Ultraviolet A Irradiation of C57BL/6 Mice Suppresses Systemic Contact Hypersensitivity or Enhances Secondary Immunity Depending on Dose

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Ultraviolet radiation is the most common environmental carcinogen humans are exposed to. It is now known that in order for skin cancers to develop, both genetic damage and immunosuppression is required. Ultraviolet-induced immunosuppression is therefore a key contributor to the development of skin cancer. Little is known about the relative contributions of the different ultraviolet spectra (A and B), however. Therefore detailed ultraviolet dose–response curves for systemic suppression of contact hypersensitivity in two mouse strains were determined to examine the relative contributions of each of these spectral components of sunlight to primary and secondary immunity. Whereas ultraviolet B caused a linear dose-related immunosuppression in both C57BL/6 and Balb/c mice, only C57BL/6 mice were immunosuppressed by medium doses of ultraviolet A. At higher ultraviolet A doses, C57BL/6 mice were protected from immunosuppression, suggesting a genetic predisposition to ultraviolet-A-induced immunomodulation. Surprisingly, we found that, in contrast to primary immunosuppression, low dose ultraviolet A enhanced the secondary immune response, whereas ultraviolet B caused antigen-specific tolerance. When ultraviolet A and ultraviolet B were combined to mimic sunlight (solar-simulated ultraviolet), immunosuppression and tolerance were only observed over a narrow dose range as the memory-enhancing effect of low dose ultraviolet A and the immunoprotective effect of higher dose ultraviolet A prevented the suppressive effects of ultraviolet B. These studies suggest that complex relationships between ultraviolet dose, immunomodulation, spectra, and genetic background are likely to be important for skin cancer induction. We also describe for the first time that low doses of ultraviolet A are able to enhance secondary immunity, which has important implications for vaccination strategies. ***Key words: immunomodulation/immunosuppression/skin cancer/sunlight/ultraviolet. J Invest Dermatol 119:858–864, 2002***

The ultraviolet (UV) wavelengths in sunlight are the prime etiologic agents responsible for causing both melanoma (Armstrong and Kricker, 1993; Klein-Szanto *et al*, 1994) and epithelial skin cancer (Kricker *et al*, 1995; Li *et al*, 1995). Sunlight is made up of both UVB (290–320 nm) and UVA (320–400 nm) with the UVB component being at a much lower intensity than UVA. UV radiation-induced suppression of the immune system is an important step in carcinogenesis as it prevents the natural defence against skin cancer. The Food and Drug Administration (FDA, *Federal Register*, Vol. 64, no. 98, Friday May 21, 1999, pages 27666–27693) have recently acknowledged that both UV-induced genetic mutation and immunosuppression are required to develop skin cancer (Donawho and Kripke, 1991). Therefore, to understand and protect against skin cancer, it is necessary to determine the doses of UV that influence the immune system as well as the role of different wavebands in sunlight. Controversy surrounds the relative roles of UVA compared to UVB, however, and comparative dose–responses have not been established. UVA can induce immunosuppression in both mice and humans (Halliday *et al*, 1998; Damian *et al*, 1999; Nghiem *et al*, 2001), and in animal models UVA has been shown to contribute to the development of both squamous cell carcinoma (Kelfkens *et al*, 1992) and melanoma (Ley, 1997). Others have also found that UVA does not alter immunity, however (Skov *et al*, 1997; Dittmar *et al*, 1999). Conversely, there is evidence that high doses of UVA can protect mice and humans from UVB-induced immunosuppression (Reeve *et al*, 1998; Skov *et al*, 2000; Garssen *et al*, 2001). The experiments described here aimed to clarify the roles of UVA and UVB in UV-induced systemic suppression and tolerance to contact sensitization by constructing dose–response curves for UVA, UVB, and solar-simulated UV radiation (ssUVR). The results show complex interactions dependent on dose, spectra, and genetic background.

Manuscript received May 20, 2002; revised June 19, 2002; accepted for publication July 9, 2002

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Abbreviations: CHS, contact hypersensitivity; MEdD, minimal edematous dose; ssUVR, solar-simulated ultraviolet radiation; Th, T helper.

MATERIALS AND METHODS

Mice Female C57BL/6 and Balb/c mice aged 7–8 wk at the start of irradiations were used in these experiments (Animal Resource Center, Perth, WA, Australia), which were conducted with the approval of the Sydney University animal ethics committee.

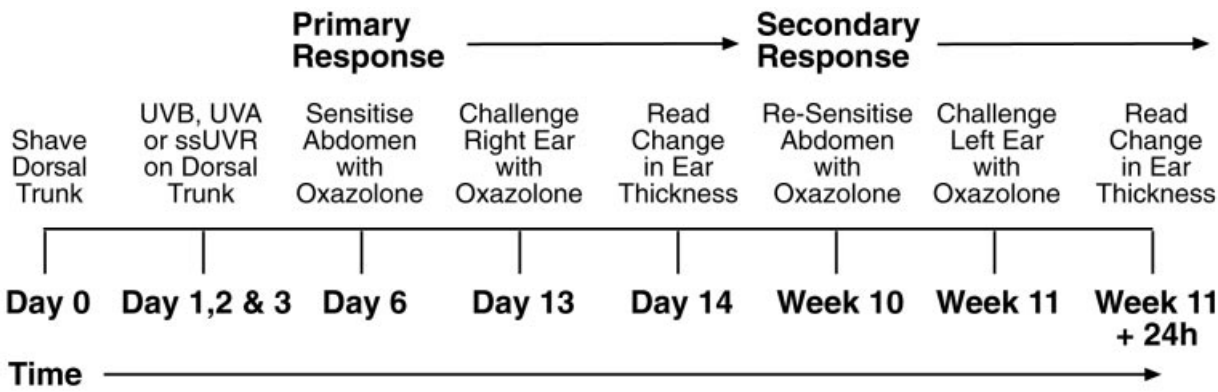

Figure 1. Experimental design showing time line and order of procedures.

