[Frontiers in Bioscience 11, 394-411, January 1, 2006]

Spectral and dose dependence of ultraviolet radiation-induced immunosuppression

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1. ABSTRACT

Ultraviolet radiation (UV) wavelength and dose dependence has been demonstrated for a number of cutaneous endpoints such as erythema, pigment darkening, DNA damage, and photocarcinogenesis. More recently, a number of *in-vitro* and *in-vivo* models of UV immunosuppression have implicated UVA (320-400nm) in immune protection as well as immune suppression. While the wavelength dependencies for immunosuppression within UVB have been well established in mice, the exact role of specific UVA wavelengths has been less clear. Moreover, in humans, the spectral dependence of UV immunosuppression is even less well established. This review firstly outlines the established UV action spectra for a variety of cutaneous effects. The waveband and dose dependence of UV immunosuppression and its mechanisms are explored with a focus on *in-vivo* models. Finally, since UV immunosuppression along with DNA damage is thought to play a central role in the development of skin cancer, a clearer understanding of the immunosuppressive potential of discrete UV wavebands will allow a more rational approach to our understanding and prevention of skin cancer.

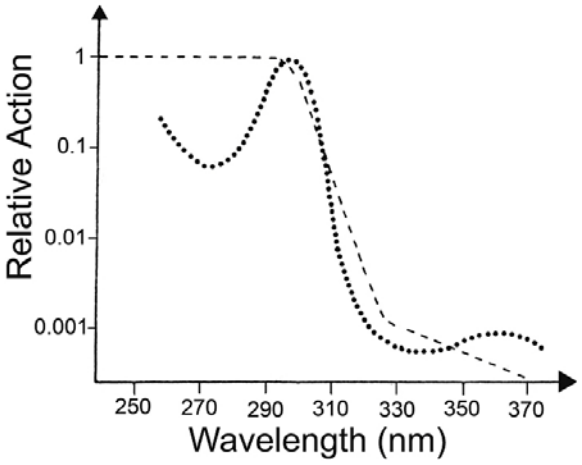

Figure 1. Comparison of human erythema action spectra. Erythema action spectra of the International Commission on Illumination (CIE) (-----) (14) and Anders *et al.* (.....) (17) are shown. Reproduced and adapted with permission from Photochemistry and Photobiology (17).

2. INTRODUCTION

UVA (320-400nm) and UVB (290-320nm) account for 5% of the solar radiation that reaches the Earth's surface (1), with UVA being generally regarded as less biologically active than UVB although it is approximately 20 times as abundant and able to penetrate deeper to the dermis. Radiation at shorter wavelengths (lambda) possesses higher energy per photon (E) by Planck's law (E=hc/lambda), increasing the likelihood that a chromophore or a photon absorbed by a molecule would initiate a photochemical process (Stark-Einstein law and Grotthus-Draper law) (1).

Recently however, there has been increasing interest and evidence for the role of longer, less energetic UVA wavelengths in various cutaneous effects such as deoxyribonucleic acid (DNA) damage and immunosuppression (2). This paper reviews the wavelength dependence of a variety of cutaneous effects of UV, subsequently focussing on UVA immunosuppression and its mechanisms, with an emphasis on *in-vivo* models of cutaneous immunity.

3. WAVELENGTH DEPENDENCE OF THE CUTANEOUS EFFECTS OF ULTRAVIOLET RADIATION

3.1. Action spectra

Ultraviolet radiation of different wavelengths has variable efficacy in producing a variety of threshold effects such as erythema, immediate and delayed pigment darkening in humans. Studies which utilise narrowband sources of radiation that centre around specific wavelengths allow the construction of UV action spectra, but are not often performed because of equipment cost and the time-consuming nature of such experiments. The use of broadband UV sources for the determination of action spectra has inherent weaknesses in that potential interactions between different wavebands may confound results (3, 4). Action spectra can aid the identification of key chromophores, or molecules that play a crucial role in initiating a photochemical reaction, such as UV-induced erythema (DNA damage) (5, 6) and immunosuppression (urocanic acid) (7).

Action spectrum studies that use a single exposure to narrowband or monochromatic UV sources depend on the strict definition of firstly, the cutaneous response or observational threshold to be measured, and secondly, the time of assessment of the response (8). It is likely that different action spectra would be obtained if these and other factors such as body site vary, thus altering the threshold for these photobiological reactions. Other factors such as single exposure *versus* multiple exposure protocols will affect the interpretation and application of derived action spectra (9).

3.2 Erythema

Ultraviolet radiation causes erythema in human skin with a delay in onset between 8-24 hours after an acute single exposure (10). The erythemal effects of UV radiation on human skin depend on a variety of factors including the UV source spectra and dose, anatomical site, skin pigmentation, previous UV exposure (11), epidermal thickening (12) and concomitant use of non-steroidal antiinflammatory drugs (NSAIDs) (13).

Published action spectra for erythema in humans are generally based on the elicitation of minimally perceptible erythema observed visually at 24 hours after exposure, otherwise known as determining an individuals' minimal erythemal dose (MED).

A reference action spectrum for erythema based on the statistical analysis of 8 published studies of MED in normal human subjects (14) has since been shown to be a valid predictor of erythemal effectiveness for wavelengths between 300-400nm (15) and for UV sources of differing bandwidths (16). Anders *et al.*, used an excimer pumped dye laser to determine an erythema action spectrum between 294-374nm in normal human subjects (17). This was similar to the International Commission on Illumination (CIE) action spectrum (14) with maxima in the UVB range declining from 300-330nm, but differed because there was a secondary maximum in the UVA region around 362nm (Figure 1).

3.3. Pigmentation

Ultraviolet radiation, predominantly at longer wavelengths (300-400nm), causes darkening of the skin immediately after exposure. This is maximal 60 seconds after exposure, and is known as immediate pigment darkening (IPD). Persistent pigment darkening (PPD) occurs 2-4 hours after UV exposure. Both IPD and PPD tend to occur more noticeably in those of darker skin types (18).

