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Cyclobutane pyrimidine dimer formation is a molecular trigger for solar-simulated ultraviolet radiation-induced suppression of memory immunity in humans

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We tested the hypothesis that DNA is a target for solar-simulated ultraviolet radiation (ssUVR)-induced suppression of the reactivation of memory immunity in humans. T4N5 liposomes contain the DNA repair enzyme T4 endonuclease V. This cleaves DNA at the site of ultraviolet radiation (UVR)-induced cyclobutane pyrimidine dimers (CPD), initiating DNA repair. It has previously been used to show that CPDs are a key molecular trigger for UVR-induced immunosuppression in mice. To determine whether CPD formation is involved in UVR immunosuppression in humans, nickel-allergic volunteers were irradiated with a range of doses of ssUVR. T4N5 or empty liposomes were then applied after irradiation. Nickel-induced recall immunity was assessed by reflectance spectrometry. T4N5 liposomes inhibited immunosuppression and prevented ssUVR from reducing the number of epidermal dendritic cells. T4N5 liposomes also reduced macrophage infiltration into irradiated epidermis. These studies show that enhanced removal of CPDs from human skin protects from immunosuppression, hence demonstrating that these photolesions are an important molecular event in ssUVR-induced immunosuppression in humans. CPDs also triggered loss of dendritic cells and infiltration by macrophages. It is possible that these changes to antigen presenting cells contribute to ssUVR induced suppression of recall immunity to nickel in humans.

Introduction

The ultraviolet (UVR) wavelengths of sunlight are the prime etiological agents responsible for causing both melanoma and epithelial skin cancer in humans.[1] The UVR wavelengths that reach the Earth's surface are defined as: UVB (290–320 nm), UVAII (320–340 nm) and UVAI (340–400 nm). Both UVB and UVA cause immunosuppression and gene mutations.[2,3] Both of these biological events are likely to be important for skin cancer induction in humans.

DNA is a major UVB-absorbing cellular chromophore in the skin. A predominant UVB-induced photolesion in DNA is the cyclobutane pyrimidine dimer (CPD). If unrepaired prior to cell division, CPDs can cause mutations in DNA, predisposing to gene disregulation and skin cancer.[4,5] T4 endonuclease V (T4N5) is a CPD-specific excision repair enzyme. Topical application of this enzyme encapsulated in liposomes has been shown to enhance repair of CPDs[6–8] and prevent systemic suppression of both delayed (DTH) and contact hypersensitivity (CHS) responses in mice.[9] This suggests that UVB-induced CPD formation in DNA is an important molecular event in mediating immunosuppression in mice. T4N5 liposomes have also been shown to partially protect from local immunosuppression in mice.[10] The immunoprotective effects of T4N5 liposomes include prevention of damage to dendritic cells (DC) in the epidermis.[8,10,11] UVB-induced damage to DC is likely to be an important cellular event in UVB-induced immunosuppression.[12] T4N5 liposomes have been shown to reduce the incidence of skin cancer in humans with xeroderma pigmentosum.[13] This is a rare genetic disease in which a defect in DNA repair is associated with the development of multiple skin cancers early in life, at a rate several thousand-fold greater than that in the normal population.

We have developed a method for studying UVR-induced immunosuppression in humans based on suppression of the reactivation of memory or recall responses to the contact allergen nickel.[14–16] There is little known about the mechanisms responsible for UVR-induced immunosuppression in humans. In this study we tested whether CPDs are involved in UVR-induced suppression of memory immunity to nickel in humans by enhancing CPD repair with topical T4N5 liposomes. We also examined whether CPDs lead to a loss of DC from irradiated human epidermis.

Materials and methods

Subjects

Volunteers were recruited from the local university (students and staff), general population and hospital staff. All subjects were recruited with approval from both the Central Sydney Area Health Service and the University of Sydney Ethics Committees. Written informed consent was provided prior to entry into the study. Subjects were excluded from the study if they had abnormal skin at the test site, a history of sun exposure to the lower back within six weeks prior to entry into the study, were less than 18 years of age, pregnant, lactating, or were photo-sensitive or immune suppressed through illness or medication use.

A group of 20 (one male, 19 females) healthy, nickel-allergic volunteers of Fitzpatrick skin types I to IV were recruited for the immunosuppression study. Five subjects were excluded from the final set of results due to insufficient nickel CHS reactions (lack of confluent induration) at non-irradiated sites making it impossible to assess immunosuppression. This left 15 subjects suitable for analysis. The average age of the subjects used for the final set of results was 38.9 ± 2.8 years. An additional 12 age and skin-type matched volunteers (4 males, 8 females) were used for determination of minimum erythemal doses (MED). An additional 4 volunteers donated skin samples for immunohistochemistry.

