Ultraviolet-Radiation-Induced Erythema and Suppression of Contact Hypersensitivity Responses in Patients with Polymorphic Light Eruption

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Ultraviolet-radiation suppresses cell-mediated immunity in healthy humans. It has been postulated that, in the short term, this immunosuppression prevents autoimmune responses to ultraviolet-radiation damaged skin. Patients with polymorphic light eruption (PLE) demonstrate abnormal responses to ultraviolet-radiation suggestive of an immune response to an ultraviolet-radiation-induced antigen. We investigated whether PLE patients (n = 22) were resistant to ultraviolet-radiation-induced immunosuppression compared to skin-type, aged-matched controls (n = 23). Groups of patients and controls (six subjects per group) received a single dose of solar-simulated ultraviolet-radiation of either 0, 0.6, 1 or 2 minimal erythema doses (MED). Erythema was quantified using a reflectance meter and all volunteers were sensitised on the irradiated site with dinitrochlorobenzene. Contact hypersensitivity responses (CHS) to dinitrochlorobenzene were quantified after challenge using ultrasound. Ultraviolet-radiation-induced erythema was comparable in patients and controls. CHS was comparable in unirradiated patients and controls. UVR-induced a dose-dependent suppression of CHS in all volunteers but patients were more resistant to immunosuppression after 1MED. Exposure to 1MED suppressed CHS by 78% in controls but induced less suppression in patients (44%, $p < 0.01$). Our data suggest that PLE patients have a flaw in their immunoregulatory response to ultraviolet-radiation it is only apparent over a narrow dose range around 1 MED.

Key words: cell-mediated immunity/erythema/immune suppression/photodermatoses/solar simulated radiation.
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Polymorphic light eruption (PLE) is the most common of the so-called "idiopathic" photodermatoses, affecting 15% of healthy people in the UK (Pao *et al*, 1994). PLE is characterized by a delayed abnormal reaction to sunlight consisting of transient, nonscarring pruritic papules and vesicles, typically developing hours or days after sun exposure and resolving over several days without sequelae. The pathogenesis of PLE is unclear, but histologic studies suggest that an abnormal T-cell-mediated immunologic response is involved (Norris and Hawk, 1990).

It is well established that the ultraviolet component of sunlight ([UVR] $\approx$ 295–400 nm) suppresses cutaneous T-cell-mediated immunity in humans. Depletion of Langerhans cells, the principal antigen-presenting cells in the epidermis, recruitment of macrophages into the dermis and epidermis, and release of cytokines, in particular tumor necrosis factor α (TNF-α) and interleukin-10 (IL-10), are all important events in the initiation of UVR-induced immune suppression. These changes result in an alteration in antigen presentation leading to the generation of regulatory cells that inhibit cell-mediated immune responses to newly encountered antigens (reviewed by Norval, 2001). The immunopathology of UVR-exposed skin in PLE provides circumstantial evidence of a defect in the induction of immune suppression after UVR exposure. Kölgen *et al* (1999) have demonstrated that single UVR exposures, which did not induce lesions in PLE patients, were associated with a persistence of epidermal Langerhans cells and the recruitment of a different macrophage subset compared to normal skin. Similarly in PLE lesions UVR does not deplete epidermal Langerhans cells but provokes endothelial adhesion molecule expression and a T-cell-rich infiltrate similar to that normally seen during an allergic contact hypersensitivity (CHS) response (Norris *et al*, 1989, 1992). *In vitro* data have also shown that UVR exposure increases epidermal cell capacity to stimulate autologous peripheral blood mononuclear cells in PLE patients but not normal volunteers (Gonzalez-Amaro *et al*, 1991), implying that T cells in the peripheral blood of PLE patients recognize UVR-induced antigens on epidermal cells. Thus it is widely believed that PLE is provoked by a T-cell-mediated response against a cutaneous UVR-induced antigen.