The underlying mechanisms however are probably different, with IPD being a result of photo-oxidation of melanin and/or its precursors within pre-existing melanosomes making the skin darker without any increase in skin melanin

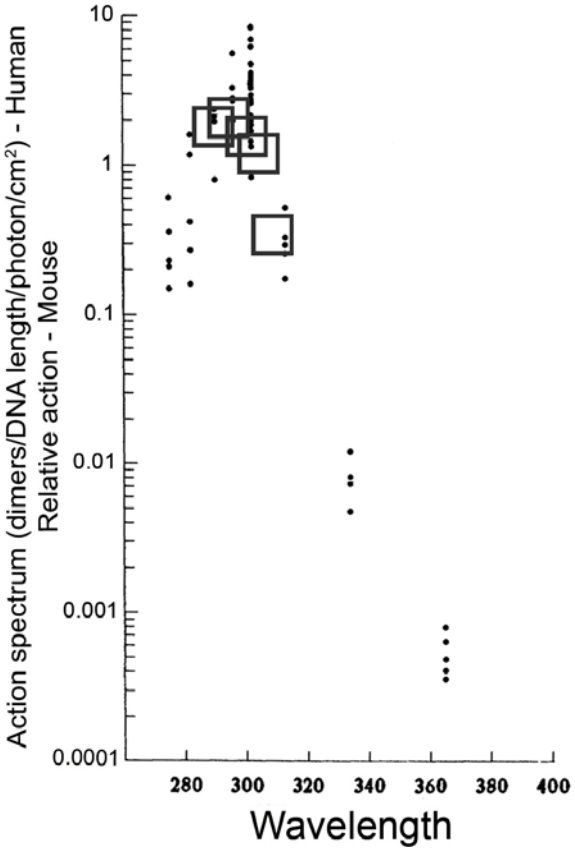

Figure 2. Action spectra for CPD formation in *in-vivo* human (solid dots) (30) and *in-vivo* murine (open squares) (29) skin. Each individual solid dot represents an individuals' actual dose-response gradient for CPD formation in-situ in humans. The mouse action spectrum (open squares) is presented as relative rather than actual data to allow comparison. Reproduced and adapted with permission from Freeman et al. (30) and Blackwell Publishing (29).

content (19) and PPD a result of greater superficial redistribution of melanin in the skin (20). The action spectrum for IPD in humans is broad within the UVA region, with a peak at 340nm, declining by 470nm (21). There is also a sharp falling-off within the UVB range (22).

The PPD action spectrum also demonstrates a broad and flat range of efficacy within the UVA range (23) when the UV-induced pigmentation is measured at 2-4 hours after exposure. It persists and peaks at 7 days after exposure (24).

3.4. Ageing and elastosis

Chronic UVB (25) as well as UVA exposure contributes to premature aging of human skin. There is evidence in humans that repeated suberythemal UVA (320-400nm) exposures cause epidermal hyperplasia, stratum corneum thickening, and dermal lysozyme deposition along elastic fibres (26) which contribute to ageing. Subsequent studies have found that shorter wavelength UVA radiation (320-345nm) is more effective than longer UVA wavelengths in causing epidermal hyperplasia. Very high single doses of broadband UVA (320-400nm) from 18.2 to 72.8 J/cm2 have also been shown to cause epidermal thickening, but not increased lysozyme deposition on elastic fibres, on biopsy 48 hours after exposure (26).

3.5. DNA damage

Ultraviolet radiation induces a number of photolesions in DNA such as cyclobutane pyrimidine dimers (CPDs), 6-4 pyrimidine pyrimidones and single-strand breaks (27), which can lead to mutations if the cell divides prior to repair of this damage. Such mutations play a major role in photocarcinogenesis (28). CPDs are the most abundant DNA photoproduct produced by UVB and UVC irradiation and action spectra have been determined for their induction in mice (29), and humans (30) (Figure 2).

The action spectrum for CPD formation in gluteal skin determined from biopsies taken immediately after monochromatic irradiation of human subjects showed peak efficiency in formation between 296-302nm (30), with a rapid fall-off of 3 decades in efficacy with longer wavelengths of 366nm (Figure 2). There was also a decrease in efficacy from this peak for those wavelengths in the UVB and UVC range down to 275nm. Longer wavelengths of 385nm and 405nm did not induce significant CPD formation with doses up to an MED of each wavelength.

Similarly, in murine studies there was a peak at 293nm (29) (Figure 2). CPDs thus occur with the greatest efficacy within the UVB and UVC wavelengths through direct photochemical effects on DNA. It is generally believed however that longer wavelength UVA causes DNA mutations via an indirect mechanism whereby it causes a photosensitising substance (such as pheomelanin, flavins, porphyrins, lipid membranes) to generate singlet oxygen radicals that cause single-strand breaks in the DNA and mutations (31).

3.6. Skin Cancer

Basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma are the three major types of invasive skin cancer that most commonly occur in humans in order of decreasing incidence. It is generally accepted that UV causes skin cancer however the precise wavelength dependence of human photocarcinogenesis has not yet been fully clarified.

With the ethical impossibility of determining an action spectrum for photocarcinogenesis in humans, studies of waveband dependence have focussed on animal models. Groups of hairless albino SKH:HR1 mice were exposed to 14 different broadband sources of chronic daily UV (from 254nm – 396nm) and monitored for the median time to development of skin cancers. Results from each UV source were then mathematically spectrally weighted by a number of existing action spectra to fit and construct an action spectrum for photocarcinogenesis in these mice (Skin Cancer Utrecht-Philadelphia –murine [SCUP-m], Figure 3) (3, 4). The peak carcinogenic wavelength was 293nm, with

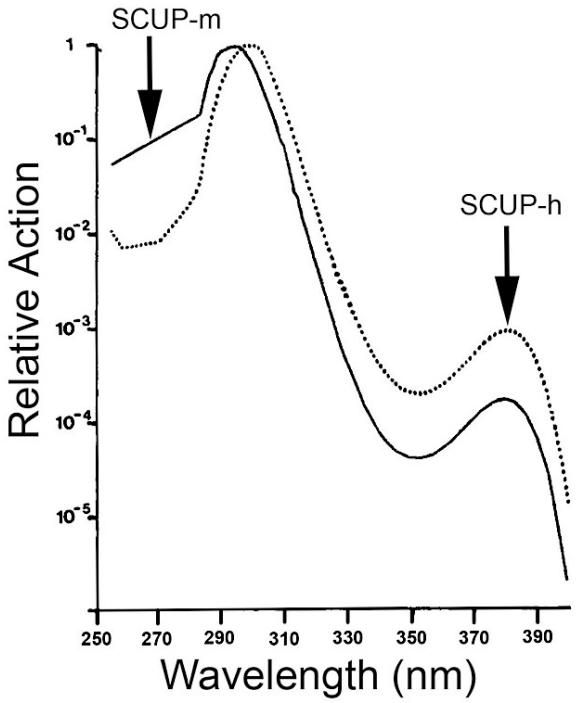

Figure 3. Comparison of action spectra for photocarcinogenesis in mice and humans. Skin Cancer Utrecht-Philadelphia – murine (SCUP-m, solid line) action spectrum for photocarcinogenesis was used to derive the SCUP-human (SCUP-h, dotted line) action spectrum. Reproduced and adapted with permission from Lippincott Williams & Wilkins (32).

a broad shoulder in the UVA at wavelengths greater than 340nm approximately 4 decades less in efficacy.

Using a mathematical correction of SCUP-m for the lower optical transmission of human compared to murine epidermis a human action spectrum for photocarcinogenesis (SCUP-human [h]) has been proposed. This red-shifted the peak effective wavelength from 293 to 299nm, with a secondary peak in the UVA at 380nm (32) (Figure 3).