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Preparation of T4N5 liposome lotion

T4N5 liposomes, provided by Applied Genetics Inc. Dermatics (Freeport, NY) were a kind gift from Dr Dan Yarosh. Purified, recombinant T4 endonuclease V enzyme was encapsulated in liposomes consisting of phosphatidylcholine, phosphatidylethanolamine, oleic acid and cholesterol hemisuccinate (2 : 2 : 1 : 5 molar ratio), using the detergent dialysis method. The liposomes were then mixed into a 1.5% hydrogel (Carbpol-941; BF Goodrich, IN) made with phosphate buffered saline. The control liposome preparation consisted of empty liposomes, without enzyme, formulated in the same manner. These two preparations were visually identical. The products were stored at room temperature and away from direct sunlight. 10 µL cm^{-2} of liposome suspension containing 2.6 µg ml^{-1} T4 endonuclease V was applied to the test sites daily using a finger cot, immediately following exposure to UV.

Ultraviolet source

The ultraviolet source was a 1000-Watt xenon arc lamp with a collimated 7.5-cm square beam (Oriel, Stratford, CT). The lamp emission was filtered by two, 280–400 nm dichroic mirrors, which attenuated the visible and infrared components of the light source. An 'atmospheric attenuation filter' (Oriel catalogue number 81017) with a spectral cut-off of 290–400 nm, was used to modify the output spectrum to more closely simulate sunlight (solar-simulated UVR, or ssUVR). The spectra was monitored at 2-nm intervals using a calibrated OL-754 spectroradiometer (Optronic Laboratories Inc., Orlando, FL). Spectral irradiance was determined at least daily with an IL 1350 broadband radiometer using SED 038 (UVA) and SED 240 (UVB) detectors (International Light, Newburyport, MA) calibrated against the xenon-arc solar simulator with the spectroradiometer. The integrated irradiance of the ssUVR at the skin surface measured at a distance of 7.5 cm was 5.1 mW cm^{-2} UVB (290–320 nm), 11.8 mW cm^{-2} UVAII (320–340 nm) and 39.2 mW cm^{-2} UVAI (340–400 nm). The spectrum is a close match to sunlight and has been published previously.[17]

Minimum erythema dose (MED) determination

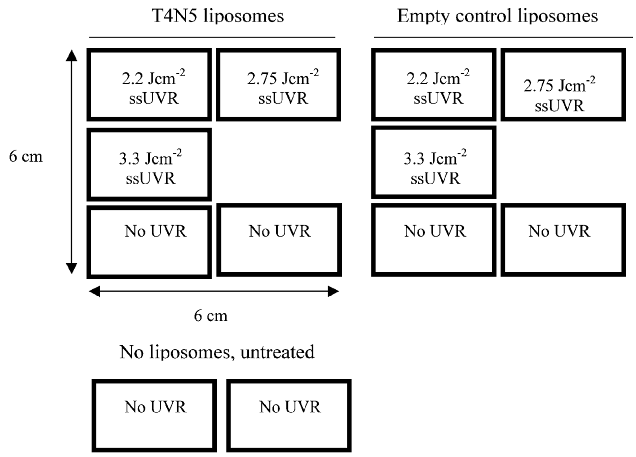
Three sets of ten, 6 × 6-mm squares on the mid-back of each volunteer were irradiated with increasing, equal increments of ssUVR. Each irradiated site was randomized to receive empty control or T4N5 liposomes applied to the skin immediately after irradiation. The third area did not receive any treatment other than ssUVR. The MED was determined visually at 22–28 h post-irradiation as the lowest dose of ssUVR that caused clearly demarcated erythema.

Nickel patch testing

To determine the degree of nickel sensitivity of each volunteer, the left side of the lower back was initially patch tested with nickel sulfate ($NiSO_4{\cdot}6H_2O$) in a petrolatum base (Trolab Hermal, Reinbeck, Germany). Nine mm Finn chambers (Epitest, Tuusula, Finland) were used to apply three concentrations of nickel sulfate (from 0.0125 to 5%) and a petrolatum-only control. The patches remained *in situ* for 48 h. The reactions were assessed 22–28 h after patch removal. Clinically, each reaction was graded from one to ten depending on the degree of vesiculation and induration. The lowest nickel concentration resulting in a uniform, erythematous reaction with papules was determined for each subject. This concentration was then used for the main study.