Abbreviations: CHS, contact hypersersitivity; PLE, polymophic light eruption; UVR, ultraviolet radiation.

In this study we have tested the hypothesis, as also suggested by Kölgen *et al* (1991), that in PLE patients the normal UVR-induced suppression of cell-mediated immunity is impaired and as a result these patients develop a T-cell-mediated response to a UVR-activated antigen (photoantigen).

Cell-mediated immunity was tested in patients and age- and skin-type-matched controls by quantitative assessment of CHS response, induced by topical application of the chemical hapten 2,4-dinitrochlorobenzene (DNCB) to the skin.

Results

Single exposures to UVR induced comparable erythema responses in PLE patients and controls but did not induce PLE lesions

Visual assessments The MED range for PLE patients was within the range of normal healthy age- and skin-type-matched controls. The distribution of MED for each treatment group is summarized in Table I. UVR exposure did not induce PLE lesions in any of the volunteers either on the MED test series sites or on the 5×5 cm test site that was used to sensitize the volunteers to DNCB.

Quantitative assessments For the MED series sites, exposure to UVR induced a dose-dependent increase in erythema in both PLE patients and controls. The mean slope of the erythema dose–response curves was 18.7 erythema units per J per cm^2 (CI 15.1–22.3) for PLE patients and 22.0 erythema units per J per cm^2 (CI 18.4–25.6) for controls with no significant difference in the slope of the respective erythema dose–response curves ($p = 0.20$).

For the 5×5 cm test sites, where the volunteers were subsequently sensitized to DNCB, no significant difference was observed in the intensity of erythema 24 h after exposure to either 0.6, 1, or 2 MED between PLE patients and controls (Fig 1).

PLE patients were more resistant to UVR-induced suppression of CHS than controls after a mild sunburn (1 MED) but not after a vivid sunburn (2 MED)

Unirradiated volunteers All unirradiated volunteers in both the patient and control groups were successfully sensitized and developed a DNCB dose-dependent CHS response to all four incremental challenge doses of DNCB. There was no significant difference in the CHS response of these two groups ($p = 0.8$).

UVR-exposed volunteers Exposure to UVR induced a dose-dependent suppression of CHS in PLE patients and controls (Fig 2). A moderate but vivid sunburn (2 MED) almost completely suppressed CHS in both patients and controls (93%). At 1 MED controls were more suppressed than PLE patients, 78% *vs* 44% ($p < 0.01$; 95% CI 6–62). Suppression of CHS was also greater after 0.6 MED in controls than PLE patients (43% *vs* 31%) but this was not statistically significant ($p = 0.5$).

Discussion

The aim of this study was to compare the relationship between UVR-induced erythema and immunosuppression in PLE patients of a defined skin-type (I/II). At present there are no functional studies of UVR-induced suppression of cell-mediated immunity in PLE patients and there is also

Table I. Volunteer demographics

Group	Controls				PLE patients			
UVR exposure (MED)	0	0.6	1	2	0	0.6	1	2
Skin type	5 II	2 I	1 I	1 I	2 I	2 I	3 I	2 I
	1 II/III	2 II	3 II	2 II	3 II	2 II	3 II	2 II
		1 I/II	2 I/II	3 II/III	1 II/III			1 II/III
								1 III
Gender	4 f	5 f	5 f	3 f	4 f	3 f	6 f	6 f
	2 m		1 m	3 m	2 m	1 m		
Age[a]	31	32	38	34	36	36	45	48
	(21–35)	(19–39)	(25–47)	(20–38)	(27–46)	(31–44)	(35–50)	(32–52)
MED (J per cm^2)[b]	6.4	4.3	4.8	4.3	3.4	3.9	3.4	5.4
	(5.1–7.9)	(4.0–7.9)	(3.4–6.3)	(2.7–5.4)	(2.2–5.4)	(2.7–4.3)	(2.7–4.3)	(2.7–6.7)
MED (J per cm^2)[c]	6.2	5.1	4.8	4.4	4.1***	3.7*	3.6**	5.0*
	(5.1–7.3)	(3.0–7.1)	(3.7–6.0)	(3.4–5.4)	(2.9–5.3)	(2.4–4.9)	(2.9–4.2)	(3.6–6.4)