More recent studies have confirmed that ultraviolet-A1 (UVA1: 340-400nm) alone can cause SCC in hairless mice. There were fewer "signature" p53 mutations typical of UVB-induced SCC in these UVA1 tumours (33). This suggests that the mechanisms of DNA damage caused by UVA1 are indirect, probably via the generation of reactive oxygen species (34), as mentioned earlier. In addition, "signature" UVA mutations have been observed in the basal layer of human SCC and premalignant solar keratoses compared to the suprabasal location of "signature" UVB mutations (35).

A two-stage skin carcinogenesis model where human skin is grafted onto severe combined immunodeficient (SCID) or recombinase activating gene-1 (RAG-1) knockout mice could be used to study the carcinogenic effects of discrete UV wavebands on intact human skin in an immunodeficient environment. The single topical application of dimethyl-(a)benzanthracene (DMBA) as an initiator followed by 3 times weekly chronic irradiation with UVB (and stray UVC 30-50mJ/cm^2) for promotion has been successful for development of SCC (36-38) and melanoma (39) in human xenografts in this model.

While the role of UVB in BCC and SCC is well established, there has been considerable debate about the relative importance of UVB and UVA in melanoma. The reduced latitude gradient observed with melanoma compared to BCC and SCC (40) has suggested the importance of UVA in melanoma development, since relative terrestrial solar UVB irradiance decreases more rapidly than UVA as latitude increases. Melanoma case control studies on users of sunbeds which emit primarily UVA, have so far revealed conflicting results with some studies showing up to a 1.8 odds ratio in regular sunbed users (31, 34, 41, 42), while other studies have shown only little evidence of such an association (43).

An action spectrum for induction of melanoma has been constructed for the hybrid *Xiphophorus* platyfish by determining the rate of melanoma induction 4 months after a single dose of monochromatic UV radiation between 302 – 547nm (44, 45). While the 302nm waveband was the most efficacious in inducing melanoma, longer wavelengths up to 548nm in the visible were only 2 decades less effective.

More recent mammalian studies of melanoma and melanocytic hyperplasia/naevi in neonatal HGF/SF-transgenic mice, albino guinea pigs, and *Monodelphis domestica* opossums point to the UVB rather than UVA as important in initial development of melanoma (46-49). This may have been a result of using high dose UVA protocols that could have triggered photoprotection (2).

4. ULTRAVIOLET RADIATION-INDUCED IMMUNOSUPPRESSION

4.1. UV immunosuppression, skin carcinogenesis and infectious diseases

The importance of DNA damage in photocarcinogenesis is well established, and it is generally accepted that a cutaneous environment of immunosuppression is also required for the initiation, promotion and progression of skin cancer (50). The role of immunosuppression (and DNA damage) in photocarcinogenesis has stimulated great interest for over three decades since the discovery that skin cancers that develop in chronically UV-irradiated mice were highly immunogenic and failed to grow when transplanted into unirradiated syngeneic mice (51). However, these cancers were able to progress when transplanted into UV-irradiated syngeneic mice or unirradiated mice which had splenic suppressor T-cells transferred from UV-irradiated syngeneic mice (52, 53). This seminal work in immunosuppression has led to further studies in elucidating the mechanisms by which UV-induced skin cancers are able to progress in human skin.

Observations that renal transplant patients who are on systemic immunosuppressive medications are at a significantly increased risk of developing SCC particularly on sun-exposed areas (54) also support the importance of an intact cutaneous immune system in the prevention of skin cancer.

In addition, Yoshikawa *et al.* found that 92% of skin cancer patients compared to 40% of healthy normal individuals are susceptible to local UV suppression of contact hypersensitivity (CHS), with 45% of the skin cancer patients displaying hapten-specific tolerance (55). This suggests that the local CHS induction model may be a suitable one for the UV induction of tolerance to tumour antigens. A low dose UV protocol was specifically designed in this study to cause suppression in a low proportion of controls.

Susceptibility to UV immunosuppression in humans may also be important in developing adequate immune responses against infectious diseases such as the reactivation of latent herpes simplex virus (HSV) (56). People with a history of recurrent HSV-1 infections were more likely (65%) to be susceptible to local CHS UV immunosuppression than the normal population (44%) (57). Those susceptible to local CHS immunosuppression were also more likely to develop clinical HSV recurrences after 2 MED UVB exposure to their face, compared to those who were not susceptible to immunosuppression.

4.2. Experimental models of UV immunosuppression

There have been a number of experimental models used to investigate the various effects of UV on the skin's immune system. These have included *in-vitro* studies such as the mixed epidermal cell lymphocyte reaction (MECLR), observations of the various cytokines released by UV-irradiated keratinocytes, dendritic cells, lymphocytes, as well as *in-vivo* models.

4.2.1. *In-vitro*

The MECLR measures *in-vitro* proliferation of T lymphocytes exposed to allogeneic Langerhan's cells (LC's) from extracted epidermal cells. The action spectrum for suppression of the MECLR in human skin, using biopsies that were irradiated with monochromatic radiation from 254-312nm shows a broad peak within the UVC to UVB from 254-302nm, with a tailing off of approximately 2 decades in efficacy at 312nm (58). Because the action spectrum for CPD formation in human skin closely paralleled that of the MECLR, it was hypothesised that DNA damage was involved in UV-immunosuppression as measured by the MECLR.

A single UVA1 exposure of 60 J/cm^2 on human skin 3 days prior to biopsy has also been shown to suppress the MECLR by 47% (59). This was partially prevented by pre-application of a sunscreen with a UVA filter.

4.2.2. *In-vivo*

Since the first *in-vivo* model, transplantation of UV-induced skin cancers in mice (51), other models have been developed that have taken advantage of the ability of the skin's immune system to recognise epicutaneous antigens or dermally delivered antigens (afferent arm) and to then develop a hypersensitivity response when re-challenged with the same antigen (efferent arm). These two models are termed the CHS and delayed type hypersensitivity (DTH) models respectively, and are likely to be regulated by different mechanisms (60).

When antigen is applied to a UV-irradiated area, the subsequent failure to elicit an immune response in the efferent arm is termed "local" immunosuppression. In these studies of induction of hypersensitivity through UV-irradiated skin (afferent arm), an immune response cannot be subsequently elicited by that particular antigen due to specific tolerance to that antigen (61). This parallels the effect of the suppressor T-cells to UV-induced skin cancer antigens in the murine transplant studies.

In a model more commonly used in animals, antigen is applied to unirradiated skin after relatively high doses of UV-irradiation to another skin site. Subsequently, the failure to then elicit an immune response to that same antigen is termed "systemic" immunosuppression and refers to the defective induction of immunity (afferent arm) through unirradiated skin and failure of elicitation (efferent arm) because of the systemic immunosuppressive effects of UV exposure.

