DOI 10.1111/j.1365-2133.2006.07670.x

Repeated low-dose ultraviolet (UV) B exposures of humans induce limited photoprotection against the immune effects of erythemal UVB radiation

J. Narbutt, A. Lesiak, A. Sysa-Jedrzejowska, A. Wozniacka, A. Cierniewska-Cieslak,* J. Boncela,† C. Jochymski,‡ W. Kozlowski,‡ A. Zalewska, M. Skibinska§ and M. Norval¶

*Department of Dermatology and *Centre for Molecular and Medical Biophysics, Medical University of Lodz, Krzemieniecka 5, 94-017 Lodz, Poland*
†Center of Medical Biology, Polish Academy of Sciences, Lodz, Poland
‡Department of Pathology, Military Medical Institute, Warsaw, Poland
§Basildon Hospital, Basildon, Essex, U.K.
¶Medical Microbiology, University of Edinburgh Medical School, Edinburgh, U.K.

Correspondence
Joanna Narbutt.
E-mail: joanna.narbutt@onet.pl

Accepted for publication
30 August 2006

Key words
cyclooxygenase-1, cyclooxygenase-2, cytokines, photoprotection, thymine dimers, ultraviolet-induced immunosuppression

Conflicts of interest
None declared.

Summary

Background Exposure of human subjects to ultraviolet (UV) B radiation causes immunosuppression. Most experiments to date have not tested the effects of low daily doses of UVB radiation.

Objectives To ascertain whether photoprotection against several UV-induced immune effects might develop following repeated exposure.

Methods Groups of approximately 30 healthy individuals were given whole-body UVB irradiation on each of 10 consecutive days with 0.7 minimal erythema dose, or whole-body irradiation as before followed by a single erythemal UVB dose on a small body area, or irradiated only with a single erythemal UVB dose on a small body area, or were not irradiated. They were sensitized with diphenylcyclopropenone (DPCP) 24 h after the final dose, and skin biopsies collected to assess cytokine mRNA expression and the number of cells with thymine dimers and expression cyclooxygenase (COX)-1 and COX-2.

Results The contact hypersensitivity (CHS) response to DPCP was significantly lower in the three irradiated groups compared with the unirradiated controls, while cutaneous interleukin (IL)-1β, IL-6, IL-10 and tumour necrosis factor-α mRNAs, COX-1 and COX-2 and thymine dimers were all significantly higher. When the single erythemal UVB dose was given following the repeated low exposures, a slight downregulation in cytokine expression and thymine dimer formation was indicated.

Conclusions The repeated low doses of UVB protected to a limited extent against the effects of an erythemal UVB dose on cytokine expression and thymine dimer formation, but not on CHS or COX enzymes.

Solar ultraviolet (UV) radiation reaching the surface of the earth contains predominantly UVA wavelengths (315–400 nm) with a smaller component of UVB wavelengths (280–315 nm). As UVB is more effective than UVA at inducing biological damage, UVB is thought to contribute towards about 80% of the harmful effects associated with sun exposure.[1] One of these effects is suppression of cell-mediated immune responses. The process involved is complex.[2–4] It is initiated by chromophores in the upper layers of the skin that are capable of absorbing the radiation. One such chromophore is DNA[5] with the commonest photoproduct being cyclobutane pyrimidine dimers (thymine dimers, dTT). These dimers are repaired, but this process is slow with an estimated half-life of 33 h.[6] The DNA damage leads to the increased synthesis of several cytokines.[7–11] In addition, an upregulation in the production of platelet activating factor,[12] histamine and prostaglandin E_2 (PGE_2)[13,14] occurs. Cyclooxygenase (COX) enzymes are the first in a series that converts arachidonic acid into prostaglandins and thromboxane. Two isoforms of COX are found:[15] COX-1 is constitutively expressed in almost all cell types including keratinocytes, while COX-2 is induced in many tissues in response to stimuli such as injury or inflammation. UV radiation can stimulate COX-2 expression in basal keratinocytes.[16]

Modulation of the contact hypersensitivity (CHS) response is frequently used to evaluate immunity in human subjects following UV exposure.[17] In most cases the sensitizer, a hapten such as diphenylcyclopropenone (DPCP), is applied to the skin within a few days of the UV radiation, with the elicitation phase taking place several weeks later. To measure local immunosuppression, the hapten is placed directly on an irradiated skin site. A primary allergic response (PAR) can develop within 1–2 weeks of sensitization in many individuals who have not previously come in contact with the particular sensitizer. This response is thought to indicate the onset of hapten-specific sensitization.[18]

To date, most information regarding UV-induced immunosuppression in human subjects has been gathered from studying the induction phase of CHS following exposure of a small area of the body to a single UV dose, often > 1 minimal erythema dose (MED).[19–21] However, such a protocol does not mimic natural conditions as most people are exposed to the sun for short periods of time on a daily basis over the summer months with many experiencing occasional burning UV exposures on limited areas of the body. There is the possibility that, as the skin adapts to the chronic UV radiation by epidermal thickening and tanning, photoprotection against the effects of UV on the immune system may develop. In the present study, we aimed to test this possibility by repeatedly irradiating volunteers with suberythemal UVB, followed by a single erythemal UVB dose on a small body area. CHS, cytokine and COX expression, and DNA damage were then assessed in comparison with unirradiated subjects or others irradiated with the single erythemal dose only.