Solar-simulated UVR-induced suppression of nickel-induced CHS

The methodology and data analysis for this procedure has been published in detail[15] and will therefore only be described



Time course for experimental protocol

Day:	1	2	3	4	5	6	7
Protocol:	ssUVR	ssUVR	ssUVR	ssUVR + nickel patches		Nickel patches removed	Results read

Fig. 1 Experimental protocol showing the layout of the treatment groups on each volunteers back and the time course. Two 6-cm areas on each side of the lower back were divided into five regions each. Three different doses of ssUV were given to three of the regions, with the remaining two being unirradiated. The area on one side was randomly allocated to receive T4N5 liposomes and the other side received empty control liposomes. Two additional sites received neither UV nor liposomes (positive controls). This treatment was given to the same test sites daily, for four consecutive days. Nickel patches were applied on day 4, removed on day 6, and CHS was assessed 24 h after patch removal.

briefly here (Fig. 1). Volunteers were irradiated within two 6 by 6 cm areas on each side of the lower back daily for four consecutive days. These areas were randomly assigned to receive either the active T4N5 liposome or control empty liposomes. Each area was divided into five, 2 by 3 cm regions. Three regions received fixed doses of ssUVR and two were left unirradiated. The same doses of ssUVR were used in each subject; 2.2, 2.75 and 3.3 J cm^{-2} ssUVR. Fixed physical doses rather than the same MED were used in each subject as we have previously found that suppression of recall immunity to nickel is unrelated to the MED.[14] T4N5 or empty liposomes were applied to the test areas immediately after each irradiation. On day 4, after the final application of liposomes, nickel patches were applied to the irradiated test sites and to two unirradiated, untreated regions (Fig. 1). The patches were left *in situ* for 48 h. Nickel-induced erythema was assessed 22–28 h after patch removal with a reflectance spectrometer (Diastron, Hampshire, UK) as an erythema index (EI). As nickel-induced erythema was assessed 72 h after the final irradiation, any ssUVR-induced erythema had faded, however this was corrected for by subtraction from the nickel sites. Four separate measurements were performed per nickel-induced CHS reaction and also on adjacent skin. An average of the results at each test site with adjacent background skin subtracted was the nickel-induced EI, which is a measure of the CHS reaction to nickel. An average was used of the two sites that were unirradiated. Immunosuppression was calculated as EI (average of unirradiated, empty liposome treated site) − EI (test site).

Immunostaining of skin biopsies

All reagents were obtained from DAKO (Carpinteria, CA) unless otherwise specified. Punch biopsies (6 mm) were collected on day 4, immediately after the final irradiation with 3.3 J cm^{-2} ssUVR and liposome application. They were immediately snap frozen. Frozen sections were stained for dendritic cells (anti-CD11c primary antibody) and macrophages (Mac387 primary antibody) as we have described previously.[18] Mouse IgG isotype controls were used at the same protein concentrations as the primary antibodies to ensure specificity. Slides were air dried, mounted and counted by a single researcher, blinded and in triplicate to ensure accuracy. Using image analysis (Chromatic Color Image Analysis System, Leitz, Sydney, Australia), positive cells were counted along the entire length of the epidermis and expressed as cells mm^{-1}.

Statistical analysis

Statview (Abacus Concepts Inc., Berkeley, CA) was utilized for statistical analysis. Results are presented as mean +SEM and were considered significant if $p < 0.05$ using paired two-tailed Student's *t*-tests.

Results

T4N5 liposomes do not substantially affect the MED

To determine whether the T4N5 liposomes altered ssUVR-induced erythema (sunburn), a group of 12 non-nickel allergic subjects were irradiated with incremental doses of ssUVR. In the absence of any topical treatment they had an average MED of 1.8 ± 0.11 J cm^{-2} (mean ± SEM). At another site that received empty control liposomes immediately after irradiation the MED was unaltered at 1.8 ± 0.12 J cm^{-2}. Topical T4N5 liposomes applied immediately after irradiation did not appreciably affect the MED, 1.6 ± 0.10 J cm^{-2}.

Neither empty control nor T4N5 liposomes affect the nickel-induced CHS response in the absence of ssUVR

Nickel was applied to three unirradiated test sites, in duplicate in each volunteer as shown in Fig. 1. These sites received either T4N5 liposomes, empty control liposomes or no treatment for the duration of the study protocol (Fig. 2). No significant differences were found between these nickel responses ($p > 0.05$). Thus neither the T4N5 liposomes nor control liposomes altered the CHS response in the absence of ssUVR.

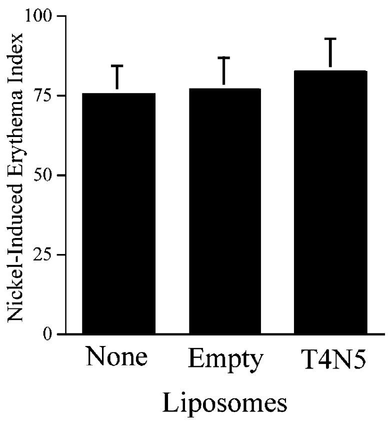


Fig. 2 Neither empty control nor T4N5 liposomes affect the nickel-induced CHS response in the absence of ssUVR. Fifteen volunteers were treated at duplicate sites for four consecutive days with nothing, empty control or T4N5 liposomes. These are the unirradiated sites shown in Fig. 1. Nickel patches were applied on day 4, removed on day 6 and the nickel-induced erythema index (measure of CHS) was assessed 24 h after patch removal by reflectance spectroscopy. The average of the duplicate sites was determined and the mean + SEM for the 15 volunteers is shown. There were no significant differences between these sites (paired two-tailed Student's *t*-test).