While the majority of human and animal studies to date have largely focussed on the induction of immunity, studies on the elicitation of recall responses have been fewer (62). In humans, local immunosuppression is a more feasible model as the doses of UV used are much lower than those used in systemic immunosuppressive protocols. The study of recall responses as opposed to induction of immunity in humans not only allows multiple UV doses to be studied in each individual, but as each individual serves as their own control, the number of subjects required for each study is far fewer (63). Since human subjects are already pre-sensitised, there is no need to sensitise subjects to new allergens as is the case in models studying the induction of immunity.

4.3. Mechanisms of UV immunosuppression

While the photoreceptors for UVA are unknown, it is generally recognised that UVB initiates immunosuppression via three possible photoreceptors: DNA damage, the isomerisation of *trans*-urocanic acid (UCA) to *cis*-UCA, or lipid membrane peroxidation (64). This then leads to a variety of cellular changes, a Th2 biased cytokine cascade, prostaglandin production, generation of reactive oxygen species, and neuropeptide release which ultimately results in the suppression of certain immunological responses (65). The exact underlying mechanisms, especially of UVA-induced immunosuppression however have not yet been completely understood in mice and even less so in humans since most studies to date have utilised UVB emitting sources of radiation.

4.3.1. Urocanic acid

Urocanic acid is considered a major UV chromophore and is located in the stratum corneum. It is

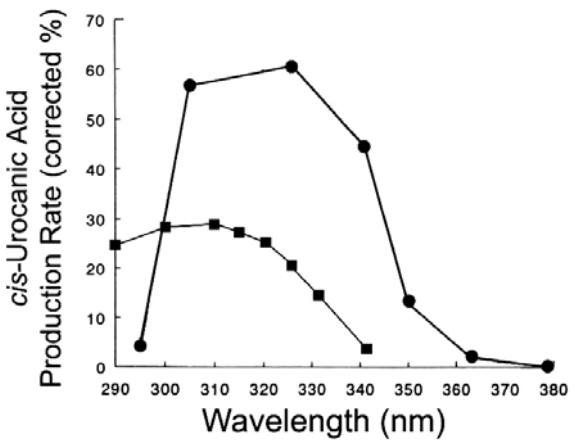

Figure 4. Action spectra for *cis*-urocanic acid formation in *in-vivo* human (circles) (67) and murine (squares) (68) skin. The data shown for humans (circles) is based on irradiations normalised to 1 MED narrowband exposure. Reproduced and adapted with permission from Blackwell Publishing (67) and Photochemistry and Photobiology (68).

formed by the enzyme histidine ammonia-lyase during keratinisation, with the *trans* isomer converting to the *cis* isomer on absorption of UV which is then thought to exert systemic immunosuppressive effects (66). The UV action spectra for the *in-vivo* photoisomerisation of *trans* to *cis* UCA in both humans (67) and mice (68) have been established (Figure 4).

The human action spectrum was determined by high-performance liquid chromatography (HPLC) analysis of non-invasive alkaline extraction of the epidermis after narrowband irradiation from 295-405nm. This showed that *in-vivo* human *cis*-UCA production extended from 295nm in the UVB to 363nm in the UVA but not beyond. The maximally efficient wavelengths were between 300-341nm. Similar to the Sk1-hairless albino murine studies (68), *in-vitro* assays of human *cis*-UCA production were most effective within the 305-326nm range (67).

The action spectrum for UV suppression of systemic CHS responses between the 250-320nm wavelengths in BALB/c mice parallels the *in-vitro* absorption spectrum of UCA. Additionally a restoration of immune responses occurs with the removal of *cis*-UCA within the stratum corneum by tape-stripping mice skin prior to UV-irradiation (7). This supports the hypothesis that *cis*-UCA is a major chromophore responsible for systemic UVB immunosuppression in mice.

The *in-vivo* UV action spectrum for *cis*-UCA production in Sk1-hairless albino mice determined from 270-340nm however shows broad efficacy across a wide range of UV wavelengths. The 270nm and 330nm wavelengths were equally efficacious, with a broad maximum between the 300-315nm wavelengths, not more than 3 times as effective as 270nm or 330nm (68). This action spectrum departs significantly from the action spectrum for UV immunosuppression in BALB/C mice which shows at least a decade less activity in short-wavelength UVA (320nm) (7). Thus there is also evidence that chromophores other than *cis*-UCA may also be important in initiating UV immunosuppression especially in the UVA waveband.

4.3.2. Langerhan's cells

Depletion of LC's from the epidermis occurs after a single UVB (69, 70), high dose (60 J/cm^2) UVA1 (340-400nm) (59), or 40-160 J/cm^2 UVA2 (335nm interference filter) (71) exposure in humans. Multiple exposures to broadband UVA or solar-simulated UV (ssUV) have also been shown to cause this in humans (26).

The time course and action spectrum for LC depletion has been studied more thoroughly in C3H/He mice with LC depletion maximal when measured between 36 hours and 4 days after a single 300nm UVB irradiation (72). The same study also showed using a UV monochromator set at 10nm half-maximal intensity bandwidths that the 260-300nm UV wavelengths were the most effective in causing LC depletion, with wavelengths from 320-400nm at least 4 times less effective.

DNA damage in LC's caused by UVB has been found to play a central role in local UV immunosuppression. Unrepaired UVB DNA damage in LC's is one of the initiating factors of UV suppression of local CHS induction (73, 74). This causes defective antigen presentation to Th2 cells in the draining lymph node which ultimately results in immunosuppression and production of suppressor T cells (75-78).

4.3.3. Suppressor T-cells

Studies of the phenotype and characteristics of UVB-induced suppressor T cells induced by local irradiation have shown that these cells are likely to be either CD3+, CD4+, DX5+, IL-4 secreting natural killer (NK) T-cells (79) or cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) + T cells that secrete high levels of IL-10, transforming growth factor (TGF)-beta and interferon (IFN)-1alpha but not IL-4 (80). Although suppressor T cells have been induced by chronic broadband UVA alone in C3H/HeJ mice, the phenotype of these cells is not known (81).

4.3.4. Lipid membrane peroxidation

More recently, membrane lipid peroxidation as a mechanism for initiation of immunosuppression has received greater attention. In mice and less convincingly in humans, free oxygen radicals generated as a result of oxidative stress from UVA (82) or UVB (83, 84) may act by inducing the production of platelet activating factor (PAF) which results in the production of systemically immunosuppressive cytokines such as PGE_2, IL-4 and IL-10 (85).

4.3.5. Mast cells

Mast cells are implicated in systemic UVB immunosuppression since the number of dermal mast cells is related to the dose of UVB required to cause 50% systemic suppression of CHS in 3 different strains of mice (86). UVB is required to form the *cis* isomer of UCA which

allows mast cells to degranulate and release histamine (87). Histamine with *cis*-UCA then induces human keratinocytes to produce prostaglandins that could help drive systemic UVB immunosuppression (88). Experiments with mast-cell depleted mice have shown that mast cells are necessary for systemic UVB suppression of CHS or DTH responses, but not local UVB suppression of CHS responses (89, 90). *In-vitro* studies with UVA and UVB irradiation of human mast cells have been difficult to interpret, with mixed effects depending on the activation state of the mast cell (91, 92).