UV source UVA, UVB, and ssUVR spectra were produced with a 1000 W xenon arc solar simulator (Oriel, Stratford, CT). For ssUVR, two dichroic mirrors that each allow wavelengths between 200 nm and 400 nm to pass through were used in conjunction with an atmospheric attenuation filter to produce a spectrum closely resembling sunlight. By changing this filter for one that blocks UVB radiation, a spectrum containing mostly UVA was produced. Alternatively, by changing the two dichroic mirrors to ones that reflect wavelengths between 260 nm and 320 nm, a spectrum containing mostly UVB was produced. The intensity (mW per cm^2) of the UV output was measured continuously using a radiometer (Solar Light Company, PA), and the timing of UV exposure was adjusted with an automated timing device so that accurate UV doses (measured in mJ per cm^2) could be delivered to individual mice. Spectral output of the solar simulator (both intensity and wavelength) was measured using an OL-754 spectroradiometer (Optronic Laboratories, Orlando, FL), which was calibrated against standard lamps for spectra and intensity and was used to calibrate the radiometer against the source. Additionally, the spectral output of the sun was measured for comparison on October 10, 2001, at midday on a cloudless day in Sydney, Australia.

UV irradiations The minimum dose to induce edema (the minimum edematous dose, MEdD) was determined by exposing groups of six to eight mice to various doses of ssUVR. The pre-UV and 24 h post-UV skin thickness was measured using a hand-held high frequency ultrasound (Longport International, Silchester, U.K.) with the minimum dose of ssUVR required to cause a significant increase in skin thickness being the MEdD. For ethical reasons, mice were not given any dose greater than the MEdD. Irradiation times were short (less than 1 min) and a combination of fans and air-conditioning were used to ensure that the mouse body temperature did not increase during irradiation.

For each experiment, seven groups of four to seven C57BL/6 or Balb/c mice each had their back-skin hair removed using animal clippers (Oster, McMinnville, TN) and a close shave electric razor (Remington, Austria). Mice were allowed 24 h to recover from any inflammatory effects of the shaving before they were placed in a black perspex animal-restraining device fitted with a quartz glass lid for exposure to various doses and wavelengths of UV radiation. Additionally, the mice ears and head were shielded from the UV with black perspex. One of six different UV doses ranging from control unirradiated (0 mJ per cm^2) to $1 \times$ MEdD were delivered to groups of mice each day for three consecutive days. UV-induced systemic immunosuppression has been produced by many groups using a variety of irradiation protocols ranging from single doses to multiple doses over the course of many weeks. The 3 d irradiation regime used in these experiments was based on previous reports by others using multiple irradiations ranging from 2 to 4 d (Noonan and De Fabo, 1990; Roberts and Beasley, 1997). The seventh group was an unirradiated irritant control. Each experiment was repeated three times with the results normalized and pooled.

Contact hypersensitivity (CHS) response To determine the UV effects on primary and secondary immunity, CHS was induced (**Fig 1**). For systemic immunosuppression (primary immunity) studies, mice that received UV exposure to the back-skin were sensitized by applying 50 μl of a 2% wt/vol solution of oxazalone (4-ethoxymethylene-2-pheyl-2-oxazolin-5-one; Sigma Chemical, St. Louis, MO) dissolved in acetone. This hapten was applied to the shaved abdomen 3 d after the final UV exposure, with positive control unirradiated mice being sensitized in the same way. To assess the primary CHS, 5 μl of the 2% oxazalone solution was applied to the right ear of the mice 7 d later. After a further 24 h, the difference in the thickness between the right challenged and left unchallenged ears was measured using engineers' callipers (Mitutoyo, Japan). The increase in ear thickness of negative control unirradiated, unsensitized but challenged only mice (irritant control) were subtracted from the test groups.

For evaluation of the effects of UV on the secondary immune response, these same groups of mice were rested for 8–10 wk (**Fig 1**). Memory and/or regulatory T cell activity induced by the primary sensitization was detected by a second contact sensitization without further UV irradiation. Thus, the mice were re-sensitized by applying

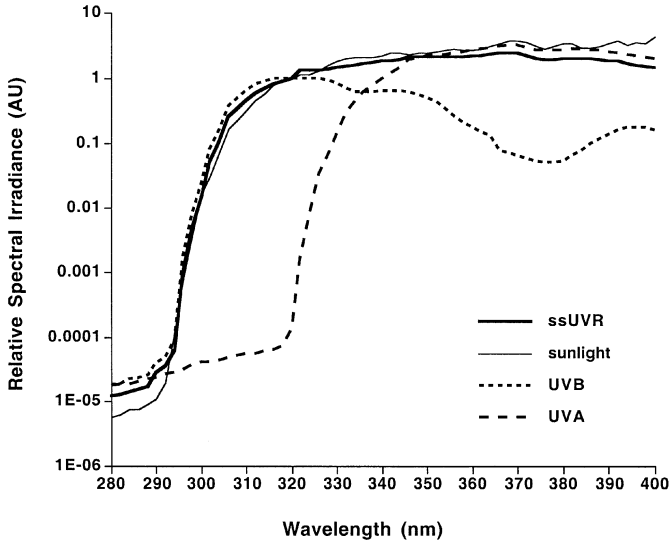

Figure 2. The Oriel 1000 W xenon arc solar simulator emits a spectrum of UV radiation that closely approximates sunlight. The spectral output of the Oriel solar simulator was measured using an OL-754 spectroradiometer at 2 nm intervals (*thick solid line*). The outputs of the UVA only spectrum (*dashed line*) and the UVB only spectrum (*dotted line*) were also determined (at 2 nm intervals). These were compared to the spectral output of midday sun on a typical cloudless spring day on October 10, 2001, in Sydney, Australia (*thin solid line*). The log of the relative spectral irradiance was then plotted against wavelength for the UV spectrum (280 nm to 400 nm). The UVB, ssUVR, and sunlight spectra were normalized to a relative spectral irradiance unit of 1 (arbitrary units, AU) at 320 nm, whereas the UVA spectrum was normalized to 1 at 340 nm. Integration was used to calculate the total area under each curve as well as the UVB and UVA component areas.