Materials and methods

Subjects

The study included 140 healthy subjects of either phototype II or III, as assessed by Fitzpatrick score.[22] These are the most commonly occurring skin types in Poland and include subjects who do not burn easily and develop a tan on sun exposure. The volunteers were recruited following advertising in the local newspapers and within the University of Lodz. They were without any skin or other disease and were not receiving any medication. Subjects exposed to high doses of sunlight or sunlamps within 2 months prior to the study, or previously sensitized with DPCP, were excluded. The experimental procedures were conducted in the winter months to decrease any influence of natural sunlight. Each volunteer gave written informed consent before entry into the study and underwent a thorough physical examination and full blood count. The experimental plan was approved by the Local Ethics Committee of the Medical University of Lodz and was conducted according to the Declaration of Helsinki principles.

Phototesting and ultraviolet B irradiation

Phototesting of each volunteer was undertaken approximately 1 week before the study began using a Waldmann Medizintechnik UV 109 device (Waldmann Medizintechnik, Villingen-Schwenningen, Germany) containing TL-12 tubes (Philips, Eindhoven, the Netherlands) emitting 58.6% UVB and 41.4% UVA. The test was performed with an incremental dose series on six squares (1 × 1 cm) on the back. The MED was defined as a just perceptible erythema 24 h later. From this value, 0.7 MED was calculated for each individual although it is recognized that, due to undulations on the body surface and variations in MED with site, some areas may receive slightly higher and others slightly lower doses.

The volunteers were divided into five groups as shown in Table 1: 40 nonirradiated individuals served as the control group (group A), 30 subjects were given whole-body UV irradiation for 10 consecutive days with a dose of 0.7 MED (group B), 30 subjects were given whole-body UV irradiation for 10 consecutive days with a UV dose of 0.7 MED followed 24 h later by a single UV dose of 3 MED (left buttock, 10 × 10 cm) (group C), 30 subjects were irradiated with a single UV dose of 3 MED (left buttock, 10 × 10 cm) (group D) and 10 individuals were irradiated on the left buttock (10 × 10 cm) with a single UV dose of 4 MED (group E). The TL-12 lamps, with an erythema effectiveness irradiance for UVB (280–315 nm, biologically weighted with the CIE erythema action spectrum) of 0.64 mW cm^{-2}, were used for the whole-body exposures, and the phototesting device was used to irradiate the buttock. Measurement of the intensity of the lamps was performed using a type 1 UV meter calibrated against a spectrophotometer (Waldmann Medizintechnik). For erythema, pigmentation, PAR and CHS measurements, all 140 subjects were included. For the cytokine mRNAs (n = 40),

Table 1 Characteristics of subjects in groups A–E

Group	Number of volunteers	Mean age, years (range)	Sex, F/M	Phototype, II/III	MED, J cm^{-2}, mean (range)
A: unirradiated	40	25.0 (18–36)	17/23	19/21	0.18 (0.09–0.21)
B: 10 × 0.7 MED, whole body	30	30.0 (19–38)	19/11	14/16	0.16 (0.09–0.19)
C: 10 × 0.7 MED, whole body (C1) + 3 MED, 10 × 10 cm (C2)	30	27.8 (19–32)	16/14	18/12	0.15 (0.07–0.17)
D: 3 MED, 10 × 10 cm	30	29.4 (18–37)	16/14	11/19	0.14 (0.09–0.19)
E: 4 MED, 10 × 10 cm	10	30.6 (19–34)	4/6	5/5	0.14 (0.07–0.19)

MED, minimal erythema dose.

COX proteins (n = 21) and dTT (n = 21), not all the subjects in each group were used on ethical grounds, as these assessments required the collection of skin biopsies, up to three per person in some instances.

Erythema and pigmentation

Erythema and pigmentation were quantified using the UV Optimise 555 device (Chromo-Light, Espergaerde, Denmark) before and after irradiation, but before sensitization, on the buttock skin in the UV-exposed groups (groups B–E). The mean values of three readings were calculated.

Sensitization and elicitation of contact hypersensitivity

Sensitization and elicitation of CHS were performed using DPCP (Fluka Chemie GmbH, Buchs, Switzerland) as described by Narbutt *et al.*[23] In brief, the subjects were sensitized on the left irradiated (groups B–E, 24 h after the final UVB exposure) or nonirradiated (group A) buttock skin. The sensitization site was assessed for the development of a PAR, including its length of time, severity and size. Three weeks later, all the volunteers received an antigenic challenge on the unirradiated upper inner left arm skin using a series of 20 μL acetone containing 0.4, 0.8, 1.6, 3.2 and 6.4 μg DPCP. The highest concentration of DPCP was applied only if no PAR was observed. The CHS response was evaluated after 48 h by a subjective visual scoring system: 0, no reaction; 1, macular erythema; 2, erythema with infiltration; 3, erythema with infiltration and papules or vesicles; 4, bullous reaction. This score was determined by one dermatologist who did not know the group assignations.