ssUVR-induced immunosuppression is reduced by T4N5 liposomes

The nickel-induced erythema index from two representative individuals is shown in Fig. 3. These individual examples illustrate

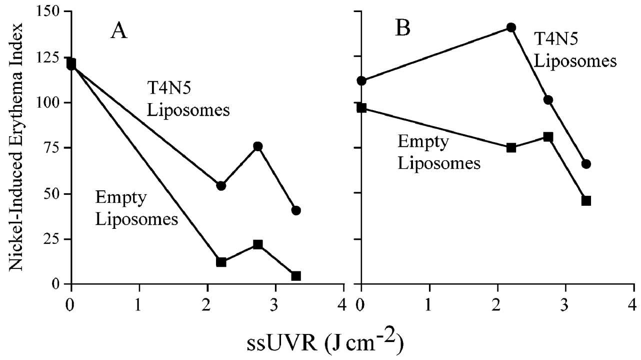


Fig. 3 Representative examples of T4N5 liposomes reducing ssUVR-induced suppression of nickel-induced CHS. Two individual subjects (A and B) were exposed to four consecutive daily doses of ssUVR prior to application of nickel patches. Some areas were treated with empty control liposomes, others received T4N5 liposomes as shown in Fig. 1. The nickel-induced erythema index was measured by reflectance spectroscopy.

extremes of the range of responses with subject A showing a large reduction in the response even at the lowest ssUVR dose tested, while subject B required the highest dose tested for suppression to be observed. In both subjects however the T4N5 liposomes reduced the level of immunosuppression. Immunosuppression was calculated by subtracting the response at each ssUVR dose from the unirradiated control in each volunteer. Pooled data from the 15 volunteers showed that 2.75 and 3.3 but not 2.2 J cm^{-2} ssUVR caused levels of immunosuppression that reached statistical significance at sites treated with empty control liposomes. T4N5 containing liposomes inhibited this immunosuppression, as significant immunosuppression was not induced by any ssUVR dose at the test sites receiving active liposomes (Fig. 4). At a daily dose of 2.75 J cm^{-2} ssUVR, immunosuppression was almost halved from 25.6 ± 10.5 to 14.1 ± 7.9 EI units by the addition of DNA repair enzymes to the test site.

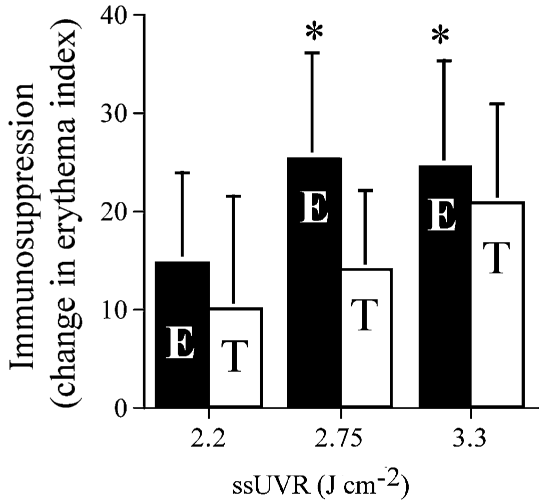


Fig. 4 ssUVR-induced immunosuppression is reduced by T4N5 liposomes. Fifteen volunteers were exposed to four consecutive daily doses of ssUVR prior to application of nickel patches. Some areas were treated with empty control liposomes (E), others received T4N5 liposomes (T) as shown in Fig. 1. Nickel-induced erythema was measured by reflectance spectroscopy. The mean of duplicate sites that received no ssUVR or liposomes (results shown in Fig. 2) was the positive, unirradiated control to which all other sites were compared and from which the irradiated sites were subtracted to determine the level of immunosuppression (change in erythema index). Immunosuppression is shown as the mean + SEM. The filled in bars labelled E represent the sites treated with empty liposomes, the open bars labelled T represent the sites treated with T4N5 liposomes. * significance; $p < 0.05$, using a paired two-tailed Student's *t*-test (n = 15), treatment site compared to the positive unirradiated control.

T4N5 liposomes inhibit ssUVR-induced reduction of epidermal CD11c+ cells and infiltration by Mac387+ cells

Biopsies from sites receiving 3.3 J cm^{-2} ssUVR daily, both with T4N5 and empty liposomes, and from an untreated, unirradiated control site, were taken from four subjects immediately after the final irradiation and application of liposomes. CD11c+ and Mac387+ cells were detected by immunostaining and counted by light microscopy and image analysis. The CD11c+ cell results for each site are presented as a percentage of the untreated, unirradiated control in the same individual to enable the effect of ssUVR to be determined independently of individual variation in control values (Fig. 5). ssUVR significantly reduced the number of epidermal CD11c+ cells compared to unirradiated control sites ($p < 0.05$; $n = 4$). T4N5 liposomes inhibited this effect of ssUVR as there was a significant increase in CD11c+ cells in the samples treated with ssUVR and T4N5 liposomes compared to the samples which received ssUVR and control liposomes. Fig. 6 shows an example of the protective effect of T4N5 liposomes on CD11c+ cells in irradiated skin.