[a]Age expressed as median (range).
[b]Just perceptible median MED (range) expressed as total UVR dose (290–400 nm).
[c]Just perceptible mean MED (95% CI) expressed as total UVR dose (290–400 nm). Note that the differences in MED between patients and controls were not significant (*$p > 0.1$) in the 0.6 and 2 MED groups but were significant (***$p < 0.01$; **$p = 0.04$) in the 0 and 1 MED groups.

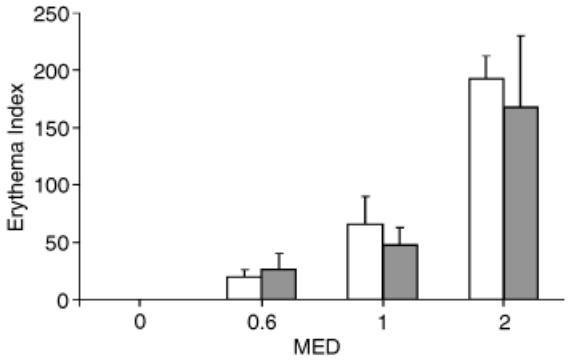

Figure 1
UVR-induced erythema responses were comparable in PLE patients and controls after an equivalent MED challenge. Erythema was quantified using a reflectance meter (Diastron) as described in *Materials and Methods*. The mean increase in erythema (erythema index + SD) for each UVR treatment group is shown. There was no significant difference in the intensity of erythema between PLE patients (■) and age- and skin-type-matched controls (□) 24 h after exposure to 0.6 MED ($p > 0.4$), 1 MED ($p > 0.17$) or 2 MED ($p > 0.37$).

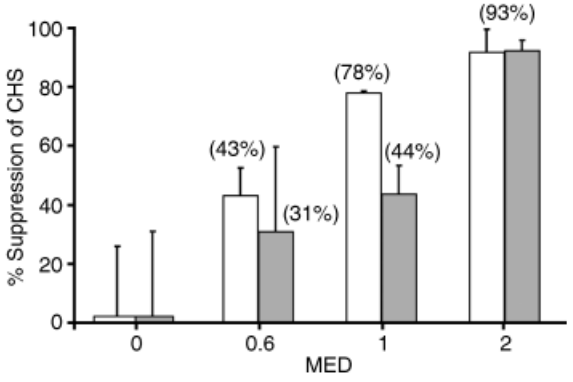

Figure 2
PLE patients were more resistant to immunosuppression than controls after a mild sunburn (1 MED), but a vivid sunburn (2 MED) almost completely suppressed both groups. The percentage suppression of CHS was calculated as described in *Materials and Methods*. The figures in parentheses represent percentage suppression of CHS and are the means ± SEM. After 1 MED, controls were more suppressed than PLE patients ($p < 0.01$; 95% CI 6–62). Suppression was also greater after 0.6 MED in controls but this was not statistically significant ($p = 0.5$). All volunteers were substantially suppressed after 2 MED.

some conflict as to whether they do (Kölgen *et al*, 1999; Boonstra *et al*, 2000) or do not (Diffey and Farr, 1986; Mastalier *et al*, 1998) have a lower MED threshold than normal controls. Ethical considerations prompted us to design the experiments using erythemally weighted UVR doses (multiples of MED), rather than a set scale of physical UVR doses, to ensure that no volunteer received a severe (blistering) sunburn. The MED range of our PLE patients, determined visually, was within that expected for healthy skin types I/II (Table I). Quantitative assessments of the degree of erythema, determined using reflectance spectroscopy, also showed that erythema responses were similar in both groups. The intensity of erythema was comparable after an equivalent MED exposure (Fig 1) and over the physical UVR dose range used for MED determination. This supports data from Diffey and Farr (1986) who reported that the erythema dose–response curves for UVB and UVC in PLE patients were indistinguishable from controls.