Assessment of interleukin (IL)-1β, IL-6, IL-10 and tumour necrosis factor-α mRNA expression by relative quantitative reverse transcription–polymerase chain reaction

The control samples consisted of 3-mm punch biopsies from 10 subjects in group A, 15 in group C (before irradiation began) and 15 in group D (before irradiation began), making 40 in total. The irradiated samples consisted of two more biopsies from the same 15 individuals in group C (C1: 24 h after 10 days of 0.7 MED UVB, and C2: 24 h after 3 MED UVB) and one more biopsy from the same 15 individuals in group D (24 h after 3 MED UVB). The samples (50 mg) were dissolved in Fenozol reagent and total mRNA prepared using a total RNA Prep Plus Kit (A&A Biotechnology, Gdynia, Poland). The first strand of cDNA was synthesised according to the manufacturer's instructions (Promega, Madison, WI, U.S.A.) using (dT)20 primer with 2 μg of total RNA. Then mRNAs for tumour necrosis factor (TNF)-α, interleukin (IL)-10, IL-6 and IL-1β were determined by relative quantitative reverse transcription–polymerase chain reaction (RT–PCR) using β-actin mRNA as an internal standard,[24] using the following primers: 5′-GTAGCCCATGTTGTAGCAAACC-3′ and 5′-GAGGACCTGGGAGTAGATGAGG-3′ for TNF-α, 5′-GAGAACAGCT GCACCCACTTCC-3′ and 5′-CTGGGTCTTGGTTCTCAGCTTGG-3′ for IL-10, 5′-ACCTGAACCTTCCAAAGATGG-3′ and 5′-GACT GCAGGAACTCCTTAAAGC-3′ for IL-6, 5′-CTCGCCAGTGAAAT-GATGG-3′ and 5′-GCATCTTCCTCAGCTTGTCC-3′ for IL-1β and 5′-CAGCAGATTCAAGCAGCTATGG-3′ and 5′-GTCTGTGG-TGCTGATCTCATCC-3′ for β-actin. The RT–PCR assay contained 5 μL cDNA sample, 10 × *Taq* polymerase buffer (Epicentre Biotechnologies, Madison, WI, U.S.A.), 2 mmol L^{-1} $MgCl_2$, 1 × PCR enhancer, deoxyribonucleoside triphosphate mix, and 25 pmol of each primer in 50 μL reaction volume. The samples were denatured at 95 °C for 5 min, then cooled on ice before 1.25 U *Taq* DNA polymerase (Epicentre Biotechnologies) was added. The reaction was performed in 25–30 extension cycles consisting of a 30-s denaturation step at 94 °C, a 30-s annealing step at 60 °C and a 40-s polymerase extension step at 72 °C. Finally, each reaction was terminated with a 10-min elongation step at 72 °C. The final products were electrophoresed in 7% polyacrylamide gels using the genetic size marker 100 bp DNA Ladder (Promega). Bands were visualized by UV radiation, and the results were recorded photographically and analysed densitometrically using LKB Ultrascan XL Enhanced Laser Densitometer (Pharmacia LKB, Uppsala, Sweden). Concentrations of mRNAs were normalized in each sample relative to β-actin mRNA. Usually, four to seven bands were analysed for each sample and the mean value was calculated. The mean ± SD was then calculated for each group.

Expression of cyclooxygenase (COX)-1 and COX-2, and assessment of DNA damage (thymine dimers)

Three-millimetre biopsies were collected from buttock skin of seven unirradiated individuals (group A), seven individuals in group C (C1: 24 h after 10 days of 0.7 MED UVB, and C2: 24 h after 3 MED UVB), and seven individuals in group D (24 h after 3 MED UVB). The samples were put in liquid nitrogen immediately, and stored at −80 °C until analysis.

For COX-1 and COX-2 assessment, 4-μm frozen sections were collected on to Superfrost Plus slides (Menzel, Braunschweig, Germany), fixed in acetone for 10 min and air dried. Endogenous peroxidase was blocked by incubating for 5 min with 3% hydrogen peroxide before washing in phosphate-buffered saline (PBS) and incubation for 30 min in 10% normal horse serum in Tris–HCl–PBS and 1% bovine serum albumin (BSA). After rinsing, the sections were incubated overnight at 4 °C with the COX-1 monoclonal antibody (mAb) (dilution 1 : 40, NCL-COX-1, clone 12E12; Novocastra, Newcastle upon Tyne, U.K.) or COX-2 mAb (dilution 1 : 50, NCL-COX-2, clone 4H12; Novocastra). Detection was performed with LSAB+ System-HRP (Dako Cytomation, Glostrup, Denmark). High-sensitivity diaminobenzidine (Dako Cytomation) chromogenic substrate system was used. The slides were counterstained with haematoxylin and dehydrated. Negative controls were performed by omitting the primary

antibody. The presence of brown colour at the sites of the target antigens (in cellular membrane, cytoplasm) indicated positive immunoreactivity. The number of COX-1+ or COX-2+ cells in the epidermis was counted by a computer-image program (SIS Analysis; Olympus, Tokyo, Japan). At least two sections were examined from each subject at × 400 magnification, and the mean ± SD number of positive cells mm^{-2} for each group calculated.

For the assessment of dTT, 4-μm cryostat sections were collected on to Superfrost Plus slides (Menzel), fixed for 1 h in 4% buffered formalin at room temperature and air dried. The sections were treated for 15 min of microwave oven heating in Target Retrieval Solution (citrate buffer, pH 6.0; Dako Cytomation) at 800 W and then transferred to distilled water. Endogenous peroxidase was blocked by incubation for 30 min with 0.3% hydrogen peroxide in methanol. Slides were washed in PBS and incubated for 30 min with 10% normal rat serum in Tris–HCl–PBS and 1% BSA. After rinsing, the sections were incubated overnight at 4 °C with the dTT mAb (dilution 1 : 4000, clone KTM53; Kamiya Biomedical Company, Seattle, WA, U.S.A.). Detection, colour development and counterstaining were performed as outlined above for COX. Negative controls were performed by omitting the primary antibody. Stained sections were examined at × 400 magnification throughout the whole of the epidermis. Presence of brown colour at the target antigen in cellular nucleus indicated positive immunoreactivity. The number of dTT+ nuclei was counted by a computer-image program (SIS Analysis). At least two sections were examined from each subject and the mean ± SD number of positive cells mm^{-2} for each group calculated.