4.3.6. Keratinocytes

It is known that UVB-irradiated keratinocytes produce many different cytokines including IL-1, IL-6, IL-8, tumour necrosis factor (TNF)-alpha and neuropeptides (93). This helps create an environment permissive to immunosuppression. There have been reports that like UVB, UVA1 can stimulate human keratinocytes to produce IL-10 (94), but others have found that UVA alone did not (95). The latter result may have been due to the induction of IL-12 which is known to have a Th1 promoting effect through suppressing IL-10 production (96).

4.3.7. Cytokines

Interleukin-10 in particular plays a key role in immunosuppression since it interferes with antigen-presenting cell function (97), inhibits IL-12 secretion (98), inhibits T cell activation and is also anti-inflammatory (99). The cellular source of IL-10 however has been less clear with both NK T-cells (79) and infiltrating CD1a-, DR+, CD11b+, CD36+ macrophages (100) being proposed as possible sources.

Interleukin-12 protects against immunosuppression (101) by inducing DNA repair (102), suppressing the secretion of IL-10 (96) and overcoming UV-induced tolerance (103).

5. WAVELENGTH DEPENDENCE IN VARIOUS *IN-VIVO* MODELS OF UV-INDUCED IMMUNOSUPPRESSION

5.1. Human studies

Investigations of *in-vivo* UV-induced immunosuppression in humans have been based largely on three models of skin immunity: local suppression of the induction or elicitation arms of CHS, and suppression of the elicitation arm of DTH. Only a few studies have adopted systemic immunosuppression as an immunological endpoint because of the relatively large body surface areas and doses of UV that subjects are required to be exposed to (104-107). An action spectrum for UV immunosuppression in humans has not yet been established using any of these models with monochromatic radiation sources. Instead many recent studies have focussed on the use of various sunscreen preparations with broadband UV sources that have indirectly allowed the study of UVA and its controversial role in immune suppression.

5.1.1. Suppression of CHS elicitation

The use of nickel as a recall antigen has been established as a model for the UV suppression of local CHS elicitation responses in humans in our laboratory (63). ssUV is immunosuppressive in a dose-dependent fashion as measured by reflectance spectrometry when subjects are given suberythemal doses (108) or mildly erythemal doses ranging from 0.6-2.0 J/cm^2 ssUV (109, 110) for 4 days before challenge with nickel. This has also been shown by other groups using suberythemal ssUV doses containing 70 mJ/cm^2 UVB daily for five consecutive days prior to nickel challenge and subsequent clinical scoring of nickel responses and measurement using a reflectance spectrometer (111). Results from studies using predominantly UVB or UVA sources have however been less clear.

Early studies testing simultaneous single graded doses individualised from 0.5 to 4 MED of UVB failed to show significant clinical suppression of nickel hypersensitivity reactions when subjects were challenged immediately after the dose or 4-7 days after the irradiation (112). Instead the same group then used a chronic irradiation protocol with 4 daily whole body exposures per week for 3 weeks of predominantly UVB (230-365nm) or UVA (300-430nm) sources giving a mean cumulative dose of 5.3 J/cm^2 or 205.2 J/cm^2 respectively, before challenging subjects with nickel in irradiated and unirradiated areas (104). The nickel reactions were quantified using a clinical scoring system. There was evidence of local and systemic immunosuppression in the UVB-irradiated subjects since there was a significant reduction between the clinical score of the nickel reactions at the irradiated site compared to the unirradiated test site, as well as the unirradiated test site and the nickel reaction before irradiation, respectively. UVA however did not produce any detectable local or systemic immunosuppression.

Damian *et al.* studied the time-course of local UV-immunosuppression in groups of nickel allergic subjects exposed to ssUV and broadband UVB and UVA (113). Immunosuppression was observed as early as 24 hours after a single dose of 1.2 J/cm^2 ssUV and continued to be suppressive when 1.2 J/cm^2 was given four times per week for up to 3 weeks. This ssUV time-course paralleled that for UVB in subjects given up to 5 daily doses of 144 mJ/cm^2 UVB. In contrast, the time-course for UVA immunosuppression was acute and transient, with immunosuppression evident only in skin exposed to one to three daily UVA doses of 1.9 J/cm^2 prior to nickel challenge. Chronic UVA exposures for up to 4 weeks (4 times per week) failed to cause significant suppression of nickel CHS which suggested that chronic UVA exposure could trigger immune protective mechanisms.

Using a similar nickel model, exposure to a higher dose of 17.8 J/cm^2 UVA (320-400nm) for 4 consecutive days also failed to cause significant local suppression of the nickel response (109). Subjects were also irradiated with graded doses of ssUV (0.6-2.0 J/cm^2) for 4 consecutive days to test the effect of an additional daily dose of 17.8 J/cm^2 UVA irradiation (either 320-400nm or 330-400nm UVA sources) on the ssUV immunosuppressive dose-responses. UVA (320-400nm or 330-400nm) augmented ssUV immunosuppression since

significant immunosuppression was observed at lower ssUV doses than those areas not receiving additional UVA. Although the dose-responses for 320-400nm and 330-400nm were performed in different groups which limited comparisons, the authors suggested that the 320-330nm waveband in particular contributed more toward this augmentation because the 320-400nm UVA source augmented the ssUV immunosuppression to a greater degree than the 330-400nm UVA source.

Thus ssUV, UVB and UVA have all been shown to cause significant immunosuppression using the nickel CHS model in humans in a variety of irradiation protocols. While single doses of ssUV and UVA given 24 hours prior to challenge with nickel are able to cause significant immunosuppression, with the apparent disappearance of immunosuppression seen with more chronic UVA (113), little else is known about the dose-response, time-course and effects of discrete UV wavebands using this model in humans.

5.1.2. Suppression of DTH elicitation

Using the response to tuberculin (purified protein derivative) delivered intradermally in subjects who had been previously vaccinated with Bacillus Calmette-Guérin (BCG), low suberythemal doses of ssUV from fluorescent lamps (290-360nm) of 1.3 J/cm^2 to 2.1 J/cm^2 given daily for 5 consecutive days (114), or single doses of ssUV (290-400nm) of 3.4 and 5.7 J/cm^2 given 24 or 48 hours prior to challenge (115) significantly suppress the human DTH elicitation response.