We also found that the CHS responses of unirradiated PLE patients were identical to controls ($p = 0.8$) and exposure to solar simulated UVR induced a dose-dependent suppression of CHS in both groups, leading to almost complete immunosuppression (93%) after 2 MED. PLE patients, however, were substantially less suppressed than controls after 1 MED (44% and 78%, respectively, $p < 0.01$). Less suppression was also seen in PLE patients after 0.6 MED but this did not reach significance ($p = 0.5$) (Fig 2). Our results suggest that PLE patients may be resistant to UVR-induced immunosuppression compared to controls but we need to be cautious in interpreting the results. It is possible that PLE patients were less immunosuppressed after a 1 MED exposure simply because the physical dose of UVR required to induce erythema in PLE patients was slightly lower than controls: 3.6 J per cm^2 and 4.8 J per cm^2, respectively ($p = 0.04$; 95% CI 0.08–2.39) (Table I). The dose–response for immunosuppression is quite steep between 0.6 and 1 MED, so a small difference in physical dose may have confounded the results. If PLE patients are resistant to suppression of CHS compared to controls then a further study with a larger number of patients is required to confirm our findings. At present, our data suggest that resistance to immunosuppression is only apparent over a modest dose range (around 1 MED) and can be overcome if higher UVR doses are given. This may explain why PLE lesions are provoked by low UVR exposures but are rarely observed after a severe sunburn.

Although our data imply that PLE patients have a flaw in their immunoregulatory response to UVR exposure and would seem to support those of Kölgen *et al* (1999), there are important differences between our functional data and Kölgen's histologic findings. First, in Kölgen's study, a high dose of nonsolar UVR (6 MED) depleted epidermal Langerhans cells in healthy controls and led to the recruitment of dermal $CD11b^+/CD68^-$ macrophage-like cells. In PLE patients, epidermal Langerhans cells were retained and the infiltrating macrophage-like cells were $CD11b^+/CD68^+$, implying that PLE patients are resistant to immunosuppression after a high dose of UVR. In contrast, we find that the highest UVR dose used in our study (2 MED) is highly immunosuppressive in both patients and controls (Fig 2). Furthermore, our previous studies have also shown that single exposures of 0.75 MED or 1 MED of solar simulated UVR do not significantly deplete epidermal Langerhans cells in healthy human subjects (Novakovic *et al*, 2001), and so a retention of epidermal Langerhans cells in our PLE patients would not explain their resistance to suppression of CHS (dermal macrophages were not assessed). Our group has also reported that Langerhans cells are present in the epidermis of UVR-provoked PLE lesions (Norris *et al*, 1989) but the time-course data from this study suggested that they migrated from the dermis after irradiation. Kölgen *et al* (1999) took biopsies at a single time point after UVR (48 h) so it is not clear if they are observing a retention or recruitment of Langerhans cells. Further work is under way in our laboratories to clarify this point and to establish if an aberrant infiltration of $CD11b^+/$

$CD68^+$ macrophage-like cells occurs in PLE after exposure to low dose solar UVR.