Statistical analysis

The Mann–Whitney test, Wilcoxon pair test and χ^2 test were used to analyse the results, with $P < 0.05$ being considered statistically significant.

Results

Repeated suberythemal ultraviolet (UV) B exposure caused erythema and pigmentation development, and was slightly protective against the erythemal effects of a subsequent high UVB dose

Clinical examination revealed no visible erythema after 10 days of suberythemal whole-body UVB but a slight pigmentation was evident. Irradiation with 3 or 4 MED UVB on the small body area caused well-defined oedematous erythema. Table 2 shows the detailed results for the measurement of erythema and pigmentation in groups B–E. In all four groups, the erythema values were significantly higher ($P < 0.005$) following the UV exposure than before irradiation. In group C, after 10 days of irradiation with suberythemal UVB followed by irradiation with 3 MED on the small body area, the erythema value was significantly lower than in group D ($P < 0.0001$) and group E ($P < 0.0001$). Suberythemal UVB on 10 consecutive days led to a significant increase in pigmentation ($P = 0.001$ for group B and 0.0019 for group C), while the additional erythemal UVB dose decreased the pigmentation value in group C ($P = 0.000004$). Following erythemal UVB, pigmentation was significantly lower in group D compared with group C ($P = 0.0004$). The decrease in pigmentation observed in groups D and E may have resulted from the intense erythema and oedema which cause changes in the optical properties of the skin.

Repeated suberythemal ultraviolet (UV) B exposures suppressed the occurrence of primary allergic response and contact hypersensitivity, and did not protect against the immunosuppressive effects of a subsequent erythemal UVB dose

PAR was detectable a mean of 8.8 days after sensitization. The response ranged from erythema with definite borders

Table 2 Erythema and pigmentation following ultraviolet (UV) exposure in groups B–E (mean ± SD, n = 30 in groups B–D and n = 10 in group E)

	Group B: 10 × 0.7 MED, whole body	Group C: 10 × 0.7 MED, whole body + 3 MED, 10 × 10 cm	Group D: 3 MED, 10 × 10 cm	Group E: 4 MED, 10 × 10 cm
Erythema				
% value light reflectance before UV	28.1 ± 5.8	26.0 ± 5.3	29.2 ± 5.8	25.2 ± 4.6
% value light reflectance after 10 × 0.7 MED	36.6 ± 7.0	37.8 ± 6.7	–	–
% value light reflectance after 3 or 4 MED (10 × 10 cm)	–	48.9 ± 7.4	62.6 ± 9.9	60.4 ± 7.1
Pigmentation				
% value light reflectance before UV	20.6 ± 5.7	21.6 ± 7.1	21.1 ± 8.0	23.2 ± 6.2
% value light reflectance after 10 × 0.7 MED	24.0 ± 5.8	24.9 ± 6.7	–	–
% value light reflectance after 3 or 4 MED (10 × 10 cm)	–	22.1 ± 8.3	13.9 ± 9.0	17.4 ± 8.1

MED, minimal erythema dose.

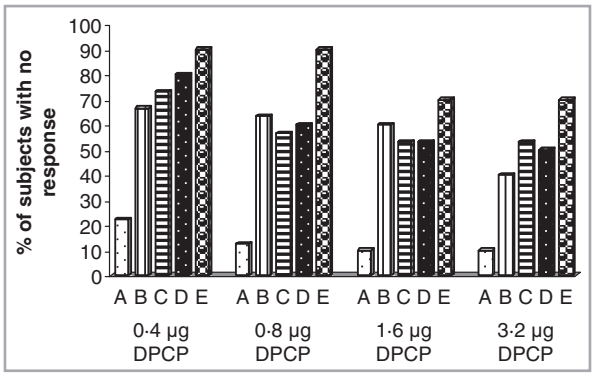

Fig 1. Percentage of subjects with no contact hypersensitivity response to the challenge dose of 0.4, 0.8, 1.6 and 3.2 μg diphenylcyclopropenone (DPCP) in groups A–E.

to strong erythema with oedema, and vesicles in some cases, but without blistering. The diameter of the PAR varied between 9 and 19 mm. The percentage of subjects in the unirradiated group with PAR (60%) was significantly higher than in all the irradiated groups (26.7%, 33.3%, 26.7% and 10% in groups B, C, D and E, respectively; $P < 0.05$ for all comparisons). There were no statistical differences between the irradiated groups B–E with respect to the occurrence of PAR, its length of time, severity or diameter.

The CHS response at the highest concentration of DPCP was not analysed statistically as the eliciting dose of DPCP was applied only in the volunteers in whom PAR did not develop. UV radiation in all the groups suppressed the CHS response, and it was observed at all the DPCP concentrations ($P < 0.05$ for all comparisons, data not shown). As analysis of the visual assessment of the CHS revealed statistically significant differences between the unirradiated group and any of the irradiated groups at any of the DPCP concentrations and because, in the majority of the irradiated volunteers, the CHS was assessed as 0 (absent), we decided to simplify the analysis by treating the CHS response as a binominal trait where 0 represented no reaction and 1 represented a response. These results are shown in Figure 1. Statistical analysis of the CHS response showed no significant differences between subjects in group B and group C ($P > 0.05$), or between subjects in group C and group D ($P > 0.05$).

