, chronic SSUV and UVA delivered to large surface areas of the body caused not only local but also systemic suppression of DTH to recall antigens in humans. With our low-dose UV protocol, using SSUV doses 2.5 times lower and UVA doses 15 times lower than Moyal's, delivered to a total irradiated surface area 200 times smaller, systemic immunosuppression did not occur. The final nickel patch test reactions adjacent to irradiated skin were not smaller than the initial, pre-irradiation reactions. Cooper *et al* (1992) used similar, suberythemal doses of UVB as in our study, delivered to a comparable surface area, and found no evidence of systemic suppression of the induction of CHS. Only when a much larger, 4 MED dose of unfiltered UVB was used was there reduced immunization through a distant, unirradiated site.

Previous work done by our group suggested that, in humans, acute low-dose UV exposure may be more immunosuppressive than chronic exposure. We found that 1 wk exposure to suberythemal SSUV caused greater suppression of DTH to tuberculin purified protein derivative in humans than 1 mo exposure (Damian *et al*, 1998). This differs from our findings that acute and chronic SSUV were equally able to suppress CHS, and supports previous suggestions that CHS and DTH responses are differentially modified by UV exposure (Kripke and Morison, 1986; Kim *et al*, 1990), possibly because of different penetration of UVB and UVA into the skin. Again, our results with chronic UV and both CHS and DTH to recall antigens suggest that there are adaptive mechanisms within the skin which limit the immunosuppressive effects of long-term UV exposure. Although subject numbers were small in this chronic UV study, the levels of immunosuppression induced after four exposures to both SSUV and UVA were comparable with the levels of immunosuppression detected in the larger, acute study.

We conclude that local CHS responses to recall antigens can be significantly suppressed by even small, suberythemal doses of UV, and that the immune effects of UVA are dependent on the amount, wavelength, and timing of UVA exposure.

We are grateful for the kind assistance of our volunteers. We also thank Mr Frank Wilkinson (Commonwealth Scientific and Industrial Research Organization) for spectral measurement of the UV source. This project was supported by the NSW Cancer Council of Australia, and a scholarship was provided for Dr. Diona Damian by the National Health and Medical Research Council.

REFERENCES

Aberer W, Schuler G, Stingl G, Honigsmann MD, Wolff K: Ultraviolet light depletes surface markers of Langerhans cell. *J Invest Dermatol* 76:202–210, 1981

Adams RM: Patch testing—a recapitulation. *J Am Acad Dermatol* 5:629–643, 1981

Applegate LA, Scaletta C, Panizzon R, Frenk E: Evidence that ferritin is UV inducible in human skin: part of a putative defense mechanism. *J Invest Dermatol* 111:159–163, 1998

Baadsgaard O, Lisby S, Wantzin GL, Wulf HC, Cooper KD: Rapid recovery of Langerhans cell autoreactivity, after in vivo ultraviolet A, but not ultraviolet B exposure of human skin. *J Immunol* 142:4213–4218, 1989

Bestak R, Halliday GM: Chronic low-dose UVA irradiation induces local suppression of contact hypersensitivity, Langerhans cell depletion and suppressor cell activation in C3H/HeJ mice. *Photochem Photobiol* 64:969–974, 1996

Black HS: Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. *Photochem Photobiol* 46:213–221, 1987

Burren R, Scaletta C, Frenk E, Panizzon RG, Applegate LA: Sunlight and carcinogenesis: expression of p53 and pyrimidine dimers in human skin following UVAI, UVAI + II and solar simulating radiations. *Int J Cancer* 76:201–206, 1998

Cooper KD, Fox P, Neisis G, Katz SI: Effects of ultraviolet radiation on human epidermal cell alloantigen presentation: initial depression of Langerhans' cell-dependent function is followed by the appearance of T6-DR+ cells that enhance epidermal alloantigen presentation. *J Immunol* 134:129–137, 1985

Cooper KD, Oberhelman L, Hamilton TA, *et al*: UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, $CD1a^{-}DR^{+}$ epidermal macrophage induction, and Langerhans cell depletion. *Proc Natl Acad Sci USA* 89:8497–8501, 1992

Damian DL, Halliday GM, Barnetson R StC: Broad-spectrum sunscreens provide greater protection against ultraviolet radiation-induced suppression of contact hypersensitivity to a recall antigen in humans. *J Invest Dermatol* 109:146–151, 1997

Damian DL, Halliday GM, Taylor CA, Barnetson R StC: Ultraviolet radiation induced suppression of Mantoux reactions in humans. *J Invest Dermatol* 110:824–827, 1998

Diffey BL, Robson J: A new substrate to measure sun protection factors throughout the ultraviolet spectrum. *J Soc Cosmet Chem* 40:127–133, 1989