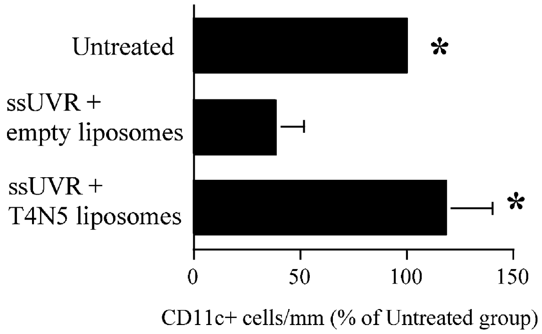


Fig. 5 T4N5 liposomes inhibit ssUVR-induced reduction of epidermal CD11c+ cells. 6-mm punch biopsies were obtained from an unirradiated, untreated control site, a site treated for four days with 3.3 J cm^{-2} per day ssUVR and empty liposomes, and a site treated for four days with 3.3 J cm^{-2} per day ssUVR and T4N5 liposomes. Biopsies were snap frozen and sections were immunostained for CD11c. Using image analysis (Chromatic Color Image Analysis System, Leitz, Sydney, Australia) cells were counted along the entire length of the epidermis and expressed as cells/mm. For each individual, the irradiated sites were expressed as a percentage of the untreated site. * significance; $p < 0.05$, compared to the ssUVR + empty liposome treated site using a paired two-tailed Student's t-test ($n = 4$).

No Mac387+ cells were found in the control unirradiated epidermis of any sample. These cells infiltrated the epidermis of each irradiated sample (26.21 ± 22.95 cells mm^{-1}) although there was considerable variation in the level of infiltration. In order to determine the effect of T4N5 liposomes independently of the level of infiltration, each T4N5 liposome and ssUVR treated site was expressed as a percentage of the site exposed to ssUVR with empty liposomes in the same individual (Fig. 7). The T4N5 liposomes significantly reduced ssUVR-induced inflammation by these cells as this group was significantly lower than the samples treated with ssUVR and control empty liposomes.

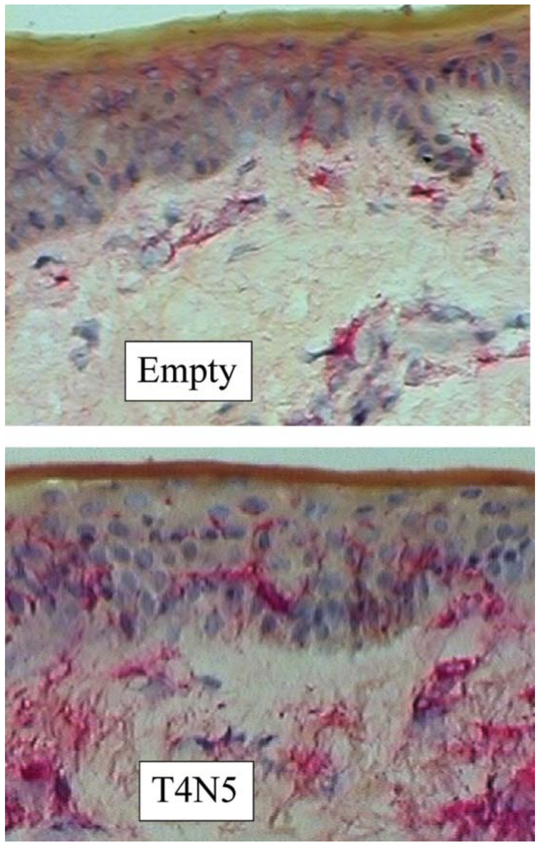


Fig. 6 Photomicrographs of CD11c+ cells in ssUV-irradiated skin treated with empty control or T4N5 liposomes. Empty-section of skin irradiated with ssUVR and treated with empty control liposomes. T4N5-section of skin irradiated with ssUVR and treated with T4N5 liposomes. The CD11c+ cells can be seen as pink dendritic cells stained with the substrate new fuchsin.