A Multitest® kit containing tetanus toxoid, diphtheria toxoid, streptococcal, tuberculin, Candida albicans, Trichophyton, and Proteus antigens allows simultaneous intradermal delivery of 7 different antigens to irradiated and unirradiated areas. The diameter of the response to each of the 7 antigens was then measured individually and summed to give a cumulative diameter score indicative of immune reactivity. Both local and systemic immunosuppression was observed with a large area (1200cm^2), high dose, chronic irradiation protocol in subjects exposed to ssUV (290-390nm) at an average of 1.5 MED each dose (with corresponding 7.5 J/cm^2 UVA) over 10 occasions, or to broadband UVA (320-400nm) of 29 J/cm^2 each dose over 12 occasions (105). The average ssUV immunosuppression ranged between 53-68% for both local and systemic suppression, and between 62-67% for both measures of UVA immunosuppression. This study was then extended, exposing subjects to a UVA1 (340-400nm) source under the same conditions, giving the same cumulative UVA1 dose of 352 J/cm^2 over 12 occasions which also caused 58-62% immunosuppression of both local and systemic DTH responses (106).

Subsequent studies by the same group using the same model have also tested the effects of high single acute doses of UV. A single acute UVA (320-400nm) exposure of 60 J/cm^2 caused systemic immunosuppression of 20% and local immunosuppression of 46% (116).

5.1.3. Suppression of CHS induction

Local UV-induced suppression of the induction of CHS has also been studied in humans. Low dose UVB (with peak emissions at 300 and 310nm) of 144 mJ/cm^2 per day administered for 4 consecutive days before sensitisation with 2,4-dinitrochlorobenzene (DNCB) on the irradiated areas caused suppression of the CHS response in 40% of healthy skin type II and III subjects, and 50% of healthy darker-skinned volunteers of African descent when they were rechallenged 30 days after initial sensitisation (55, 117). These studies using low dose UVB contrast with a report that high dose UVB of 4 MED does not cause local suppression of the induction of CHS after rechallenge with DNCB (118).

Kelly *et al.* however have found that exposure to acute single doses of 2 or 3 MED of ssUV over a 25cm^2 area 24 hours before sensitisation with DNCB causes 93 or 97% suppression of local CHS responses respectively when rechallenged 3 weeks later (107, 119).

By using a similar acute single erythemal dose protocol as Kelly *et al.* outlined above, Skov *et al.* showed that 3 MED of UVB (mean dose of 207 mJ/cm^2) or high dose UVA1 (mean dose of 171 J/cm^2) exposure over a 10cm^2 area 3 days prior to sensitisation with DPCP caused suppression of CHS induction only in the UVB exposed group, as measured by reduction of clinical scores and skin thickness on rechallenge 3 weeks later when compared to an unirradiated control group (120). UVA1 given for 3 consecutive days (mean cumulative UVA1 dose of 222 J/cm^2) prior to 3 MED UVB irradiation (mean UVB dose of 192 mJ/cm^2) afforded partial protection from UVB immunosuppression (121). When the UVA1 was given alone for the 3 consecutive days, there was no observed local immune enhancement or suppression of the induction of CHS (121). Thus, although UVA1 may have an interactive protective effect, there have been no direct studies that have shown that the UVA1 wavelengths in this *in-vivo* model cause immunosuppression.

In contrast to UVA1, a single dose of 40-160 J/cm^2 UVA2 (335nm interference filter) 72 hours prior to sensitisation with DNCB on irradiated and DPCP on unirradiated skin, caused significant local but not systemic CHS suppression respectively when subjects were rechallenged 2-3 weeks later (71).

5.1.4. Sunscreen studies

Further evidence for the role of UVA in causing immunosuppression in humans has been gleaned from studies of the effects of ssUV exposure on subjects' *in-vivo* CHS or DTH responses with or without various sunscreens offering broad-spectrum (UVB and UVA) or narrow-spectrum (UVB only) protection.

Using different models, a large body of evidence from a number of different groups has shown that a broad-spectrum sunscreen with both UVB and UVA filters rather than a narrowband UVB sunscreen is required to fully (105, 106, 122) or partially (110, 123, 124) protect against UV immunosuppression.

Broad-spectrum sunscreens also provide complete protection from UV-induced (TL-12 lamps: 250-

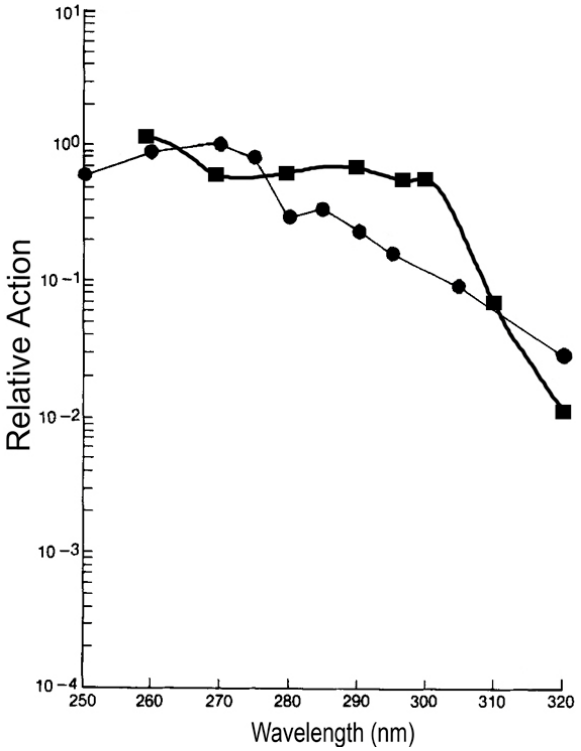

Figure 5. Action spectra for local (squares) (130) and systemic (circles) (7) suppression of contact hypersensitivity responses in mice. Reproduced and adapted with permission from Photochemistry and Photobiology (130) and The Rockerfeller University Press (7).

400nm) suppression of the MECLR in humans irradiated *in-vivo* compared to only partial protection seen with UVB filter sunscreens (125), which supports the findings of the above studies. However, the broad-spectrum sunscreen in this study reduced, but did not completely prevent the formation of *cis*-UCA, consistent with *cis*-UCA playing at least a partial role in UVA immunosuppression in humans.

Thus collectively, these sunscreen studies have provided strong indirect evidence for the role for UVA in immunosuppression in humans, but because of the difficulties in determining *in-vivo* effective waveband doses delivered through a sunscreen, they are unfortunately unhelpful in targeting the precise dose or wavelength dependence of this effect.

5.2. Murine studies

In-vivo murine studies have allowed more detailed study into understanding the various mechanisms and effects of UV immunosuppression in different models of skin immunity. Unfortunately, the elucidation of an action spectrum for UV immunosuppression in mice has not progressed into the UVA range for the past 20 years even though there have been recent *in-vivo* studies that have suggested a role for UVA (126). This highlights the difficulty and complexity of ascertaining UVA action spectra using *in-vivo* models (127).

5.2.1. Skin cancer transplant studies

While syngeneic UVB-irradiated mice can be sufficiently immunosuppressed to allow the progression of a transplanted antigenic UVB-induced skin cancer (128), it is not known whether UVA-induced skin cancers (129) display similar highly antigenic properties (51) or if UVA irradiation itself can overcome such antigenicity if it exists.