It is also important to note that we did not observe PLE lesions in any of our patients after single exposures to solar simulated UVR, a finding also reported in Kölgen's study. The most likely explanation for this is that all irradiations were on previously unexposed buttock skin. Our group has reported that lesions can be provoked by single exposures to low dose solar simulated UVR on previously sun-exposed sites (Norris *et al*, 1989) but we find that it usually requires three exposures to low doses (0.25–1.5 MED) of solar simulated UVR to induce lesions on buttock skin. We also find that successful provocation of PLE is dependent upon erythemally weighted UVR exposure (MED) and not cumulative physical UVR dose (J per cm^2) (van de Pas *et al*, 2004), perhaps implying that resistance to immunosuppression in the presence of mild inflammation is important in the pathogenesis of PLE. If, however, resistance to immunosuppression plays a role in disease expression it is probably not the only cutaneous abnormality. In our previous studies of healthy volunteers we found that skin types III/IV were 2-fold more resistant to UVR-induced suppression of CHS than skin types I/II for a given MED, and 5-fold more resistant for a given physical UVR dose (Kelly *et al*, 2000). None of these volunteers had a history of PLE, demonstrating that resistance to UVR-induced immunosuppression on its own does not give rise to PLE lesions. One explanation may be that the putative photoantigen in PLE is not commonly expressed or is rapidly cleared from the UVR-exposed skin of healthy controls. Heat shock protein 65 is elevated in PLE lesions (McFadden *et al*, 1994), but not in UVR-exposed skin from healthy controls, and is a possible photoantigen. So far no other studies have attempted to identify the nature of the photoantigen(s) in these patients. At present any investigations into possible flaws in immunoregulation in PLE are hampered by the limited data on normal responses to UVR in healthy humans. Much work is needed not only on the mechanisms and genetics of susceptibility factors to immunosuppression but also on wavelength dependence and modulation of immune function by multiple UVR exposures.

In conclusion, our study suggests that PLE patients may have a failure in immunoregulation after a mild sunburn (1 MED). We hypothesize that this resistance to UVR-induced suppression of cell-mediated immunity may lead to a T-cell-mediated response to a photoantigen but further work with a larger group of patients is needed to confirm these findings. Evidence is also required to link the appearance of lesions (multiple UVR exposures) with the induction of immunosuppression (single UVR exposure).

Materials and Methods

Volunteers Twenty-three white-skinned Caucasian patients (age range 19–52 y) who were diagnosed with PLE at the Photobiology Clinic of St John's Institute of Dermatology were randomly recruited into the study. Diagnosis was made on the basis of clinical history and examination, supported by monochromatic irradiation and solar simulated provocation tests as deemed clinically relevant. The condition was defined as a fully resolving, papular, photo-eruption occurring 1–48 h after sun exposure. Patients were screened to exclude lupus by estimation of their antinuclear and extractable nuclear antigen autoantibodies and porphyria by blood, urine, and stool porphyrin levels. Skin type was assessed by interview and erythema assessment. Twenty-three skin-type- and age-matched normal healthy controls, who had no history of photosensitivity, were also recruited into the study from staff and students working at St Thomas' Hospital. Only one volunteer (PLE patient) failed to complete the study.

Exclusion criteria for all volunteers, during or in the 6 mo prior to the study, included phototherapy, medication (oral contraceptive excepted), previous exposure of buttock skin (test site) to sunlamps or sunlight, a recent sunburn, or previous exposure to the contact allergen DNCB. Pregnant or lactating females were also excluded as were outdoor workers and people who participated in regular outdoor sport. The study was approved by the local ethical committee and adhered to the Declaration of Helsinki guidelines for use of human subjects. Each volunteer was fully informed of the procedures and gave written informed consent to participate. The volunteers' demographics are summarized in Table I.

UVR source and dosimetry Solar simulated UVR was generated by a 1 kW xenon arc solar simulator (Oriel, Stratford, CT) fitted with a WG320 1 mm thick glass filter, giving an even field of irradiance (290–400 nm) of about 15 mW per cm^2 on the skin surface at 11 cm from the source. Irradiance was routinely determined with a wide-band thermopile radiometer (Medical Physics, Dryburn Hospital, Durham, UK) calibrated against a DM150 double monochromator Bentham spectroradiometer (Bentham Instruments, Reading, UK). Eighty-eight percent of the erythemally effective energy of the source was in the UVB range with the remaining 12% in the UVA.