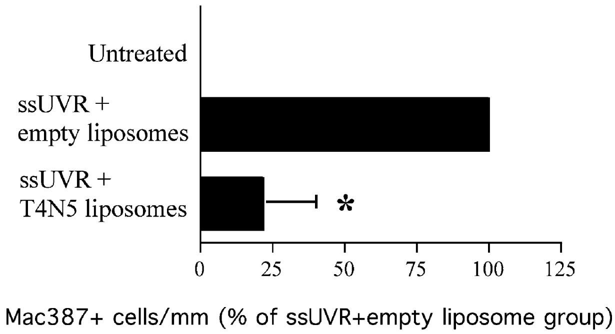


Fig. 7 T4N5 liposomes inhibit infiltration of ssUVR-irradiated epiderms by Mac387+ cells. Legend as for Fig. 4 except that the cells were immunostained for Mac387+ cells. For each individual, there were 0 infiltrating Mac387+ cells at the untreated site and therefore data was expressed as a percentage of the UVR + empty liposome treated site. * significance; $p < 0.05$, compared to the ssUVR + empty liposome treated site using a paired two-tailed Student's t-test ($n = 4$).

Discussion

The initial photobiological reaction responsible for triggering the cascade of events leading to suppression of the immune response remains controversial. There have been few studies on this in humans.[19] Here we provide evidence that CPDs are involved in ssUVR induced suppression of the recall response to nickel in humans. Our results show that the DNA-repair enzymes protect the cutaneous immune system, inhibit ssUVR-induced loss of DC from the epidermis, and prevent infiltration of the epidermis by Mac387+ macrophages.

Cyclobutane pyrimidine dimer formation was found to be involved in ssUVR-induced local cutaneous suppression of the reactivation of secondary immunity in humans. This observation supports previous studies. Topical application of T4N5 liposomes to UV-irradiated skin has been shown to decrease the number of CPDs in the epidermis and prevent suppression of primary DTH and CHS when the antigen is applied to an unirradiated site.[9] In addition these liposomes have been shown to protect from UVR suppression of primary CHS when the antigen is applied to the irradiated site[10] and reactivation of memory DTH responses to *C. albicans* in mice.[20] It therefore appears that CPDs are one of the initial photolesions that lead to suppression of primary and secondary immunity in mice, and secondary immunity in humans. Stege *et al.* showed that UVB radiation-induced suppression of recall immunity to nickel in three humans could be reduced using a different enzyme to repair CPDs, a photolyase derived from *Anacystis nidulans* that requires photoreactivation.[21] In this study the photolyase was activated by exposure to UVA, which itself suppresses recall immunity to nickel in humans.[2] While this was controlled by exposure to UVA prior to application of the photolyase, the CHS responses were only assessed semiquantitatively by clinical and histological assessment. Thus our study has used a much larger number of human volunteers, provided quantitative data on the level of protection provided by enhanced repair of CPDs, and avoided the confounding factor of requiring exposure to immunosuppressive UVA in order to activate the repair enzyme. Additionally, our study has shown that CPD repair can protect from a UV source with a good approximation to sunlight, while the study of Stege *et al.* used a UVB source. However both studies have come to the same conclusion, that CPD formation is a molecular trigger for UV-induced immunosuppression.

Langerhans cells (LC), a type of DC, are the major antigen presenting cell type found in the epidermis and are essential for the activation of primary immunity although their importance in reactivation of secondary immunity is unclear. The damaging effects of UVR on these cells is likely to be an important cellular event in immunosuppression.[12,22] Alterations in DC migration patterns and morphology occur after UVR, with a dose-dependent increase in the rate of DC migration from skin contributing to the loss of DC from UVR exposed skin.[23–25] In this study, DC were detected by immunostaining for CD11c, and ssUVR was found to decrease epidermal DC numbers. Augmenting CPD repair with T4N5 liposomes inhibited the ssUVR-induced loss of epidermal DC. Our results are consistent with previous studies. In mice, CPDs have been found in DC that migrated to lymph nodes from UVR exposed skin,[26] and T4N5 liposomes have been shown to protect DC from the damaging effects of UVR.[10] T4N5 liposomes have been shown to have direct effects on DC. Active enzymes have been demonstrated in the nucleus and cytoplasm of murine DC after topical application.[27] IL-12 also removes UV-induced DNA damage by an unknown mechanism[28] and inhibits UV from reducing LC from the skin of wild-type but not DNA repair-deficient mice.[29] This study also showed that IL-12 reduced the appearance of CPD containing LC in draining lymph nodes. Therefore, this different experimental approach has also shown that UV-induced DNA damage is a trigger for DC loss from the skin. Thus, UVR-induced DNA damage to cutaneous DC may cause them to migrate from the epidermis, and this may contribute to suppression of the reactivation of memory immunity to nickel.

It has been suggested that immunological tolerance following exposure to UVR is at least partially due to infiltration by macrophages into the epidermis in humans[30,31] and mice.[32] These inflammatory macrophages produce IL-10.[33] Presumably antigen presentation in UVR exposed skin by these macrophages in the absence of DC induces immunosuppression or tolerance. We used immunostaining with the antibody Mac387 to identify inflammatory macrophages in human skin exposed to ssUVR. Unirraditated skin did not contain any of these cells, but they infiltrated skin exposed to ssUVR. T4N5 liposomes reduced macrophage infiltration by about 75%. The mechanism by which this occurs is unknown, it is possible that enhanced CPD repair in keratinocytes reduces production of the chemokines or cytokines that attract these cells into UV exposed skin. T4N5 liposomes have been shown to reduce IL-10 production following exposure to UVR[34] and therefore are able to modulate production of such molecules in the skin.