2% wt/vol oxazolone to the shaved abdomen. CHS was then assessed by ear challenge to the previously unchallenged (left) ear 7 d later as described above.

Statistics For assessment of the MEdD, a paired Student's *t* test was used and $p < 0.05$ was considered statistically significant. Differences between the two strains was compared by a repeated measures ANOVA. For CHS experiments testing primary and secondary immune responses, experiments were repeated three times with the same result observed each time. Results were then normalized against the positive control group in each experiment and pooled for final analysis. An unpaired Student's *t* test was used to test for significance, with $p < 0.05$ considered statistically significant.

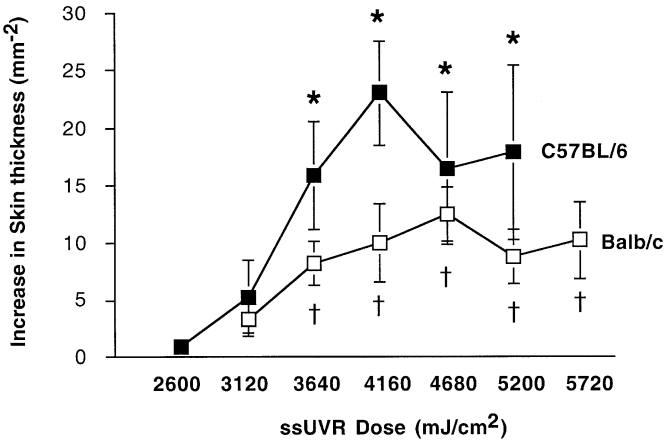

Figure 3. C57BL/6 and Balb/c mice have the same MEdD after exposure to ssUVR. Groups of six to eight C57BL/6 (■) or Balb/c (□) mice had their back shaved at least 24 h prior to exposure to various doses of ssUVR. Immediately before UV irradiation, the initial thickness of back-skin was measured using a hand-held ultrasound (the pre-UV measurement). Mice were then immediately exposed to various single doses of ssUVR. Twenty-four hours after this exposure, the back-skin was again measured using the hand-held ultrasound (the post-UV measurement). The difference between the post-UV and pre-UV measurements was calculated to be the increase in skin thickness and the minimum dose of ssUVR required to cause a significant increase in the thickness of the skin was the MEdD. A paired Student's *t* test was used to compare the difference between pre- and post-UV thickness in each individual mouse. Results are presented as mean ± SEM; *$p < 0.05$ for C57BL/6 mice, †$p < 0.05$ for Balb/c mice. A repeated measures ANOVA showed that the magnitude of the increase between the two mouse strains was significantly different; $p < 0.005$.

RESULTS AND DISCUSSION

Many previous studies on the biologic effects of UV have used banks of fluorescent tubes emitting disproportionately large amounts of UVB (47%) and less UVA (53%) than is found in sunlight (5% and 95%, respectively). The work described here used