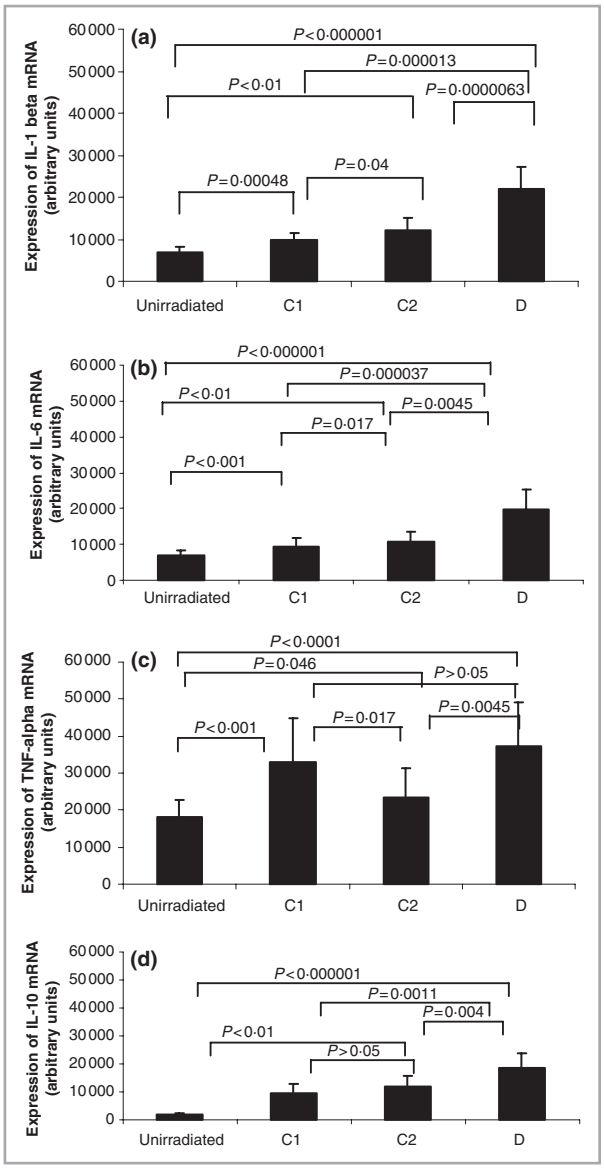

Fig 2. Expression of (a) interleukin (IL)-1β, (b) IL-6, (c) tumour necrosis factor (TNF)-α and (d) IL-10 mRNAs in skin biopsies from unirradiated subjects ($n = 40$), subjects irradiated with 0.7 minimal erythema dose (MED) ultraviolet (UV) B for 10 consecutive days (C1, $n = 15$) followed by 3 MED UVB (C2, $n = 15$), and subjects irradiated with 3 MED UVB (D, $n = 15$). The cytokine expression was normalized to the β-actin mRNA intensity and is presented in arbitrary units. The mean ± SD for each group is shown.

Repeated suberythemal ultraviolet (UV) B exposures increased the expression of interleukin (IL)-1β, IL-6, IL-10 and tumour necrosis factor-α mRNAs in skin biopsies, and slightly protected against the increased expression of these cytokines induced by a subsequent erythemal UVB dose

Following the suberythemal UVB exposures for 10 consecutive days (group C1), the expression of the mRNAs of IL-1β, IL-6, IL-10 and TNF-α increased significantly (Fig. 2a–d). After the additional exposure to erythemal UVB (group C2), there was a further, although smaller, increase in IL-1β, IL-6 and IL-10 mRNA expression and a decrease in TNF-α mRNA expression. The single erythemal UVB dose induced a large rise in the expression of all four cytokine mRNAs (group D). The increase was greater than that following the repeated suberythemal exposures and also following the repeated suberythemal exposures plus the additional erythemal exposure.

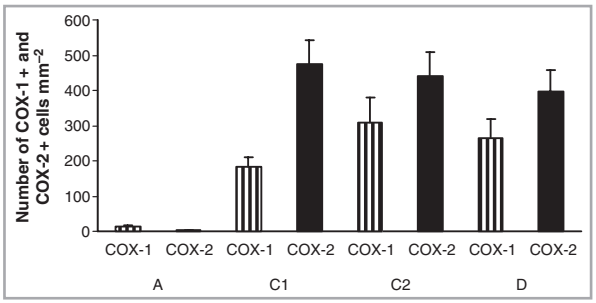

Fig 3. Number of cyclooxygenase (COX)-1+ and COX-2+ cells mm^{-2} in the epidermis of unirradiated subjects (A, n = 7), subjects irradiated daily with 0.7 minimal erythema dose (MED) ultraviolet (UV) B for 10 consecutive days (C1, n = 7) followed by 3 MED UVB (C2, n = 7), and subjects irradiated with 3 MED UVB (D, n = 7). The mean ± SD for each group is shown.

