Sunlight-Induced Immunosuppression in Humans Is Initially Because of UVB, Then UVA, Followed by Interactive Effects

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Solar-simulated ultraviolet radiation (ssUV) suppresses immunity in humans. The ultraviolet B (UVB) waveband is recognized as immunosuppressive; however the relative significance of UVA to ssUV immunosuppression is unknown. We created dose and time–response curves for UVB-, UVA-, and ssUV-induced suppression of memory immunity to nickel in humans. UVB caused immunosuppression within 24 h. UVA immunosuppression required 48 h and was normalized by 72 h. UVB alone accounts for ssUV immunosuppression at 24 h, but both UVB and UVA contributed at 48 h. By 72 h neither waveband accounted for ssUV immunosuppression. An interaction between these wavebands was therefore the major contributor. To confirm this dose–response curves were used to determine immune protection factors (IPF) for sunscreens with nickel challenge 72 h following ssUV. A sunscreen with good UVA protection had an IPF twice that of a poor UVA protector, despite providing similar protection from sunburn. Thus UVA was a major contributor to ssUV-induced immunosuppression at 72 h but only with the cooperation of UVB. Hence, UVB initiates immunosuppressive signals within 24 h, followed by UVA at 48 h, then an interaction between UVB and UVA. By 72 h following ssUV exposure, neither UVB nor UVA, but an interaction between them is the major cause of sunlight-induced immunosuppression.

Key words: immunosuppression/skin cancer/sunlight/sunscreens/ultraviolet
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Skin cancer continues to be a major worldwide healthcare issue and is largely caused by ultraviolet (UV) radiation in sunlight (de Gruijl, 1999). Not only does UV induce genetic mutations (Wikonkal and Brash, 1999), but also the importance of UV-induced suppression of cell-mediated immunity is well established in photocarcinogenesis (Beissert and Schwarz, 1999). The increased incidence of non-melanoma skin cancers in chronically immunosuppressed transplant populations further supports the role of cell-mediated immunity in tumor surveillance (Lindelof *et al*, 2000).

The relative roles of the UVA (320–400 nm) and UVB (290–320 nm) wavebands in sunlight-induced cutaneous immunosuppression are unclear, particularly in humans. Although it is generally accepted that UVB suppresses both contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses in humans and animal models, the role of UVA is more controversial. In some studies UVA has been found to suppress the induction of local primary immunity in mice (Bestak and Halliday, 1996), but others have suggested that UVA does not suppress the immune system, but protects from UVB (Reeve *et al*, 1998). UVA has also been shown to suppress the reactivation of memory immunity in mice (Nghiem *et al*, 2001) and humans (Damian *et al*, 1999; Moyal and Fourtanier, 2001; Kuchel *et al*, 2002). Recent studies have shown that immunomodulation is dependent on the dose of UVA used; with moderate doses being immunosuppressive and higher doses immunoprotective (Byrne *et al*, 2002).

Sunscreen studies have further delineated the role of UVA in solar-simulated ultraviolet (ssUV) -induced immunosuppression. Studies in mice (Bestak *et al*, 1995) and humans (Damian *et al*, 1997; Kelly *et al*, 2003; Wolf *et al*, 2003) have determined that broad-spectrum sunscreens that protect from both UVA and UVB provide better defense against ssUV suppression of CHS than UVB only protective sunscreens. Similarly, sunscreens with higher UVA protection factors have improved protection of the elicitation of DTH responses than sunscreens with low levels of UVA protection (Fourtanier *et al*, 2000). We have recently demonstrated that immune protection by sunscreens does not correlate with protection of erythema, and that the level of sunscreen UVA protection is important for determining the level of immune protection from ssUV (Poon *et al*, 2003). This indicates that the UVA waveband within ssUV, either independently or in a manner that it dependent on UVB, is an important contributor to ssUV-induced immunosuppression.