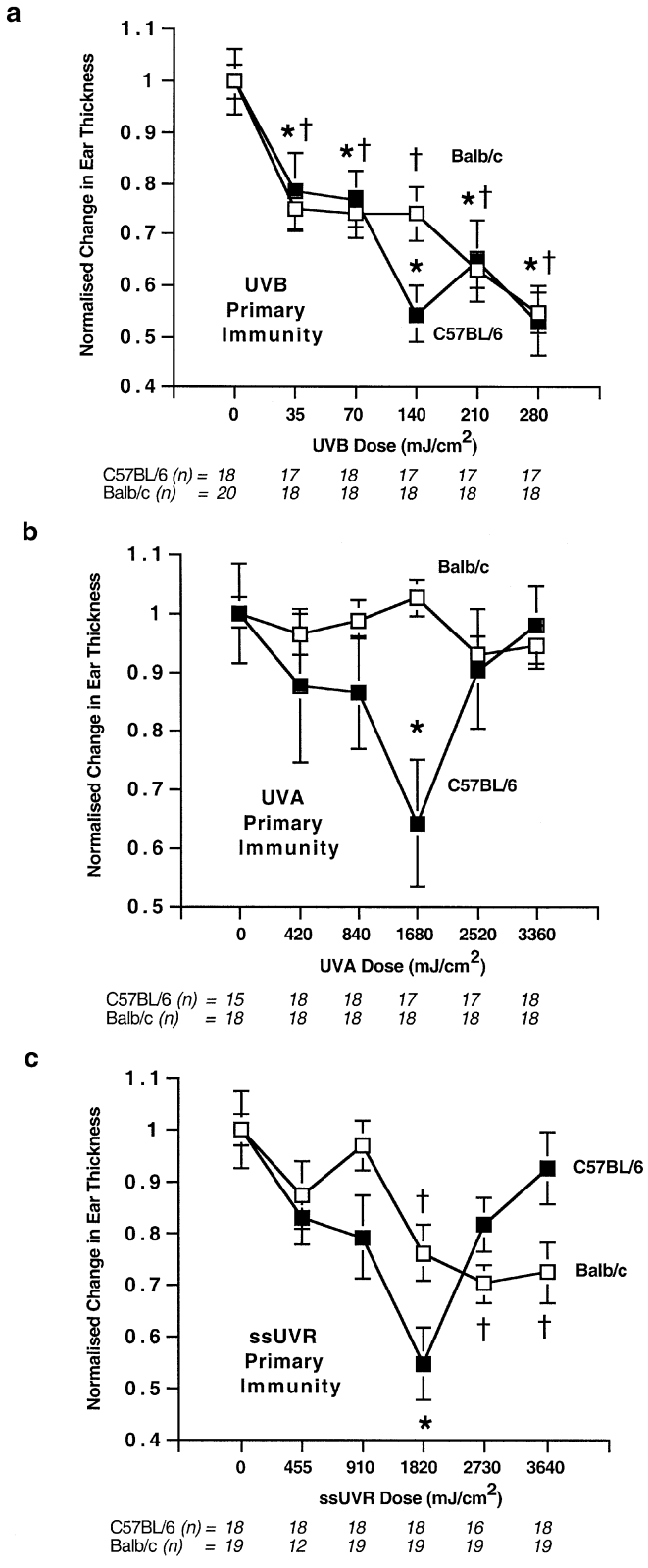

Figure 4. UV Modulation of primary immunity. The primary immune response was suppressed in both C57BL/6 (■) and Balb/c(□) mice after exposure to the UVB component of sunlight (*a*). C57BL/6 mice were significantly immunosuppressed by 1680 mJ per cm^2 of UVA, but recovered at higher UVA doses. Balb/c mice, however, were unaffected by any UVA dose (*b*). Exposure of C57BL/6 mice to ssUVR mirrored the biphasic dose–response seen for UVA only experiments, but Balb/c mice did not show a recovery from immunosuppression at higher ssUVR doses (□). In each individual experiment, groups of four to seven mice had their back-skin shaved 24 h prior to exposure to various doses of ssUVR, or the relative UVA or UVB components. The UVB and UVA doses are those components of ssUVR at the appropriate point. Mice were exposed for three consecutive days and then rested for three more days before being sensitized to oxazolone and then ear challenged 7 d later. Twenty-four hours following challenge, CHS was assessed by measuring ear swelling. Within each dose–response experiment the change in ear swelling for each mouse that received UV radiation was normalized to the mean of the control unirradiated group. Each experiment was repeated three times, with the same result observed each time, and normalized values were pooled for final analysis and presentation. The total number of each mouse strain pooled from the three experiments (the *n* value) is shown below the axis. Mean ± SEM is shown. An unpaired Student's *t* test was used for statistical analysis; *$p < 0.05$ for C57BL/6 mice, †$p < 0.05$ for Balb/c mice compared to unirradiated control mice.

a xenon arc solar simulator, which provided a better mimic of the solar spectrum comprising 5.9% UVB and 94.1% UVA (**Fig 2**). When using wavebands within the solar spectrum, it was important for this study that the shape of the waveband remained similar to that band within the solar spectrum. When the UVB component was blocked using a UVB/UVC blocking filter, the UVA spectrum closely approximated the UVA component of sunlight with a sharp cut-off at 320 nm (**Fig 2**). The percentage of contaminating UVB present in the UVA spectrum was less than 0.001%, which was determined by integrating the area under the spectral curve. Similarly, by changing the dichroic mirrors, a spectrum containing mostly UVB wavelengths was produced with the UVA wavelengths being severely attenuated relative to ssUVR (**Fig 2**). The relative spectral irradiance of the UVB spectrum between 290 and 320 nm was a very good approximation of the UVB component of the solar spectrum (**Fig 2**). For wavelengths greater than 320 nm not completely removed, there was a one log reduction in intensity by 340 nm and a one and a half log reduction in intensity compared to the solar-simulated spectrum by 370 nm (note that the figure is on a log scale). Therefore, a wavelength distribution similar to that found in sunlight (ssUVR) as well as the two component spectra (UVA and UVB) could accurately be delivered to mice. The delivery of this high intensity output to immobilized single mice therefore enabled more accurate determination of dose–responses than has previously been described.

The lowest dose of ssUVR that caused a significant increase in skin thickness (MEdD) was found to be the same in both mouse strains (**Fig 3**). In humans, the minimum erythema dose (MED) is commonly used to assess the biologic endpoint of erythema or redness of the skin and is also used to determine the skin type of individuals. Because mouse skin is pink, however, changes in skin color cannot be easily detected. Therefore, instead of erythema, the edemal component of sunburn is commonly used as a way of measuring sunburn in mice (Cole *et al*, 1983). We established the use of a hand-held high frequency ultrasound to accurately determine the MEdD. This is conventionally measured using callipers, but we found the ultrasound gave more reproducible results. The MEdD was 3640 mJ per cm^2 of ssUVR, being made up of 280 mJ per cm^2 UVB and 3360 mJ per cm^2 UVA (**Fig 3**). Although the MEdD was the same for both C57BL/6 and Balb/c mice, the magnitude of the response between the two strains was significantly different ($p < 0.005$; repeated measures ANOVA), with C57BL/6 mice showing a greater response than Balb/c mice. All irradiations used for immunosuppression were below the MEdD so that the biologic changes associated with sunburn did not confound the immunosuppression studies. This also meant that this study used ssUVR doses that could be experienced in everyday situations by humans. As the MEdD was indistinguishable between the two mouse strains, they can be considered to be of a similar "skin type". MEdD does not correlate with sensitivity to immunomodulation, however (Damian *et al*, 1997), so this issue does not complicate interpretation of the immunosuppression studies.