Repeated suberythemal ultraviolet (UV) B exposures increased the number of cyclooxygenase (COX)-1+ and COX-2+ cells in skin biopsies, and did not protect against the increased number induced by a subsequent erythemal UVB dose

In unirradiated skin biopsies, COX-1+ cells were observed only occasionally, mainly in the basal layer of the epidermis, and no COX-2+ cells were seen. The number of both COX-1+ and COX-2+ cells increased significantly following UVB exposure ($P < 0.05$ for all comparisons, Fig. 3). In irradiated skin, the COX-1+ cells were located in both the basal layers and the upper part of the epidermis, while the COX-2+ cells were present mainly in the suprabasal layer of the epidermis (Fig. 4). The additional erythemal UVB dose following the repeated suberythemal doses (group C2) induced almost the same number of COX-1+ and COX-2+ cells as the single erythemal UVB dose on its own (group D) (Fig. 3), indicating that photoprotection for this factor had not developed.

Repeated suberythemal ultraviolet (UV) B exposures increased the number of thymine dimer cells in skin biopsies, and slightly protected against the increased number induced by a subsequent erythemal UVB dose

In unirradiated skin, only a few dTT cells were observed (15 mm^{-2}), scattered within the epidermis, and the staining was considered weak. The number increased significantly following UV exposure ($P < 0.05$ for all comparisons, Fig. 5). The highest was seen after irradiation with the single erythemal dose (group D) and this number was significantly greater than that following the repeated suberythemal doses followed by the single erythemal dose (group C2) ($P = 0.00001$). The dTT+ cells were found mainly in the upper parts of the epidermis following the repeated suberythemal doses of UVB, while they were present throughout the epidermis following the single erythemal dose.

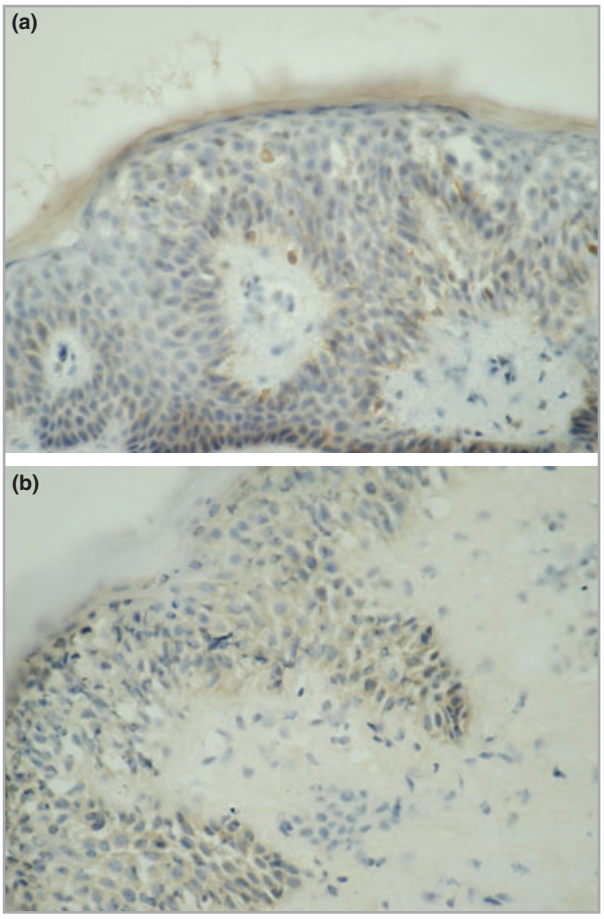

Fig 4. Expression of cyclooxygenase (COX)-1 (a) and COX-2 (b) in human epidermis after 0.7 minimal erythema dose (MED) ultraviolet (UV) B for 10 consecutive days followed by 3 MED UVB as shown by immunohistochemistry (original magnification × 400).

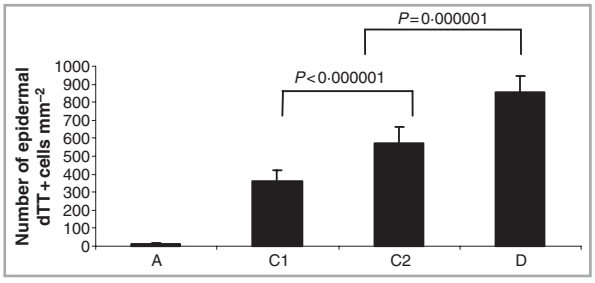

Fig 5. Number of thymine dimers (dTT+) cells mm^{-2} in the epidermis of unirradiated subjects (A, n = 7), subjects irradiated daily with 0.7 minimal erythema dose (MED) ultraviolet (UV) B for 10 consecutive days (C1, n = 7) followed by 3 MED UVB (C2, n = 7), and subjects irradiated with 3 MED UVB (D, n = 7). The mean ± SD for each group is shown.

Discussion

In a previous study we attempted to simulate the natural exposure of subjects with skin phototypes II/III to summer sunlight by whole-body irradiation with 0.3 MED solar-simulated

radiation (SSR) daily for up to 30 days.[23] Their immune responses to DPCP were then assessed. We found that both the PAR and the CHS were reduced. These effects were dependent on the cumulative UV dose as the downregulation became more apparent as the number of days of exposure increased. Therefore photoadaptation of this immune response had not occurred, despite the development of some pigmentation in the skin.