This study shows that ssUVR-induced CPD formation in DNA is at least one key molecular mechanism that leads to ssUVR-induced suppression of the reactivation of memory immunity to nickel in humans. UVR-induced CPDs appear to disrupt antigen presentation in the skin by reducing the number of DC and causing macrophage infiltration. It is likely that CPD-induced DC depletion and macrophage infiltration are at least partially responsible for ssUVR-induced immunosuppression in humans, although other molecular and cellular events cannot be discounted.

Abbreviations

CHS, contact hypersensitivity; CPD, cyclobutane pyrimidine dimer; DTH, delayed type hypersensitivity; EI, erythema index; MED, minimum erythemal dose; ssUVR, solar-simulated ultraviolet radiation; T4N5, T4 endonuclease V; UVR, ultraviolet radiation.

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References

1 B. K. Armstrong and A. Kricker, The epidemiology of UV induced skin cancer, *J. Photochem. Photobiol. B: Biol.*, 2001, **63**, 8–18.
2 D. L. Damian, G. M. Halliday and R. S. Barnetson, Low-dose UVA and UVB have different time courses for suppression of contact hypersensitivity to a recall antigen in humans, *J. Invest. Dermatol.*, 1999, **112**, 939–944.
3 N. S. Agar, G. M. Halliday, R. S. Barnetson, H. N. Ananthaswamy, M. Wheeler and A. M. Jones, The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: A role for UVA in human skin carcinogenesis, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 4954–4959.
4 D. E. Brash, A. Ziegler, A. S. Jonason, J. A. Simon, S. Kunala and D. J. Leffell, Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion, *J. Invest. Dermatol. Symp. Proc.*, 1996, **1**, 136–42.
5 Y. Matsumura and H. N. Ananthaswamy, Toxic effects of ultraviolet radiation on the skin, *Toxicol. Appl. Pharmacol*, 2004, **195**, 298–308.
6 D. Yarosh, L. G. Alas, V. Yee, A. Oberyszyn, J. T. Kibitel, D. Mitchell, R. Rosenstein, A. Spinowitz and M. Citron, Pyrimidine dimer removal enhanced by DNA repair liposomes reduces the incidence of UV skin cancer in mice, *Cancer Res.*, 1992, **52**, 4227–31.
7 D. B. Yarosh, Liposomes in investigative dermatology [Review], *Photodermatol., Photoimmunol. Photomed.*, 2001, **17**, 203–212.
8 A. A. Vink, F. M. Strickland, C. Bucana, P. A. Cox, L. Roza, D. B. Yarosh and M. L. Kripke, Localization of DNA damage and its role in altered antigen-presenting cell function in ultraviolet-irradiated mice, *J. Exp. Med.*, 1996, **183**, 1491–1500.
9 M. L. Kripke, P. A. Cox, L. G. Alas and D. B. Yarosh, Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 7516–7520.
10 P. Wolf, P. Cox, D. B. Yarosh and M. L. Kripke, Sunscreens and T4N5 liposomes differ in their ability to protect against ultraviolet-induced sunburn cell formation, alterations of dendritic epidermal cells, and local suppression of contact hypersensitivity, *J Invest. Dermatol.*, 1995, **104**, 287–292.
11 A. A. Vink, D. B. Yarosh and M. L. Kripke, Chromophore For UV-induced immunosuppression-DNA, *Photochem. Photobiol.*, 1996, **63**, 383–386.
12 T. Schwarz, Photoimmunosuppression, *Photodermatol., Photoimmunol. Photomed.*, 2002, **18**, 141–145.
13 D. Yarosh, J. Klein, A. O'Connor, J. Hawk, E. Rafal and P. Wolf, Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study, *Lancet.*, 2001, **357**, 926–929.
14 D. L. Damian, G. M. Halliday and R. S. Barnetson, Broad-spectrum sunscreens provide greater protection against ultraviolet-radiation-induced suppression of contact hypersensitivity to a recall antigen in humans, *J. Invest. Dermatol.*, 1997, **109**, 146–151.
15 D. L. Damian and G. M. Halliday, Measurement of ultraviolet radiation-induced suppression of recall contact and delayed-type hypersensitivity in humans, *Methods*, 2002, **28**, 34–45.