European Cosmetic Toiletry and Perfumery Association: *Colipa Sun Protection Factor Test Method*. Ed. Macmillan R. Brussells, p. 21, October, 1994

Fitzpatrick TB: The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 124:869–871, 1988

Grabbe S, Steinbrink K, Steinert M, Luger TA, Schwarz T: Removal of the majority of epidermal Langerhans cells by topical or systemic steroid application enhances the effector phase of murine contact hypersensitivity. *J Immunol* 155:4207–4217, 1995

Hersey P, Hasic E, Edwards A, Bradley M, Haran G, McCarthy WH: Immunological effects of solarium exposure. *Lancet* 1:545–548, 1983

Hersey P, MacDonald M, Burns C, Schibeci S, Matthews H, Wilkinson FJ: Analysis of the effect of a sunscreen agent on the suppression of natural killer cell activity induced in human subjects by radiation from solarium lamps. *J Invest Dermatol* 88:271–276, 1987

Kim T-Y, Kripke ML, Ullrich SE: Immunosuppression by factors released from UV-irradiated epidermal cells: selective effects on the generation of contact and delayed hypersensitivity after exposure to UVA or UVB radiation. *J Invest Dermatol* 94:26–32, 1990

Kripke ML, Morison WL: Studies on the mechanism of systemic suppression of contact hypersensitivity by UVB radiation. II. Differences in the suppression of delayed and contact hypersensitivity. *J Invest Dermatol* 86:543–549, 1986

de Laat A, Kroon ED, de Gruijl FR: Cell cycle effects and concomitant p53 expression in hairless murine skin after longwave UVA (365 nm) irradiation: a comparison with UVB irradiation. *Photochem Photobiol* 65:730–735, 1997

LeVee GJ, Oberhelman L, Anderson T, Koren H, Cooper KD: UVAII exposure of human skin results in decreased immunization capacity, increased induction of tolerance and a unique pattern of epidermal antigen-presenting cell alteration. *Photochem Photobiol* 65:622–629, 1997

Memon AA, Friedmann PS: Studies on the reproducibility of allergic contact dermatitis. *Br J Dermatol* 134:208–214, 1996

Moyal D, Courbiere C, Le Corre Y, Lacharriere O, Hourseau C: Immunosuppression induced by chronic solar-simulated irradiation in humans and its prevention by sunscreens. *Eur J Dermatol* 7:223–225, 1997

Murphy GM, Norris PG, Young AR, Corbett MF, Hawk JLM: Low-dose ultraviolet-B irradiation depletes human epidermal Langerhans cells. *Br J Dermatol* 129:674–677, 1993

Polla L, Margolis R, Goulston C, Parrish JA, Granstein RD: Enhancement of the elicitation phase of the murine contact hypersensitivity response by prior exposure to local ultraviolet radiation. *J Invest Dermatol* 86:13–17, 1986

Reeve VE, Bosnic M, Boehm-Wilcox C, Nishimura N, Ley RD: Ultraviolet A radiation (320–400 nm) protects hairless mice from immunosuppression induced by ultraviolet B radiation (280–320 nm) or *cis*-urocanic acid. *Int Arch Allergy Immunol* 115:316–322, 1998

Sayre RM, Cole C, Billhimer W, Stanfield J, Ley RD: Spectral comparison of solar simulators and sunlight. *Photodermatol Photoimmunol Photomed* 7:159–165, 1990

Sjovall P, Christensen OB: Local and systemic effect of ultraviolet radiation (UVB and UVA) on human allergic contact dermatitis. *Acta Derm Venereol (Stockh)* 66:290–294, 1986

Skov L, Hansen H, Barker JNWN, Simon JC, Baadsgaard O: Contrasting effects of ultraviolet-A and ultraviolet-B exposure on induction of contact hypersensitivity in human skin. *Clin Exp Immunol* 107:585–588, 1997

Vermeer M, Schmieder GJ, Yoshikawa T, van den Berg JW, Metzman MS, Taylor JR, Streilein JW: Effects of ultraviolet B light on cutaneous immune responses of humans with deeply pigmented skin. *J Invest Dermatol* 97:729–734, 1991

Wang Y, Rosenstein B, Goldwyn S, Zhang X, Lebwohl M, Wei H: Differential regulation of *P53* and *Bcl-2* expression by ultraviolet A and B. *J Invest Dermatol* 111:380–384, 1998

Webber LJ, Whang E, De Fabo EC: The effects of UVA-I (340–400 nm), UVA-II (320–340 nm) and UVA-I+II on the photoisomerisation of urocanic acid *in vivo*. *Photochem Photobiol* 66:484–492, 1997