In the present study, broadband UVB lamps were used rather than SSR but, as the UVB waveband is the most biologically active and has similar immunological effects as SSR, we assume that the changes induced by such lamps mimic closely those following exposure to natural sunlight. We wished to establish whether photoprotection against several effects of an erythemal UVB dose was generated by pre-exposure of subjects to suberythemal whole-body UVB radiation for 10 days. We estimate that the dose of UVB given daily was equivalent to being in the sun for about 35 min around midday on a clear sky summer day in mid-Europe. The repeated irradiations resulted in a small but significant degree of protection against the erythemal effects of the subsequent high UVB dose. This might be expected as the subjects were all of phototype II/III (the commonest skin type in Poland) with the ability to tan to some extent in response to solar UV radiation.

The repeated low UVB doses resulted in the suppression of both the PAR and CHS, indicating a lack of photoadaptation and corroborating our previous study.[23] In addition, these exposures did not offer any protection against the downregulating effects of a subsequent erythemal UVB dose on PAR and CHS. In one of the few previous reports to monitor CHS after several exposures to low doses of UVB, Cooper *et al.*[19] showed that irradiation of a small body area with 0.7 MED UVB on four consecutive days caused suppression of the CHS response in 68% of subjects. Damian *et al.*[25] demonstrated that the recall response to nickel in nickel-allergic volunteers was suppressed by exposure of a small area of the skin to suberythemal SSR and that this effect was maintained even after 4 weeks of repeated UV exposures. Long-term UVB phototherapy given to patients with psoriasis was reported to impair subsequent sensitization to DPCP.[26]

Following UV radiation of human skin, there is an upregulation in the expression of various cytokines,[10, 27–33] induced by various mediators such as calcitonin gene-related peptide, α-melanocyte stimulating hormone, platelet activating factor, histamine and PGE_2.[3] The vast majority of the studies monitoring cytokine changes has been performed either *in vivo* following a single erythemal dose of UVB, or *in vitro* in keratinocyte cultures. As far as we are aware, the situation *in vivo* following repeated suberythemal exposures has not been examined previously. We found that the mRNAs of the four selected cytokines, IL-1β, IL-6, IL-10 and TNF-α, were increased as a result of the 10 daily suberythemal UVB exposures. However, the rise for each cytokine was significantly less than that following the single erythemal UVB exposure, indicating that the extent of the change was not dependent on the cumulative dose of UVB. It was also noted that a small but significant photoprotection against the mRNA induction of all four cytokines occurred in the group irradiated daily for 10 days before receiving the single erythemal UVB exposure (group C2, Fig. 2a–d) compared with the group irradiated with the single erythemal UVB dose (group D, Fig. 2a–d).

PGE_2 is the predominant prostaglandin formed in the human epidermis, and its production is increased after UV exposure.[34] Of the two major forms of COX, COX-2 is induced by both UVB and UVA-II radiation.[35] The COX enzymes have important effects on apoptosis and proliferation in the skin,[36–38] and PGE_2 has been shown to play a critical role in UV-induced systemic immunosuppression in mice.[13,16] It was demonstrated by Athar *et al.*[39] that the extent of COX-2 protein expression in the epidermis of UVB-irradiated mice strongly correlated with dose; after several weeks of exposure, COX-2 was present in all the layers of the epidermis but most strongly in the basal layer. We found a similar pattern of expression of COX-2 in human skin following the 10 daily suberythemal exposures to UVB. The UVB radiation increased COX-1 and COX-2 protein expression even when no clinical signs of erythema were present. When the numbers of COX-1+ and COX-2+ cells in the epidermis of subjects irradiated with the single erythemal UVB dose were compared with the numbers in subjects preirradiated with 10 daily suberythemal doses followed by the erythemal dose, no evidence for the development of photoprotection was obtained.

One of the major absorbers of UV radiation in the skin is DNA, and DNA photoproducts, such as dTT, are recognized as critical molecular triggers for local and systemic suppression of CHS.[7,40] IL-12, given either before or after the UV exposure, can prevent this effect.[41–44] DNA damage is associated with many biological changes in the skin other than immunomodulation, including erythema and sunburn cell formation,[45,46] and it also enhances melanogenesis.[47] In the present study, all the UV protocols increased the number of dTT+ epidermal cells, most after the single erythemal UVB dose. The number in the skin of subjects irradiated with the 10 daily doses of suberythemal UVB before administration of the single erythemal dose was about 34% less than this figure. Thus some photoprotection against DNA damage had been generated by the repeated pre-exposures. As erythema correlates with DNA damage, the small degree of photoprotection observed against erythema may explain this result. However, the protection was by no means absolute as, in the repeatedly exposed subjects, the subsequent single erythemal UV dose increased the number of dTT+ cells substantially, from about 363 to about 573 mm^{-2}. In confirmation of this result, Sheehan *et al.*[48] showed that dTT steadily accumulated during 12 daily suberythemal SSR exposures of volunteers with skin types II and IV, and that a tan may not be the major factor in photoprotection against erythema.

In conclusion, we have shown that repeated exposures of individuals to suberythemal UVB resulted in the generation of slight erythema and pigmentation, the suppression of PAR and CHS, and an increase in the mRNA expression of four cytokines, in COX-1 and COX-2 proteins, and in dTT. The repeated

irradiations protected to a limited extent against the subsequent effects of an erythemal UVB dose on erythema, cytokine expression and dTT formation, but not on the suppression of PAR and CHS or the expression of the COX enzymes. Thus sun protection measures are recommended, even in subjects who have developed a tan.

Acknowledgments

This study was funded by the European Union project QTL-CT-2001-0022IHA-UV and the Medical University of Lodz projects 503-1019-1 and 502-11-352. The dTT monoclonal antibody was kindly provided by Professor H. van Loveren.