Few studies have addressed the comparative importance of UVB and UVA in sunlight, or ssUV-induced immunosuppression. This is an unresolved issue, particularly in humans. To address this we have adapted our model of repetitive exposure ssUV-induced suppression of the

Abbreviations: CHS, contact hypersensitivity; DTH, delayed-type hypersensitivity; IPF, immune protection factor; LC, Langerhans cells; MISD, minimum immunosuppressive dose; SPF, sun protection factor; ssUV, solar simulated ultraviolet radiation; UV, ultraviolet radiation; UVA-PF, UVA protection factor.

reactivation of memory immunity to nickel in humans (Damian and Halliday, 2002) to a single irradiation protocol. This has enabled us to determine time and dose–response curves for UVA-, UVB- and ssUV-induced immunosuppression. We have also used sunscreens with low and high UVA protective capability to remove the contribution of UVB from ssUV-induced immunosuppression. These studies show that UVB initiates the earliest immunosuppressive signals from ssUV, within 24 h, and that UVA is also immunosuppressive, but not until 48 h following exposure. By 72 h following exposure an interaction between these two wavebands makes ssUV much more suppressive than either waveband independently.

Results

Time course and dose–response of UV-induced immunosuppression UVB caused a dose-related immunosuppression. Antigen challenge 24 h after irradiation caused significant immunosuppression at 500 mJ per cm^2 UVB, whereas antigen challenge 48 and 72 h after irradiation caused significant immunosuppression at 300 and 500 mJ per cm^2 (Fig 1). Therefore the biological changes caused by UVB that lead to immunosuppression occurred within the first 24 h following irradiation and were maintained for at least 72 h.

The response to UVA differed from UVB. The doses used were the relative amounts present in ssUV that corresponded to each UVB dose, 7.7 times the UVB doses. UVA did not cause significant immunosuppression at any dose tested when antigen was applied 24 h after irradiation (Fig 1). Antigen challenge 48 h following UVA exposure caused significant immunosuppression at 2310 and 3850 mJ per cm^2. When the time between UVA exposure and antigen challenge was increased to 72 h, significant immunosuppression was not observed at any dose tested. Therefore the biological changes caused by UVA that lead to immunosuppression take longer to develop than for UVB, and also recover more rapidly so that immunosuppression is only observed over a narrow time frame between irradiation and antigen challenge.

The response to ssUV differed from both UVB and UVA. The ssUV doses contained the amounts of UVB and UVA that had been tested separately (i.e., 1740 mJ per cm^2 ssUV comprised of 200 mJ per cm^2 UVB and 1540 mJ per cm^2 UVA). Antigen challenge 24 or 48 h following ssUV caused significant immunosuppression only at the highest dose tested, 4350 mJ per cm^2. In contrast, when the time between ssUV exposure and antigen was increased to 72 h, significant immunosuppression was observed at all doses tested; even the lowest dose caused significant immunosuppression (Fig 1). Thus ssUV-induced immunosuppres-