16 J. M. Kuchel, R. S. Barnetson and G. M. Halliday, Nitric oxide appears to be a mediator of solar-simulated ultraviolet radiation-induced immunosuppression in humans, *J. Invest. Dermatol.*, 2003, **121**, 587–593.
17 J. M. Kuchel, R. S. C. Barnetson and G. M. Halliday, Ultraviolet A augments solar-simulated ultraviolet radiation-induced local suppression of recall responses in humans, *J. Invest. Dermatol.*, 2002, **118**, 1032–1037.
18 A. Patel, G. M. Halliday, B. E. Cooke and R. S. Barnetson, Evidence that regression in keratoacanthoma is immunologically mediated: a comparison with squamous cell carcinoma, *Br. J. Dermatol.*, 1994, **131**, 789–98.
19 M. S. Duthie, I. Kimber and M. Norval, The effects of ultraviolet radiation on the human immune system [Review], *Br. J. Dermatol.*, 1999, **140**, 995–1009.
20 D. X. Nghiem, N. Kazimi, D. L. Mitchell, A. A. Vink, H. N. Ananthaswamy, M. L. Kripke and S. E. Ullrich, Mechanisms underlying the suppression of established immune responses by ultraviolet radiation, *J. Invest. Dermatol.*, 2002, **119**, 600–608.
21 H. Stege, L. Roza, A. A. Vink, M. Grewe, T. Ruzicka, S. Grether-Beck and J. Krutmann, Enzyme plus light therapy to repair DNA damage in ultraviolet-B-irradiated human skin, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 1790–1795.
22 G. Stingl and P. R. Bergstresser, Dendritic cells: A major story unfolds, *Immunol. Today*, 1995, **16**, 330–333.
23 G. W. Dandie, G. J. Clydesdale, I. Jacobs and H. K. Muller, Effects of UV on the migration and function of epidermal antigen presenting cells, *Mutat. Res.*, 1998, **422**, 147–154.
24 G. W. Dandie, G. J. Clydesdale, F. J. Radcliff and H. K. Muller, Migration of Langerhans cells and gamma delta(+) dendritic cells from UV-B-irradiated sheep skin, *Immunol. Cell Biol.*, 2001, **79**, 41–48.
25 A. M. Moodycliffe, I. Kimber and M. Norval, The effect of ultraviolet-B irradiation and urocanic acid isomers on dendritic cell migration, *Immunology*, 1992, **77**, 394–399.
26 Y. Sontag, C. L. H. Guikers, A. A. Vink, F. R. Degruijl, H. Vanloveren, J. Garssen, L. Roza, M. L. Kripke, J. C. Vanderleun and W. A. Vanvloten, Cells with UV-specific DNA damage are present in murine lymph nodes after *in vivo* UV irradiation, *J. Invest. Dermatol.*, 1995, **104**, 734–738.
27 D. Yarosh, C. Bucana, P. Cox, L. Alas, J. Kibitel and M. Kripke, Localization of liposomes containing a DNA repair enzyme in murine skin, *J. Invest. Dermatol.*, 1994, **103**, 461–468.
28 A. Schwarz, S. Stander, M. Berneburg, M. Bohm, D. Kulms, H. van Steeg, K. Grosse-Heitmeyer, J. Krutmann and T. Schwarz, Interleukin-12 suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair, *Nat. Cell Biol.*, 2002, **4**, 26–31.
29 A. Schwarz, A. Maeda, K. Kernebeck, H. van Steeg, S. Beissert and T. Schwarz, Prevention of UV radiation-induced immunosuppression by IL-12 is dependent on DNA repair, *J. Exp. Med.*, 2005, **201**, 173–179.
30 K. D. Cooper, L. Oberhelman, T. A. Hamilton, O. Baadsgaard, M. Terhune, G. Levee, T. Anderson and H. Koren, UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans-relationship to dose, CD1a-DR+ epidermal macrophage induction, and Langerhans cell depletion, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 8497–8501.
31 C. Hammerberg, N. Duraiswamy and K. D. Cooper, Active induction of unresponsiveness (tolerance) to DNFB by *in vivo* ultraviolet-exposed epidermal cells is dependent upon infiltrating class II MHC(+) CD11b(bright) monocytic/macrophagic cells, *J. Immunol.*, 1994, **153**, 4915–4924.
32 R. Sluyter and G. M. Halliday, Enhanced tumor growth in UV-irradiated skin is associated with an influx of inflammatory cells into the epidermis, *Carcinogenesis*, 2000, **21**, 1801–1807.
33 K. F. Kang, C. Hammerberg, L. Meunier and K. D. Cooper, CD11b(+) macrophages that infiltrate human epidermis after *in vivo* ultraviolet exposure potently produce IL-10 and represent the major secretory source of epidermal IL-10 protein, *J. Immunol.*, 1994, **153**, 5256–5264.
34 C. Nishigori, D. B. Yarosh, S. E. Ullrich, A. A. Vink, C. D. Bucana, L. Roza and M. L. Kripke, Evidence that DNA damage triggers interleukin 10 cytokine production in UV-irradiated murine keratinocytes, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 10354–10359.