Results from earlier murine studies demonstrated that single doses of both UVB and UVA radiation induce antigen-specific immunosuppression as well as tolerance (Halliday *et al*, 1998), which is consistent with results obtained in humans (Damian *et al*, 1999) but inconsistent with studies showing that high dose UVA protects from the suppressive effects of UVB (Reeve *et al*, 1998). To resolve this issue, dose–response studies were performed that revealed that UVA and UVB, although both immunosuppressive, display different dose–responses in the different mouse strains. C57BL/6 mice showed positive control ear swelling responses of 21.4 ± 1.6 with irritant controls of $3.6 \pm 0.6 \times 10^{-2}$ mm. For C57BL/6 mice, low doses of the UVB portion of sunlight were sufficient to suppress systemic immunity (**Fig 4*a***). This dose (35 mJ per cm^2) was the lowest delivered to mice demonstrating that UVB is potently immunosuppressive. The amount of UVA contaminating this UVB dose was 66 mJ per cm^2 UVA, which was 25-fold lower than the minimum immunosuppressive UVA dose (**Fig 4*b***). The level of immunosuppression increased linearly with UVB dose, which is consistent with other studies exploring the dose–response effects of UVB on systemic immunosuppression (Noonan and De Fabo, 1990). Medium dose UVA (1680 mJ per cm^2) was also able to suppress CHS in C57BL/6 mice (**Fig 4*b***). This corresponded to approximately half the UVA dose present in the MEdD of ssUVR. It is unlikely that traces of UVB were responsible for this UVA-induced systemic immunosuppression in C57BL/6 mice as the percentage of contaminating UVB in the UVA spectrum was less than 0.001%, thus delivering less than 0.0014 mJ per cm^2 UVB. Moreover, if this low dose of UVB was immunosuppressive, then the Balb/c mice would also be expected to be immunosuppressed by 1680 mJ per cm^2 of UVA. Interestingly, as the dose of UVA was increased, C57BL/6 mice recovered from immunosuppression (**Fig 4*b***). These two spectra were then combined to produce ssUVR (**Fig 4*c***). Because UVB and UVA were both able to suppress primary immunity to oxazolone at low doses, ssUVR also suppressed immunity at these doses. At higher doses, however, although the UVB portion remained suppressive, the UVA component was not, and therefore appeared to protect C57BL/6 mice from the immunosuppressive effects of UVB. Therefore the dose–response for ssUVR was similar to that of UVA but different to the dose–response for UVB in this mouse strain.

Balb/c mice showed positive control ear swelling responses of 27.4 ± 1.4 with irritant controls of $5.9 \pm 1.0 \times 10^{-2}$ mm. Balb/c mice were also suppressed by low doses of UVB and displayed a similar dose responsiveness to C57BL/6 mice (**Fig 4*a***). In contrast to C57BL/6 mice, however, Balb/c mice were unaffected by any dose of UVA (**Fig 4*b***). Because of this, when the two spectra were combined to form ssUVR, UVA protection from the immunosuppressive effects of UVB was not observed; rather, higher doses of ssUVR continued to be immunosuppressive (**Fig 4*c***). Also, because low dose UVA was unable to suppress the CHS response in Balb/c mice (**Fig 4*b***), the magnitude of ssUVR-induced suppression in Balb/c was less than in C57BL/6 mice (24% and 55% suppression, respectively).

In experiments to test UVB susceptibility to systemic immunosuppression, Noonan and Hoffman (1994) showed that, compared to Balb/c mice, C57BL/6 mice required much lower doses of UVB to become systemically immunosuppressed, and the mice were therefore classed as having a low and high UVB susceptibility, respectively. This study used a UVB spectrum emitted from unfiltered FS40 sunlamps having a peak at 313 nm and containing mostly UVB (60%–65%) but also wavelengths below 290 nm. This contrasts with our UVB spectrum mimicking the UVB portion of the solar spectrum peaking at 320 nm, with essentially no wavelengths in the UVC region (below 290 nm). Our study showed no differences in susceptibility to UVB-induced systemic immunosuppression between C57BL/6 and Balb/c mice, probably because of the spectrum used. Therefore, previous reports of a genetic susceptibility to UVB is probably dependent on the spectra used and possibly the type of immune response being studied. An earlier study by Noonan *et al*, however, showed that the dose–response curves for UVB-induced local and systemic immunosuppression were the same, with Balb/c mice requiring 6.4 times more UVB than C57BL/6 to attain identical systemic immunosuppression (Noonan and De Fabo, 1990). Therefore it is likely that genetic susceptibility to UV is very dependent on spectrum and dose, and our spectrum, which closely matched the UVB portion of sunlight, did not differentiate between these strains.