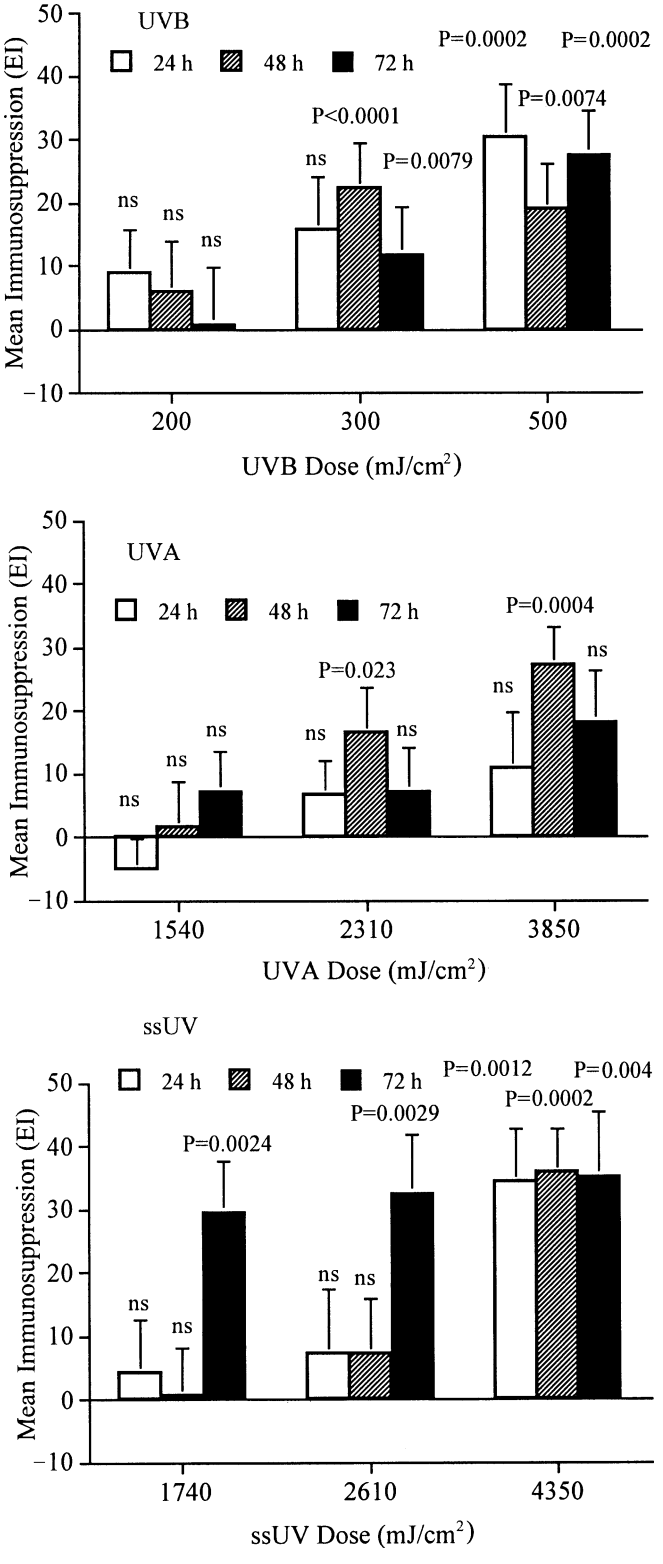

Figure 1
Single-exposure ultraviolet (UV) immunosuppression time course and dose response. In separate experiments groups of 15 nickel-allergic volunteers were irradiated with a single exposure to UVB, UVA or solar-simulated UV (ssUV). Nine different areas on the lower back of each volunteer received one of three UV doses at each of 24 h (*open columns*), 48 h (*hatched columns*) and 72 h (*closed columns*) prior to nickel challenge to generate dose and time responses for each of the three UV spectra. The UVA and UVB doses used were their relative contributions to the ssUV dose. The resultant erythema from the nickel CHS response at each irradiated site was read 72 h after nickel challenge using an erythema meter and subtracted from an unirradiated positive control site to calculate immunosuppression (erythema index). Mean ± SEM are shown for the 15 volunteers. A paired two-tailed Student's *t* test was used to compare each test site with the unirradiated control to determine whether the immunosuppression at each dose was significant, and this is shown by the p value centerd above each column.

sion is considerably greater when antigen is not applied until 72 h following ssUV exposure.

The shape of the spectral curve for UVB closely matched the UVB portion of the ssUV spectrum and the shape of the spectral curve for UVA closely matched the UVA portion of the ssUV spectrum above 340 nm (Fig 2). Thus it is reasonable to compare the results from the different spectra. But, as UVB, UVA, and ssUV were tested in different experiments on separate groups of volunteers, the magnitude of the responses cannot be directly compared between experiments as the groups may have different sensitivities to UV. Relationships within an experiment, however, can be analyzed further. At 24 h ssUV was suppressive only at the highest dose tested (4350 mJ per cm^2, made up of 500 mJ per cm^2 UVB and 3850 mJ per cm^2 UVA). UVB also caused significant immunosuppression only at the highest dose of 500 mJ per cm^2, the relative component of the highest ssUV dose. UVA was not immunosuppressive at any dose tested at 24 h. At 48 h the ssUV dose response was not significantly different from the 24 h dose response ($p = 0.893$, repeated-measures ANOVA). UVB remained significantly immunosuppressive at 48 h with no significant difference from the dose response at 24 h ($p = 0.537$, repeated-measures ANOVA). UVA, however, became immunosuppressive at 48 h with the UVA dose response being significantly greater than at 24 h ($p = 0.040$, repeated-measures ANOVA). Thus ssUV-induced immunosuppression 24 h following antigen challenge is primarily caused by UVB, but at 48 h is because of both UVB and UVA being independently immunosuppressive.

At 72 h the ssUV-induced dose response increased significantly compared with 24 h ($p = 0.007$, repeated-measures ANOVA) and 48 h ($p = 0.016$). This contrasts with the UVB dose response at this time, which did not significantly change compared with either the 24 h ($p = 0.322$) or 48 h UVB dose responses ($p = 0.409$, repeated-measures ANOVA). Additionally UVA was not immunosuppressive at any dose at this time, and the UVA dose response decreased so that it was no longer significantly different from the 24 h dose response ($p = 0.205$). Thus, as ssUV immunosuppression increased at this time, whereas the response to the UVB waveband did not, and the response to UVA was not suppressed, an interaction between these wavebands appeared to be the major cause of immunosuppression at 72 h.