5.2.2. Suppression of CHS

The action spectrum for local UV suppression of CHS induction in female C3H/HeN mice has been determined for wavelengths between 260-320nm using a xenon-arc lamp and monochromator as the narrowband UV source (130) (Figure 5). Separate groups of mice were irradiated with graded UV doses centred around different wavelengths for 4 days before sensitisation with DNFB and then subsequent rechallenge 5 days after sensitisation with the endpoint being measured by ear swelling. Assuming single-hit kinetics for immunosuppression, the action spectrum was constructed from the dose that caused 50% immunosuppression (ID_{50}) as calculated by linear regression of dose-responses. This showed peak effectiveness at 260nm UV, with a broad shoulder at wavelengths from 270-300nm, being approximately 2-3 times less effective. The efficiency of the longer UVB wavelengths then drops off dramatically with approximately one decade less efficiency at 310nm and two decades less at 320nm, when compared to 260nm UV.

A very similarly shaped action spectrum has also been shown for the systemic suppression of CHS induction using a xenon-arc lamp with interference filters to deliver different doses of narrowband UV from 250-320nm to female BALB/c mice (7) (Figure 5). The mice were sensitised to 2-chloro-1,3,5-trinitrobenzene (TNCB) 5 days after a single or 2 consecutive day split-dose irradiation, and then measured for the suppression of ear swelling when rechallenged 5 days after sensitisation. Similar to the local CHS induction model, the peak was between 260-270nm with a broad shoulder between 280-295nm, dipping more steadily to one decade lower at 305nm and almost two decades lower at 320nm.

The dose-response curves of local and systemic suppression of CHS induction have been shown to be identical in both C57BL/6 and BALB/c mice even though there is a genetically determined difference in susceptibility to immunosuppression (131). This concurs with the concept that immunosuppression is initiated by similar mechanisms such as DNA damage in both *in-vivo* models. The action spectrum within the UVA range has not yet been elucidated however.

While earlier studies often failed to show significant immunosuppression with broadband UVA sources on local CHS induction possibly due to the low (eg. 70 mJ/cm^2) single doses used (130), other studies have shown that UVA1 can also be immunosuppressive albeit with single doses as high as 50 J/cm^2 (132). Narrowband UVB at 311nm also required a relatively high dose of 5 J/cm^2, compared to only 0.5 J/cm^2 of 280-360nm UV in the

same model to suppress local CHS induction (132). In contrast a super-high single dose of 700 J/cm^2 UVA enhanced the CHS response in BALB/c and C3H- mice by 31-105%, with no evidence of immune suppression (133).

In Skh:HR2 hairless mice, 19-39 J/cm^2 of broadband UVA delivered 24 hours or immediately before 3 MED of ssUV or UVB or topical *cis*-UCA application, protected at least partially against systemic immunosuppression (134). Subsequent studies have shown that high doses of 39 J/cm^2 broadband UVA protect against UVB immunosuppression via the induction of haem-oxygenase-1 and downstream production of carbon monoxide (CO) in Skh:HR-I mice (135). Thus, immune protection has been observed in mice as well as humans when there is UVA exposure prior to an expected immunosuppressive UVB exposure.

Dose-response studies performed on female C57BL/6 mice using a 3 consecutive daily UVA (320-400nm), UVB (290-320nm with some contaminating UVA) or ssUV (UVA+UVB) exposure protocol for suppression of systemic CHS responses, have also helped clarify the role of UVA in immune suppression and protection (136). Low doses of UVA in this study (cumulative dose: 1.3-2.5 J/cm^2) did not cause immunosuppression, with medium doses (cumulative dose: 5.0 J/cm^2) causing approximately 35% immunosuppression. Interestingly, higher UVA doses (cumulative dose: 7.6-10.1 J/cm^2) failed to cause immunosuppression, possibly through an immune-protective mechanism. This data concurs with the hypothesis that the UVA dose-response may be biphasic, affording immune protection at higher cumulative doses (113, 134). The ssUV dose-responses in this study paralleled those for UVA, whereas a relatively greater importance of UVB instead has been observed in humans (113). Hence, the exact effect of UVA on skin immune responses is still controversial as it has been shown to protect as well as suppress cutaneous immune responses, depending on dose.

There have been relatively fewer studies using animal models for the study of UV immunosuppression of CHS elicitation. TNCB-immune BALB/c mice required a single dose of at least 700 mJ/cm^2 of UVB to cause significant local suppression (137). Although the mechanisms for suppression of induction and elicitation of the CHS immune response are thought to be similar (101), there may be a different wavelength dependence (UVA1 (132) and UVA2 (109, 126) respectively) for the two processes.

5.2.3. Suppression of DTH

Broadband UVA locally suppresses the elicitation of DTH recall responses to *Candida albicans* in C3H/HeNCr mice in a dose-dependent manner at doses greater than 6 J/cm^2 UVA (126). Equivalent doses of UVA (320-400nm) and ssUV (290-400nm) suppressed the elicitation of DTH to a similar degree, but the same or higher doses of UVA1 (340-400nm: 8-16 J/cm^2) failed to cause immunosuppression. The authors suggested that the UVA2 (320-340nm) wavelengths were responsible for local suppression of the elicitation of DTH in mice. It may also be possible that lower doses of UVA1 are immunosuppressive, and that this effect is reversed and becomes immune protective with higher doses of UVA1.

In contrast to the local model of CHS induction in C3H/HeN mice which required a dose of at least 50 J/cm^2 UVA1 for suppression, the local suppression of DTH induction to HSV only required a small dose of approximately 100 mJ/cm^2 of the same broadband UVA1 (132). The same pattern was also seen for narrowband 311nm UV and broadband 280-360nm UV (132) and highlights the different dose sensitivities of CHS and DTH models.

Similar to the human studies (121) and CHS studies in mice (134), systemic suppression of UVB DTH induction to *Listeria monocytogenes* has also been shown to be protected by single doses of 18 J/cm^2 UVA (320-400nm) or 30 J/cm^2 UVA1 (340-400nm) 1 day prior to UVB exposure (138).

5.2.4. Sunscreen studies

Studies using sunscreens with different UVA filters have also shown indirectly that UVA is immunosuppressive in both local (139) and systemic (140) models of CHS induction in mice.

6. SUMMARY

While both UVB and ssUV have quite consistently been shown to cause dose-dependent immunosuppression in a variety of *in-vivo* models, the data for the immune effects of UVA thus far has been less clear. Although *in-vivo* human studies with UVA alone have not been as extensive as *in-vivo* murine studies of UVA immunosuppression, some generalisations can be made by considering the total cumulative UVA doses used in studies when stratified by the model of immunity used (Figure 6.A). UVA immunosuppression is consistently observed at a lower range of doses, with higher UVA doses failing to cause immunosuppression (human CHS induction and elicitation models). These higher doses seem to activate protective mechanisms against UVB immunosuppression and have only been demonstrated with the CHS induction model in humans so far.