C57BL/6 mice are prone to T helper 1 (Th1) immunity in response to antigen (as measured by interferon-γ), whereas Balb/c mice are more likely to produce a Th2-type response [as measured by interleukin-4 (IL-4) secretion] (Kelso *et al*, 1991). This genetic predisposition to specific types of immune responses may partially explain some of the contrasting results with the two strains presented here. This is especially true considering that UV irradiation can switch from a Th1- to a Th2-type immune response (Simon *et al*, 1994; Garssen *et al*, 1999). Therefore,

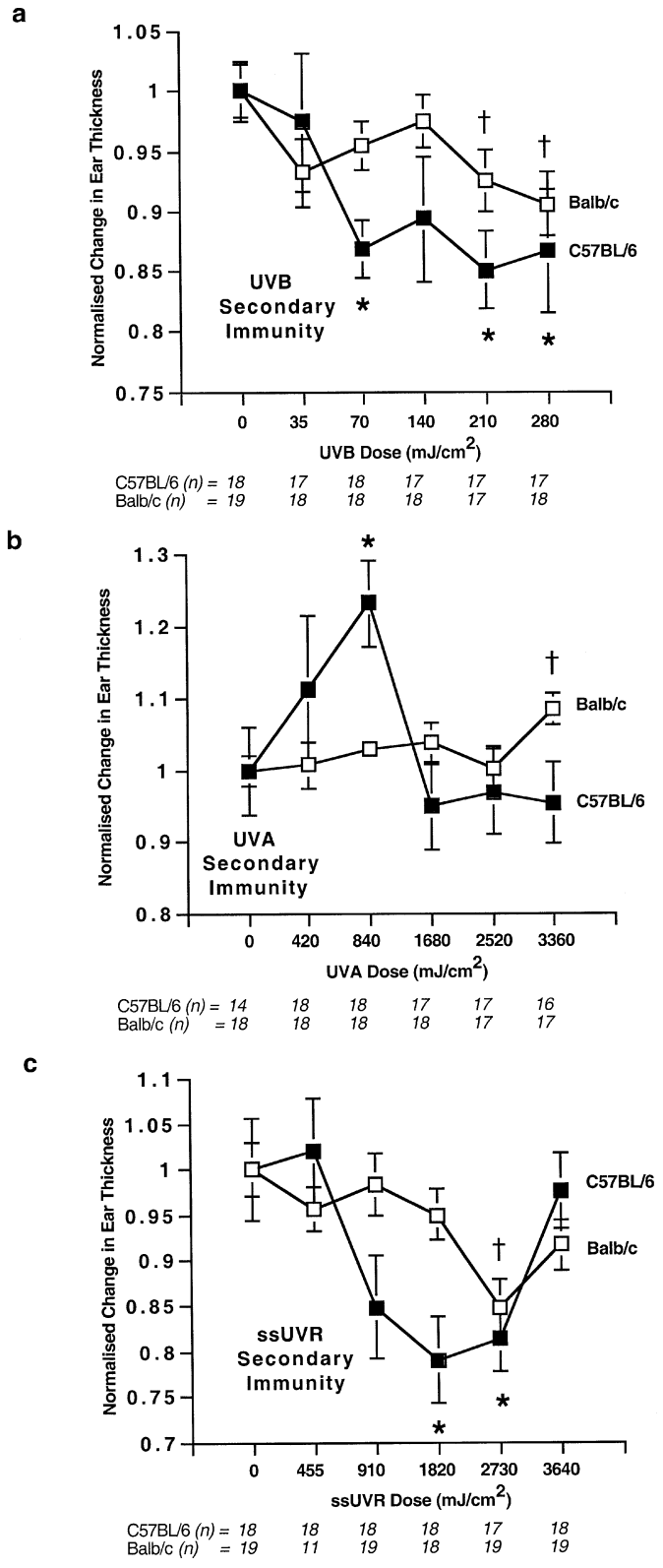

Figure 5. UV Modulation of secondary immunity. The secondary immune response was suppressed by UVB radiation in both Balb/c(□) and C57BL/6 (■) mice in a linear dose-related manner (*a*). Low dose UVA radiation enhanced the memory response in C57BL/6, whereas higher UVA doses were required in Balb/c mice (*b*). Both C57BL/6 and Balb/c mice became tolerant to oxazolone after exposure to low doses of ssUVR, but recovered at higher doses (□). Eight to 10 wk after the primary CHS experiment, the groups of C57BL/6 or Balb/c mice were re-sensitized on their abdomen and CHS was assessed 7 d later by ear challenge. The remainder of the legend is the same as for **Fig 4**.

C57BL/6 may be more sensitive to UV-induced immunomodulation of Th1 immunity than Balb/c mice.

It has not been previously examined whether there is a genetic susceptibility to ssUVR or UVA, and, despite the lack of evidence for a genetic susceptibility to UVB-induced immunosuppression in this study, a difference *was* found between C57BL/6 and Balb/c mice with regard to their susceptibility to both ssUVR and the UVA portion of the solar spectrum. These results suggest that there is a genetic dependence on the ability of UVA to modulate immunity and that the mechanisms underlying UVA and UVB immunosuppression may differ. Indeed, although we show here that Balb/c mice are not suppressed by subedemal doses of UVA and C57B/6 mice have a bell-shaped dose–response curve, Nghiem *et al* (2001) have shown using a delayed type hypersensitivity assay that C3H/HeN mice display a linear dose–response to UVA-induced immunosuppression. These results indicate that the possible reasons why previous studies have found UVA to be immunosuppressive (Halliday *et al*, 1998; Nghiem *et al*, 2001) or protective (Reeve *et al*, 1998) could be due to (i) the different doses of UVA being used, (ii) the assay, or (iii) the genetics of the irradiated host.

The mechanism of UVB-induced immune suppression is thought to involve alterations to various cytokines, which lead to systemic changes in immune cells. Alterations to IL-4, IL-10, IL-12 (Rivas and Ullrich, 1994), tumor necrosis factor (Vincek *et al*, 1993), and *cis*-urocanic acid (De Fabo and Noonan, 1983) levels are all thought to be involved in this process. These changes affect antigen presentation (Kripke and McClendon, 1986) and T cell activation, including the generation of suppressor T cells in UVB-exposed mice (Schmitt *et al*, 1995). Despite evidence that *cis*-urocanic acid is produced following exposure to UVA, however (El-Ghorr and Norval, 1999), the precise mechanism by which UVA causes immunomodulation is unknown.