Immune protection factor (IPF) for sunscreen with good UVA protection is higher than for sunscreen with poor UVA protection In further studies, sunscreens with different UVA-protective capacities were tested for immunoprotection with antigen applied 72 h following ssUV exposure. Both sunscreens absorb greater than 95% of UVB (Fig 3). Whereas sunscreen B maintains good absorption up to 380 nm, sunscreen A shows a sharp drop at about 320 nm to 60%, and another sharp drop to 30% at about 370 nm and therefore does not provide as good a protection from UVA as sunscreen B.

None of the sunscreens had any significant effect on the nickel reaction as there was no significant difference between unirradiated untreated positive controls and unirradiated sunscreen-treated controls (paired Student's *t* test).

The minimum immunosuppressive doses (MISD) for unprotected sites was determined from linear regression analysis as the ssUV dose that caused 30% immunosuppression. Using the same cutoff, the MISD for the sunscreen-protected sites was determined in a similar manner (Fig 4). The IPF for each sunscreen was calculated as the ratio of MISD (sunscreen protected) to MISD (unprotected) in the same group of individuals. With ssUV irradiation 72 h prior to antigen challenge, the IPF for sunscreen A (sun protection factor (SPF) 15, poor UVA protector with a UVA protection factor (UVA-PF) of 2.3) was determined to be 10.3 (95% confidence interval 1.6–18.3). For sunscreen B

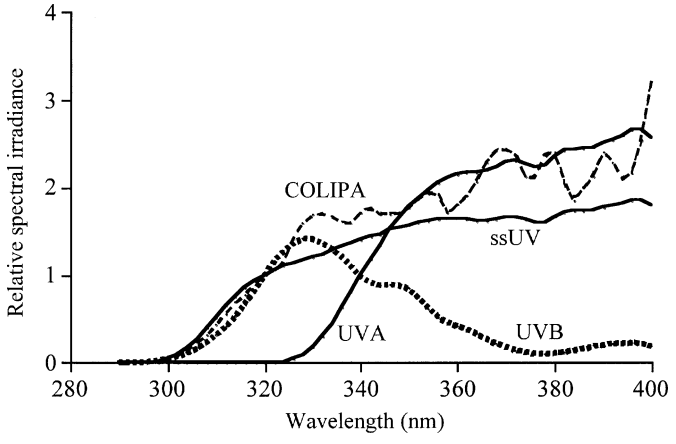

Figure 2
Spectra used in this study. An Oriel 1000 W solar simulator equipped with twin 280–400 nm dichroic mirrors and an atmospheric attenuation filter produced a solar-simulated ultraviolet spectrum that closely approximates standard sunlight as defined by COLIPA. Using a ultraviolet (UV)B/UVC-blocking filter in place of the atmospheric attenuation filter produced a UVA spectrum. Two 280–320 nm dichroic mirrors were used together with the atmospheric attenuation filter to produce the UVB spectrum. The ssUV, COLIPA, and UVB spectra were normalized to 1 at 320 nm, whereas the UVA spectrum was normalized at 340 nm to enable them to be compared.

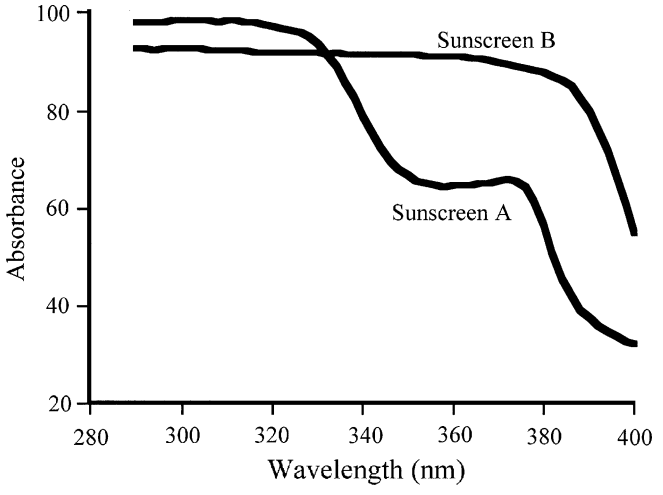

Figure 3
Sunscreen B absorbs more ultraviolet A than sunscreen A. The spectral absorbance of sunscreens A and B was obtained using a Labsphere UV-1000 SPF analyzer (Labsphere, North Sutton, New Hampshire) with sunscreen applied at 2 mg per cm^2 onto a quartz plate substrate profiled with the topography of human skin derived from casts of human test back skin.