When *in-vivo* murine studies with UVA alone are likewise considered (Figure 6.B), a similar trend is seen, with doses that do not cause immunosuppression being in a higher range than those causing suppression (CHS induction and DTH elicitation). The doses observed to protect from UVB immunosuppression are again, higher than those that fail to cause immunosuppression (CHS induction and DTH elicitation).

The murine DTH elicitation model is less sensitive to UVA1 than broadband UVA immunosuppression (126, 132). Whether these lower suppressive UVA doses (0.1-12 J/cm^2) are also similarly

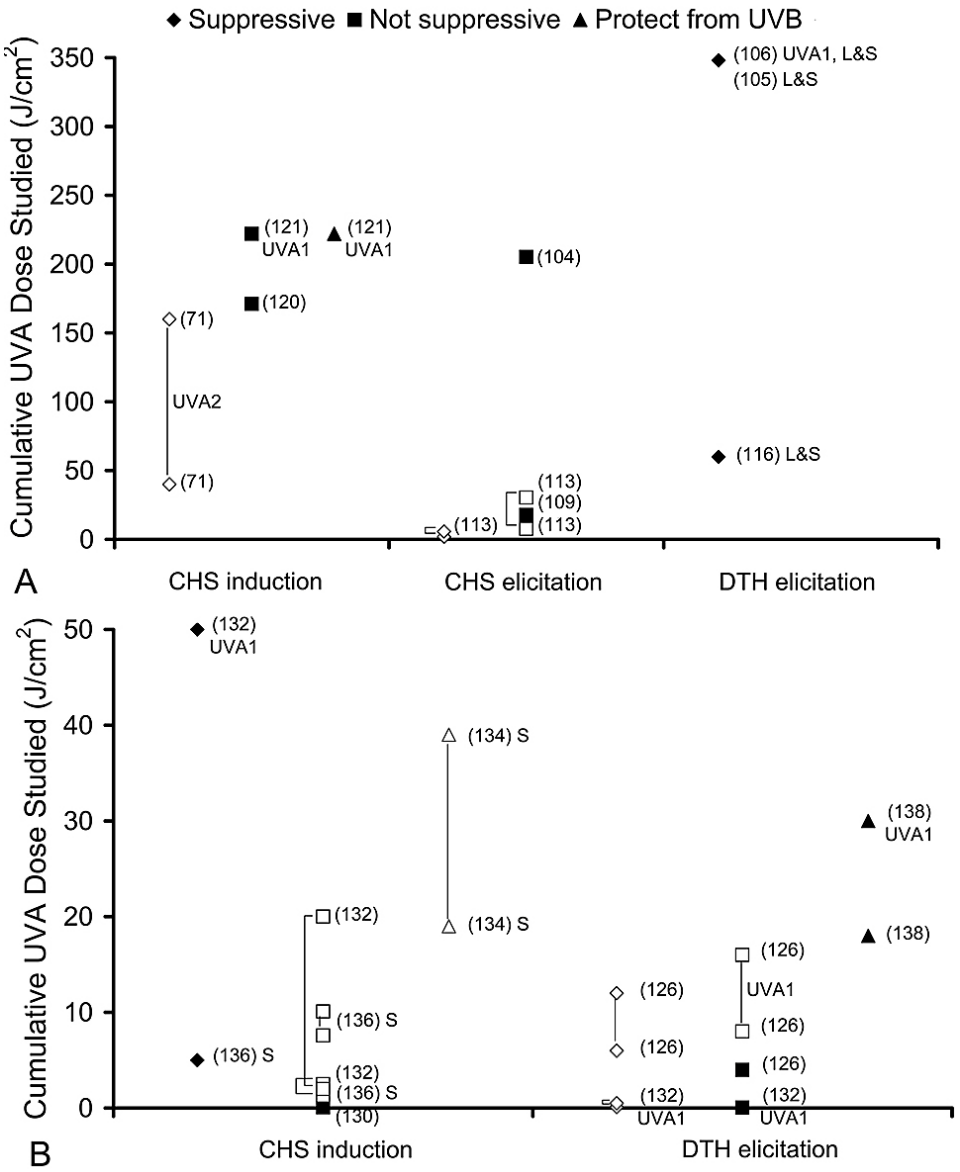

Figure 6. Doses used in (A) human and (B) murine *in-vivo* UVA immunosuppression studies. Individual studies with doses are plotted and referenced in parentheses. Solid and open symbols represent when a single dose or a range of doses were studied respectively. The UVA source used was broadband UVA (320-400nm) unless otherwise labelled as UVA1 (340-400nm) or UVA2 (320-340nm). Local (L) immunosuppression protocols were used where antigen was applied to the irradiated skin unless otherwise stated. Studies that used systemic (S) immunosuppression protocols where antigen was applied to unirradiated skin are labelled accordingly.

suppressive in a human DTH elicitation model has not yet been studied. The dose-dependency of UVA immunosuppression with the murine CHS induction model is less certain (132, 136), with the majority of studies failing to demonstrate UVA immunosuppression.

When taken in combination with the indirect evidence from human and murine sunscreen studies, it is very likely that UVA alone exerts immunosuppressive, non-suppressive, and eventually protective effects against UVB, with respectively increasing doses of UVA depending on the model of skin immunity studied.

7. CONCLUSIONS

The UV wavelength and dose-dependence of various cutaneous effects has been demonstrated by numerous action spectra. Precise investigation of the wavelength dependence of UV immunosuppression is one of the necessary steps in furthering our understanding of the mechanisms of photocarcinogenesis. Broadband UV

studies (UVB, UVA, ssUV) in the past 30 years have all produced interesting and at times conflicting observations in the various endpoints used to measure immune function. While the role of UVB wavelengths has been well established, there is now a burgeoning body of evidence that supports the role of UVA in both UV suppression and protection. The study and elucidation of the effects of pure UV narrowbands on the skin's immune system however, will hopefully provide clear scientific principles to help broaden our understanding of more complex waveband actions and interactions. Without a complete action spectrum for UV immunosuppression in any particular model of skin immunity, the interpretation of findings worldwide will always be limited by the differences in UV spectra used.

8. ACKNOWLEDGEMENTS

This work was supported by a National Health and Medical Research Council project grant. Tai Anh Phan was supported by a Postgraduate Scholarship in Dermatology administered by the Dermatology Research Foundation, University of Sydney. Diona Damian was supported by a Career Development and Support Grant from the Cancer Institute New South Wales.

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Abbreviations: CHS: contact hypersensitivity; CPD: cyclobutane pyrimidine dimer; DTH: delayed type hypersensitivity; LC: Langerhan's cell; MED: minimal erythema dose; NK: natural-killer; ssUV: solar-simulated ultraviolet radiation; UCA: urocanic acid; UV: ultraviolet radiation

Key Words: Ultraviolet Radiation, Sunlight, Immunosuppression, Immune Suppression, UVB, UVA, Skin Cancer, Review

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