Few studies have explored the complex effects of ssUVR and its component wavebands (UVA and UVB) on secondary immunity where UV-exposed mice are re-sensitized to examine long-lived regulatory cell activity. Suppression of primary immunity could be the result of either lack of activation of effector cells or activation of regulatory cells. To determine the difference between these events, mice were re-sensitized with antigen without re-exposing to the immunomodulating agent to determine whether activated regulatory cells modulate secondary immunity (see **Fig 1**). This protocol has previously been used to demonstrate the activation of regulatory cells when antigen is applied to carcinogen-treated (Halliday *et al*, 1988) or UV-irradiated skin (Katiyar *et al*, 1999). The role of UVB in inducing immune tolerance has been known for some time, and is thought to be an important event in skin tumor carcinogenesis as well as the observed lower immunization rates following UVB exposure (Cooper *et al*, 1992).

In the secondary CHS, C57BL/6 mice showed positive control ear swelling responses of 28.9 ± 1.9 with irritant controls of 6.9 ± 1.0 × 10^{-2} mm. This study has shown that C57BL/6 mice became tolerant to oxazolone re-sensitization after exposure to low doses (70 mJ per cm^2) of UVB (**Fig 5*a***). These mice displayed a linear dose–response to UVB, because the level of immunological tolerance increased with higher doses of UVB (up to 280 mJ per cm^2). This result is consistent with previous research into the effects of UVB on tolerance (Shimizu and Streilein, 1994). The effect of UVA on secondary immunity has received little research attention, however. We found that C57BL/6 mice exposed to low dose UVA (840 mJ per cm^2) developed an enhanced response to oxazolone re-sensitization (i.e., augmented memory). Whereas El-Ghorr and Norval showed a nonsignificant enhancement of primary immunity in C3H/HeN mice exposed to UVA from a Dr. Honle Light Tower source (El-Ghorr and Norval, 1999), there have been no previous reports of this UVA-induced memory enhancement occurring in any system. In contrast, medium to higher doses of UVA (from 1680 mJ per cm^2 to 3360 mJ per cm^2) had no effect on secondary immunity (**Fig 5*b***) showing that these doses of UVA did not activate regulatory cells. This result is novel, and highlights the

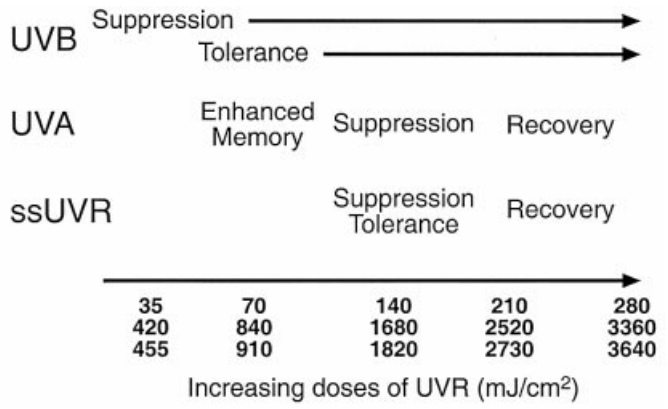

Figure 6. Summary of the complex immunomodulating effects of UVB, UVA, and ssUVR on C57BL/6 mice.

contrasting effects of UVA *versus* UVB as well as the different effects of high dose *versus* low dose UVA. When the two spectra were combined to form ssUVR, it was found that at low doses no tolerance or memory enhancement was observed (**Fig 5*c***). This is probably due to the fact that, whereas UVB was tolerogenic, UVA enhanced memory, and so the combination resulted in no observable effect (**Fig 6**). As the dose of ssUVR increased, however, C57BL/6 mice became tolerant to oxazolone re-sensitization. This is probably because UVA no longer enhanced memory at these doses, whereas UVB was tolerogenic. At the maximum dose of ssUVR tested (3640 mJ per cm^2), tolerance was no longer observed. It is possible that the protective effects of UVA during immune induction also protected mice from UVB-induced tolerance, although the mechanism of this is not understood.

In the secondary CHS, Balb/c mice showed positive control ear swelling responses of 37.2 ± 1.3 with irritant controls of $4.4 \pm 1.3 \times 10^{-2}$ mm. For Balb/c mice exposed to UVB radiation, tolerance to oxazolone re-sensitization was also observed in a linear dose-related manner, but compared to C57BL/6 mice higher doses of UVB were required (210 mJ per cm^2 compared to 70 mJ per cm^2; **Fig 5*a***). In contrast to C57BL/6 mice, low dose UVA had no effect on secondary immunity in Balb/c mice. The maximum dose of UVA (3360 mJ per cm^2), however, also enhanced memory (**Fig 5*b***), although the magnitude of this enhancement was less than that observed in C57BL/6 mice (9% compared to 23%). When the two spectra were combined, low dose ssUVR had no effect on tolerance, but Balb/c mice did become tolerant to oxazolone re-sensitization at 2730 mJ per cm^2, reflecting the effect of UVB at its relevant dose. Also, in contrast to the failure of Balb/c mice to recover from ssUVR-induced primary immunosuppression, these mice were no longer tolerant to oxazolone at higher doses of ssUVR (**Fig 5*c***), probably because the UVA component caused a slight enhancement of memory at this dose. The mechanism of this UVA-induced memory enhancement is not known. It is possible that this is a similar phenomenon to high dose UVA protection from UVB-induced immunosuppression observed previously (Reeve *et al*, 1998) and in this study; however, these events occurred at different doses.