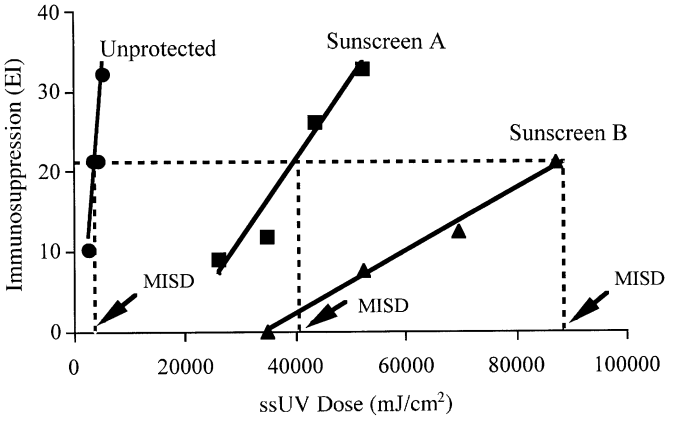

Figure 4
Immune protection factor determination for sunscreens with solar-simulated ultraviolet (ssUV) 72 h prior to antigen challenge. ssUV immunosuppression dose–response curves using four separate ssUV doses 72 h prior to nickel challenge on skin that was unprotected (*circles*) or protected with the low ultraviolet A (UVA) protecting sunscreen A (*squares*) or the high UVA protecting sunscreen B (*triangles*). Immunosuppression was calculated as the difference between an unirradiated control site and each ssUV-irradiated test site as described in the legend to Fig 2. A mean of 15 volunteers is shown. A line of best fit was obtained using linear regression analysis (*solid lines*). The minimal immunosuppressive dose was calculated from this line of best fit as that ssUV dose that gave 30% immunosuppression in unprotected skin, which was an immunosuppression of 21 erythemal index in this experiment (*dashed lines*).

(SPF 16, good UVA protector with a UVA-PF of 4.5), its IPF with a single ssUV exposure 72 h prior to antigen was 22.5 (95% confidence interval 20.3–25.1). Thus, despite having very similar SPF, sunscreen B, with about twice the UVA protection as sunscreen A, provided about double the immunoprotection as sunscreen A. This proves that UVA made a substantial contribution to ssUV-induced immunosuppression when antigen was applied 72 h following ssUV exposure, despite UVA not being autonomously immunosuppressive at this time point. This further supports the findings from the dose responses (Fig 1) that an interaction between UVB and UVA is responsible for ssUV-induced suppression to antigen challenge 72 h following ssUV exposure.

Discussion

Previously, we have demonstrated significant suppression of recall immunity to nickel using multiple daily suberythemal ssUV exposures (Damian and Halliday, 2002). This study has shown that a single ssUV exposure caused significant suppression of the nickel CHS response in a time- and concentration-dependent manner. In this study, we found ssUV to be immunosuppressive when antigen challenge was 24–72 h following irradiation. ssUV caused significant immunosuppression at doses as low as 1740 mJ per cm^2 72 h prior to antigen challenge. The average MED of subjects with the range of skin types that we tested was about 4000 mJ per cm^2 (Kuchel *et al*, 2002, 2003; Friedmann *et al*, 2004) and therefore immunosuppression occurred at ssUV doses lower than what was required to cause sunburn. We have also recently shown that a single exposure to 3420 mJ per cm^2 ssUV 24 or 48 h prior to elicitation of a DTH to tuberculin-purified protein resulted in a suppressed response (Friedmann *et al*, 2004). Thus single exposures to suberythemal doses of ssUV can suppress the reactivation of memory CHS and DTH.