Irradiation protocol The minimal dose of UVR required to induce a just visibly perceptible erythema at 24 h (minimal erythema dose, MED) was determined on the buttock skin of each volunteer using a geometric series of eight exposure doses with increments of 1.25 (dose range 1.1–10.5 J per cm^2). Quantitative measurements of erythema intensity were also made in triplicate, 24 h after UVR exposure, using a reflectance meter (Diastron, Andover, UK). For each individual, the increase in erythema (erythema index) on each UVR-exposed site was calculated by subtracting the mean background reading of adjacent nonirradiated skin. The erythema index was plotted against physical UVR dose (J per cm^2) and the erythema response was represented by the slope of the linear regression line.

One week later, PLE patients were randomly assigned to different UVR treatment groups ($n = 6$). Healthy controls were also recruited to UVR treatment groups to match the age and skin type of the patients. Each volunteer received a single UVR exposure on a 5×5 cm site on the buttock of either 0, 0.6, 1.0, or 2.0 MED. Quantitative measurements of erythema intensity were made 24 h after UVR exposure, as described above. The erythema response of each individual was represented by the mean erythema index of the irradiated site.

Induction and quantification of CHS response Full details of the sensitization and elicitation protocols are given elsewhere (Kelly *et al*, 1998). Briefly, volunteers were sensitized via buttock skin using a 12 mm Finn chamber containing 50 μL of 0.0625% DNCB in ethanol (31.2 μg per 50 μL).

Three weeks after sensitization, volunteers were challenged on the UVR-protected upper inner arm using five 8 mm Finn chambers: one contained ethanol only and four contained incremental doses of DNCB (3.125, 6.25, 12.5, and 25.0 μg per 20 μL). The elicitation sites were marked on the arm with a surgical skin marker. The chambers were taped in place for 48 h. Elicitation responses were quantified by measuring dermal thickness immediately before and 72 h after challenge using a high frequency ultrasound scanner (Quality Medical Instruments, Silchester, Reading, UK) and the percentage increase in dermal thickness was then

determined as follows:

$$\left[\frac{\text{dermal thickness at 72 h} - \text{dermal thickness at 0 h}}{\text{dermal thickness at 0 h}}\right] \times 100\%$$

The percentage increase in dermal thickness for each elicitation site was plotted *versus* DNCB challenge dose (*x* axis), and the dose–response relationship was determined using linear regression analysis. The CHS response of a given individual is represented by the slope of the linear regression line—the steeper the slope, the stronger the response (Kelly *et al*, 1998).

Calculation of UVR-induced immunosuppression The slope of the elicitation response for each of the UVR-treated individuals was also used in the formula below to calculate percentage suppression of CHS:

$$\left[1 - \left(\frac{\text{slope of elicitation response in IR subject}}{\text{mean slope of elicitation response in UR group}}\right)\right] \times 100\%$$

where IR stands for irradiated and UR stands for unirradiated.

Statistical methods Erythema responses in patients and controls were compared using quantitative data measured with the reflectance meter from (1) the nine sites used to determine each individual's MED, where erythema response was estimated for each individual by regression of erythema index against physical UVR dose (J per cm^2); and (2) the 5×5 cm test sites used for sensitization using four groups of eight individuals exposed to a single erythemally weighted UVR exposure (multiple of MED; Fig 1). For both studies, 23 observations were available on PLE patients and 20 on controls.

The percentage suppression of CHS was determined for each individual as described above using four groups of eight individuals exposed to a single erythemally weighted UVR exposure (Fig 2). Twenty-two observations were available on PLE patients and 23 on controls.

All calculations were carried out using Microsoft Excel 2000. Comparisons of erythema and percentage suppression of CHS responses in patients and controls were compared using unpaired Student's *t* tests. Results are expressed as estimates with 95% confidence intervals (CI) and p values. Significance is assumed when $p < 0.05$.

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