These memory-enhancing effects of low dose UVA have wide ranging implications for implementing immunization strategies. Further research is required to determine whether low dose UVA could be used to augment antitumor immunization strategies leading to enhanced recall responses and long-term memory to tumor-associated antigens. Furthermore, in contrast to UVB, no dose of UVA caused immunologic tolerance in either mouse strain, which also has important implications for host immune responses against tumors. The caveat, however, is that, whereas low doses of UVA enhanced memory, only slightly higher UVA doses were immunosuppressive, and it would be difficult to deliver exactly the right UVA dose to enhance memory without causing immunosuppression in an outbred population. It is still not known whether primary or secondary immunosuppression contributes more to carcinogenesis.

Because low dose UVA (840 mJ per cm^2) caused memory enhancement, medium dose UVA (1680 mJ per cm^2) caused immunosuppression, and high dose UVA (3360 mJ per cm^2) provided protection from immunosuppression, it is likely that different doses of UVA switch on different events leading to the complex dose–responses observed. Medium dose UVA as used by El-Ghorr and Norval (10,000 mJ per cm^2) may cause isomerization of *trans*-urocanic acid to *cis*-urocanic acid (El-Ghorr and Norval, 1999), which in turn may lead to tumor necrosis factor and immunosuppressive IL-10 or IL-4 being secreted (Rivas and Ullrich, 1994), perhaps from keratinocytes, infiltrating inflammatory macrophages and/or mast cells in the dermis. Another possibility is that medium dose UVA suppresses immunity via a nitric oxide or reactive oxygen dependent mechanism, which this laboratory has previously shown using 6600 mJ per cm^2 of UVA (Halliday *et al*, 1999). In the absence of higher doses of UVA, this event is sufficient to cause immunosuppression. As the dose of UVA is increased, however, this may turn on another mechanism, such as the release of interferon-γ and/or IL-12, which has been previously described to occur in response to high dose UVA (38,740 mJ per cm^2) (Shen *et al*, 1999). This change in the cytokine milieu may then counteract any immunosuppressive events caused by lower UVA doses or any UVB present. The mechanism of low dose UVA-induced memory enhancement is unknown, although it is possible that this dose enables the activation or prolonged survival of memory T cells and/or prevents regulatory $CD4^+$ T cell activation.

We found ssUVR to induce immunosuppression in a dose-related manner between 455 and 3640 mJ per cm^2 in Balb/c but not C57BL/6 mice. This Balb/c response is similar to the linear dose–response curves described for solar-simulated immunosuppression of C3H/HeN mice (Roberts and Beasley, 1995; Kim *et al*, 1998). In contrast to both these strains, C57BL/6 mice showed a biphasic dose–response. It is possible that this bell-shaped curve observed for ssUVR up to 3640 mJ per cm^2 in C57BL/6 mice is not universally observed in all mouse strains. Further dose–response studies using spectra containing larger amounts of high wavelength UVA over large dose ranges, however, are required to clarify this issue.

In conclusion, dose–response studies have revealed new intricacies in the effects of UV on immunomodulation (as summarized in **Fig 6**). The UVA portion of sunlight was found to be immunosuppressive at relatively low doses. This UVA dose was less than that contained in doses of ssUVR, which caused barely detectable sunburn. Furthermore, this UVA-induced immunosuppression only occurred in one of the two mouse strains studied, implying a genetic susceptibility to UVA-induced immunosuppression. This result has important implications for the use of solariums by humans, which claim to use ''harmless'' UVA for cosmetic tanning purposes. If certain sections of the community are found to be susceptible to UVA-induced immunosuppression, then solarium use and sunbaking in general could greatly enhance the development of skin cancer in these individuals. These results also reinforce the need for broad-spectrum sunscreen use by the community. At higher UVA doses, mice were no longer immunosuppressed, but rather were protected from solar-simulated immunosuppression, although this protection only occurred in one of the two mouse strains studied, which again suggests a genetic component to UV immunomodulation. Both these results show that UVA may have different immunologic functions at different doses and further highlights the importance of detailed UVA dose–response studies of UV-induced immunosuppression and carcinogenesis. This point is further reinforced by the discovery that even lower UVA doses enhanced secondary immunity, whereas higher UVA doses had no observable effect. At no stage in either mouse strain did UVA induce tolerance. These results imply that UVA may provide

memory enhancement to augment vaccination therapies. Considering that this effect of low dose UVA was only observed in one out of two mouse strains, however, it is unclear whether this would be effective in a large percentage of humans.

These studies have examined the effects of UVB and UVA on CHS as a model of systemic immunosuppression. Different results may have been obtained with an alternative immunologic endpoint such as delayed-type hypersensitivity. Because of the complex and long-term nature of carcinogenesis, the immune response to a developing tumor antigen is unlikely to be accurately mirrored by CHS. Nevertheless, such studies enable some hypotheses to be drawn regarding the relative roles of different doses and wavebands on UV suppression of antitumor immunity. In contrast to UVA, UVB caused both primary immunosuppression and tolerance at low doses, suggesting that UVB is a potent immunosuppressive agent, which could have profound influences on carcinogenesis and long-term memory. The dose and regime of sunlight exposure that causes cancer in humans is unknown. There is some evidence that the rate of UV exposure required for the development of melanoma and nonmelanoma skin cancer in humans differs (Armstrong and Kricker, 1993; Kricker *et al*, 1995). As ssUVR caused immunosuppression over a narrow dose range, it is possible that skin cancer induction would also be more prevalent at these moderate doses, at least for some skin cancer types in some individuals. These complex interactions between UV wavebands are therefore likely to be important for both the induction of and long-term protection against human skin cancer.

This work was supported by funds provided by the National Health and Medical Research Council of Australia and the Leo and Jenny Leukaemia and Cancer Foundation of Australia.

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