These studies have shown that within the first 24 h, ssUV-induced immunosuppression is caused by the UVB waveband, but by 48 h the UVA waveband contributes independently to ssUV immunosuppression with UVB maintaining a role. By 72 h interactive effects between UVB and UVA are the prime cause of ssUV-induced immunosuppression with the independent role of UVB diminishing and UVA no longer being independently immunosuppressive. It is therefore time dependent, which waveband within ssUV is the main cause of sunlight-induced immunosuppression. The ssUV spectrum approximated sunlight closely, and the UVA spectrum contained no detectable UVB; however, the UVB spectrum was contaminated with significant levels of UVAII.

The transient immunosuppressive effect of UVA may explain why some studies have found that UVA is immunoprotective rather than immunosuppressive (Reeve *et al*, 1998). Dose is also likely to be an important factor as we have previously shown that whereas UVA doses within the range used in this study are immunosuppressive in mice, higher doses are immunoprotective (Byrne *et al*, 2002). Previous studies of UVA-induced suppression of recall immunity to nickel in humans have shown that whereas 1942 mJ per cm^2 UVA (within the range used in this study) was immunosuppressive (Damian *et al*, 1999), 17,800 mJ per cm^2 UVA (4.5 times the highest dose used here) did not modulate immunity (Kuchel *et al*, 2002). UVA is therefore a complex immunomodulator that is both time and concentration dependent. A single exposure to 8000 mJ per cm^2 ssUV or UVA, doses nearly twice as high as those used in this study, caused similar levels of suppression of recall DTH to *Candida albicans* when the antigen challenge was 24 h following exposure in mice (Nghiem *et al*, 2001). This shows that under these conditions UVA was crucial for ssUV-induced immunosuppression and our data presented here is in agreement with this, supplying evidence that UVA is central for ssUV-induced suppression of recall immunity in humans.

Interactions between UVA and other wavebands have previously been reported. Doses of UVA too high to be independently immunosuppressive (17,800 mJ per cm^2 daily for 4 consecutive days) enhanced ssUV suppression of recall immunity to nickel in humans (Kuchel *et al*, 2002). In these experiments, whereas UVA alone 72 h prior to antigen challenge was not immunosuppressive, a sunscreen with good UVA protection provided about twice the level of immunoprotection as a sunscreen with poor UVA protection. This was despite these two sunscreens providing similar levels of protection from UVB. Thus UVA made a vital contribution to immunosuppression at 72 h via an interactive effect with UVB.

It is unclear why UVB caused immunosuppression more rapidly than UVA, with an interaction eventually occurring to cause a more profound immunosuppression. It is possible that different sequences of events are initiated by these

wavebands that take different times to develop, but eventually interact with each other. UVB initiates systemic immunosuppression in mice by forming the genetic lesion cyclobutane pyrimidine dimers (CPD) in DNA (Kripke *et al*, 1992), and we have found CPD to also be involved in ssUV-induced suppression of recall immunity to nickel in humans (Halliday *et al*, 2004). But UVA mediates much of its damage by production of reactive oxygen species (ROS) (Halliday, 2005), producing a different genetic lesion, 8-hydroxy-2′-deoxyguanine (Cadet *et al*, 2005). It is not known whether 8-hydroxy-2′-deoxyguanine initiates UVA-induced immunosuppression; however, ROS are involved in mice (Yuen *et al*, 2002) and humans (Clement-Lacroix *et al*, 1996; Kuchel *et al*, 2003). It is possible that discrete downstream events resulting from different initiating events such as distinct types of genetic damage may be responsible for the different times taken for UVB and UVA to suppress immunity to antigen, and that these may eventually interact to make ssUV more immunosuppressive than either waveband alone. In support of this, UVB and UVA cause the production of different but undefined immunosuppressive factors from keratinocytes (Kim *et al*, 1990).

Alternatively, UVA and UVB may initiate similar immunosuppressive signals, with UVA taking longer than UVB at the doses we used, and the combination of UVA and UVB compounding ssUV immunosuppression at some point. *Trans*-urocanic acid (UCA) is another photoreceptor that isomerizes to *cis*-UCA in human skin upon excitation with UVB and UVA (Kammeyer *et al*, 1995) and is a mediator of UVB-induced immunosuppression (Norval and El-Ghorr, 2002). Whether it is also involved in UVA immunomodulation is unclear. Langerhans cells (LC), dendritic antigen-presenting cells of the epidermis, are the initial cells to induce immunity in the epidermis. Damage to the function and network of LC is important for UVB-induced suppression of the induction of primary immunity (Ullrich, 2005). Although the involvement of LC in suppression of recall immunity is less clear, we find that immunosuppressive doses of ssUV reduce the number of these cells in human skin (Kuchel *et al*, 2003, 2005). UVA also reduces the number (Yuen *et al*, 2002) and function (Clement-Lacroix *et al*, 1996) of epidermal LC. It is possible that UVB and UVA both cause similar molecular and cellular changes such as UCA isomerization or LC damage but over different time courses with an eventual cumulative effect making ssUV more immunosuppressive than either waveband alone. IL-10, CPD, and transferable antigen-specific NKT suppressor cells have been shown to be at least partially responsible for both ssUV and UVA suppression of recall DTH to *C. albicans*, suggesting that at least some common suppressive mechanisms are activated by ssUV and UVA (Nghiem *et al*, 2002).

In summary, these studies show that UVB and UVA contribute to sunlight-induced immunosuppression in humans, but with different time courses. UVB causes the suppressive effects of sunlight 24 h after irradiation, whereas UVA and UVB independently are part of the cause of sunlight immunosuppression at 48 h. Whereas UVA unaided is no longer suppressive 72 h after irradiation, it cooperates with UVB to make ssUV much more immunosuppressive than either waveband alone. This interactive effect of UVA and UVB is a more potent cause of immunosuppression than either UVA or UVB alone. The different times required for UVB, UVA, and their interactions to suppress recall immunity to nickel in humans may be because of UVB and UVA activating different molecular pathways that take dissimilar times to develop, but ultimately interact. Alternatively, UVB and UVA may instigate similar biological events, but to dissimilar extents so that they take different times to develop but eventually compound each other to have a greater effect than each waveband alone.

Materials and Methods

Subjects Ethical approval for this study was obtained from both the Central Sydney Area Health Service and University of Sydney Ethics Committees in accordance with the Helsinki guidelines. Volunteers were recruited from the general population by advertisement and gave their informed consent for the studies. Exclusion criteria included sun exposure to the test sites for 4 wks prior to the study and taking any anti-inflammatory or non-steroidal medications. A total of 45 female nickel-allergic volunteers were recruited for the UV-immunosuppression trials, and 15 females were recruited for the sunscreen IPF studies. In the UVB trial the age range was 18–66 y, one with skin type I, seven skin type II, five skin type III, and two skin type IV. In the UVA trial the age range was 19–67 y, four with skin type I, eight skin type II, three skin type III. In the ssUV trial the age range was 18–66 y, three with skin type I, six skin type II, five skin type III, and one skin type IV. In the sunscreen trial the age range was 18–61 y, six skin type II, six skin type III, and three skin type IV.

UV source In these experiments, the UV source was an Oriel 1000 W solar simulator (Oriel, Stratford, Connecticut) equipped with twin 280–400 nm dichroic mirrors (Oriel) and an atmospheric attenuation filter (Oriel) to produce an ssUV spectrum. To produce a UVA-only spectrum, a UVB/UVC blocking filter (Oriel Serial no. 81017) was used instead of the atmospheric attenuation filter. Two 280–320 nm dichroic mirrors (Oriel) with the atmospheric attenuation filter produced the UVB-only spectrum. These spectra are compared with standard sunlight as defined by COLIPA (1994) in Fig 2.

Irradiance and spectra was monitored with a scanning spectrophotometer (Optronics, Orlando, Florida) and UV output monitored daily with an IL1350 broadband radiometer using SED 038 (UVA) and SED 240 (UVB) detectors (International Light, Newburyport, Massachusetts) that were calibrated against the source with the spectrophotometer. The integrated irradiance at the skin surface for the solar-simulated spectrum was 3.4 mW per cm^2 UVB, 6.9 mW per cm^2 UVA II, and 19.2 mW per cm^2 UVA I (UVA/UVB ratio of 7.7). The ssUV spectrum was a close approximation to sunlight, although it contained a lower ratio of UVA to UVB. The UVA spectrum had a sharp cutoff at 320 nm containing no detectable UVB, but was deficient in the 320–340 nm waveband compared with sunlight. The shape of the UVB spectrum from 290 to 320 nm closely matched this portion of the sunlight and ssUV spectra but was contaminated with some UVA (Fig 2).

Sunscreens The two sunscreens were purchased commercially in Australia and were both labelled as broad spectrum. Sunscreen A contained 7.5% zinc oxide, 8% octylmethoxycinnamate, and 2.5% 4-methylbenzylidene camphor. Sunscreen B contained 3% titanium dioxide, 4.2% 4-tert butyl-4-methoxybenzoylmethane, and 10.5% octocrylene. The same sunscreen application method and amount (2 mg per cm^2) used in previous *in vivo* SPF and IPF studies were used (Poon *et al*, 2003). Sunscreen was applied at least 15 min prior to irradiation and allowed to dry.

These sunscreens were chosen because we have previously found that they have similar *in vivo* SPF but different IPF and UVA-PF (Poon *et al*, 2003). Note that these IPF reported previously were determined using repeated ssUV exposures on a daily basis for

4 consecutive days. The SPF of sunscreen A was 15, but it is a poor UVA protector with a Diffey critical wavelength of 368 nm, a Boots UVA ratio of 0.33, and a UVA-PF of 2.3. From previous IPF studies using multiple UV irradiations it had an IPF of 9.5. Sunscreen B had a similar *in vivo* SPF of 16, but better UVA protection with a Diffey critical wavelength of 383 nm, a Boots UVA ratio of 0.85 and a UVA-PF of 4.5. Its IPF from previous studies was 20.7. It is important to note that this study was performed with the same UV source as the previous study where the SPF and UVA protection factors of the sunscreens were determined, but this study used a single, rather than multiple ssUV exposures to determine the IPF.

UV immunosuppression time courses and dose responses Determination of UV-induced suppression of nickel CHS in humans was as detailed previously (Damian and Halliday, 2002) except that a single exposure to UV was used. Nickel-allergic volunteers had three separate 6×2 cm areas on their lower midbacks irradiated with a single exposure 24, 48, or 72 h prior to nickel challenge. Each area was demarcated into three test sites that each received a different dose of UV. Thus, there were nine UV-irradiated test sites each of which received one of three UV doses at one of three times prior to antigen challenge. There was also a separate unirradiated control site. The areas were irradiated on different days (Tuesday, Wednesday, or Thursday) so that nickel patch testing was at the same time (Friday). Nine millimeters Finn Chambers (Epitest, Tuusula, Finland) containing nickel sulfate ($NiSO_4 \cdot 6H_2O$) in a petrolatum base were applied to each of the test sites and the unirradiated control. Each volunteer had the same concentration of nickel applied to all test sites with concentrations being individualized for each volunteer (such that the dose that produced confluent erythema without vesiculation in initial testing of that subject). These nickel patches were then removed 48 h later, with the nickel-induced CHS being quantitated after a further 24 h 72 h following nickel challenge. Objective measurement of the nickel-induced CHS responses was obtained using a reflectance spectrometer/erythema meter (Diastron, Hampshire, UK). The nickel-induced erythema index (EI) for each test site was calculated as the difference of the average of three readings taken at each test site and the average of three readings taken of adjacent skin.

This same experimental procedure was repeated on different groups of volunteers receiving different UV spectra (UVB, UVA, or ssUV). For the UVB spectrum, the UVB doses contained within the ssUV spectrum experiment were used (200, 300, and 500 mJ per cm^2 UVB). The doses used in the UVA only experiment were the UVA components of the ssUV experiment (1540, 2310, or 3850 mJ per cm^2 UVA; 7.7 times the UVB doses).

Data analysis Immunosuppression was calculated at each test site by subtracting the nickel-induced EI of the test site from that of the unirradiated control. The results of each test site in 15 volunteers for each UV spectrum were pooled, and the average immunosuppression at each test site was calculated.

To determine whether a particular dose of UVB, UVA, or ssUV caused a significant level of immunosuppression, the nickel-induced EI at the appropriate test site was compared with the unirradiated positive control in the same individual using a paired two-tailed Student's *t* test. Immunosuppression was considered significant if $p < 0.05$.

For a particular UV spectrum, differences in the dose responses at each time between irradiation and antigen challenge were analyzed by repeated-measures ANOVA.

MISD and IPF were calculated as described previously (Poon *et al*, 2003). The average immunosuppression with and without sunscreen protection in the group of 15 volunteers (calculated as described above) was plotted against ssUV dose and linear regression analysis was used to determine the dose of ssUV that caused 30% immunosuppression (compared with the unirradiated control sites). This was deemed to be the MISD. The IPF for each sunscreen was the ratio of the MISD with sunscreen protection to the MISD without protection. Ninety-five percent confidence intervals were calculated using 10,000 bootstrap samples for each sunscreen.

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