References

1 Diffey BL. Sources and measurement of ultraviolet radiation. *Methods* 2002; **28**:4–13.
2 Clydesdale GJ, Dandie GW, Muller HK. Ultraviolet light induced injury: immunological and inflammatory effects. *Immunol Cell Biol* 2001; **79**:547–68.
3 Ullrich SE. Mechanisms underlying UV-induced immune suppression. *Mutat Res* 2005; **57**:185–205.
4 Schwarz T. Regulatory T cells induced by ultraviolet radiation. *Int Arch Allergy Immunol* 2005; **137**:187–93.
5 Vink AA, Shreedhar V, Roza L *et al.* Cellular target of UVB-induced DNA damage resulting in local suppression of contact hypersensitivity. *J Photochem Photobiol B Biol* 1998; **44**:107–11.
6 Young AR, Chadwick CA, Harrison GI *et al.* The *in situ* repair kinetics of epidermal thymine dimers and 6–4 photoproducts in human skin types I and II. *J Invest Dermatol* 1996; **106**:1307–13.
7 Kibitel J, Hejmadi V, Alas L *et al.* UV-DNA damage in mouse and human cells induces the expression of tumor necrosis factor α. *Photochem Photobiol* 1998; **67**:541–6.
8 Petit-Frere C, Clingen PH, Grewe M *et al.* Induction of interleukin-6 production by ultraviolet radiation in normal human epidermal keratinocytes and in a human keratinocyte cell line is mediated by DNA damage. *J Invest Dermatol* 1998; **111**:354–9.
9 Nishigori C, Yarosh DB, Ullrich SE *et al.* Evidence that DNA damage triggers interleukin-10 cytokine production in UV-irradiated murine keratinocytes. *Proc Natl Acad Sci U S A* 1996; **93**: 10354–9.
10 Skov L, Hansen H, Allen M *et al.* Contrasting effects of ultraviolet A1 and ultraviolet B exposure on the induction of tumour necrosis factor-alpha in human skin. *Br J Dermatol* 1998; **38**:216–20.
11 Brink N, Szamel M, Young AR *et al.* Comparative quantification of IL-1beta, IL-10, IL-10r, TNFalpha and IL-7 mRNA levels in UV-irradiated human skin *in vivo*. *Inflamm Res* 2000; **49**:290–6.
12 Walterscheid JP, Ullrich SE, Nghiem DX. Platelet-activating factor, a molecular sensor for cellular damage, activates systemic immune suppression. *J Exp Med* 2002; **195**:171–9.
13 Hart PH, Jaksic A, Swift G *et al.* Histamine involvement in UVB- and *cis*-urocanic acid-induced systemic suppression of contact hypersensitivity responses. *Immunology* 1997; **91**:601–8.
14 Shreedhar V, Giese T, Sung VW, Ullrich SE. A cytokine cascade including prostaglandin E_2, interleukin-4, and interleukin-10 is responsible for UV-induced systemic immune suppression. *J Immunol* 1998; **160**:3783–9.
15 Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxidase H synthase (cyclooxygenases-1 and -2). *J Biol Chem* 1996; **271**:33157–60.
16 Tripp CS, Blomme EAG, Chinn KS *et al.* Epidermal COX-2 induction following ultraviolet irradiation: suggested mechanism for the role of COX-2 inhibition in photoprotection. *J Invest Dermatol* 2003; **121**:853–61.
17 Damian DL, Halliday GM. Measurement of ultraviolet radiation-induced suppression of recall contact and delayed-type hypersensitivity in humans. *Methods* 2002; **28**:34–45.
18 Tseng C, Hoffman B, Kurimoto I *et al.* Analysis of effects of ultraviolet B radiation on induction of primary allergic reactions. *J Invest Dermatol* 1992; **98**:871–5.
19 Cooper KD, Oberhelman L, Hamilton TA *et al.* 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 U S A* 2003; **89**:8497–501.
20 Kelly DA, Walker SL, McGregor JM, Young AR. A single exposure of solar simulated radiation suppresses contact hypersensitivity responses both locally and systemically in humans: quantitative studies with high frequency ultrasound. *J Photochem Photobiol B Biol* 1998; **44**:130–42.
21 Wolf P, Hoffmann C, Quehenberger F *et al.* Immune protection factors of chemical sunscreens measured in the local contact hypersensitivity model in humans. *J Invest Dermatol* 2003; **121**: 1080–7.
22 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 1988; **124**:869–71.
23 Narbutt J, Lesiak A, Skibinska M *et al.* Suppression of contact hypersensitivity after repeated exposures of humans to low doses of solar simulated radiation. *Photochem Photobiol Sci* 2005; **4**:517–22.
24 Al-Nedawi KN, Czyz M, Bednarek R *et al.* Thymosin beta4 induces the synthesis of plasminogen activator inhibitor 1 in cultured endothelial cells and increases its extracellular expression. *Blood* 2004; **103**:1319–24.
25 Damian DL, Barnetson RS, Halliday GM. Effects of low-dose ultraviolet radiation on *in vivo* human cutaneous recall responses. *Australas J Dermatol* 2001; **42**:161–7.
26 Friedmann PS, White SI, Parker S *et al.* Antigenic stimulation during ultraviolet therapy in man does not result in immunological tolerance. *Clin Exp Immunol* 1989; **76**:68–72.
27 Oxholm A, Oxholm P, Staberg B, Bendtzen K. Immunohistological detection of interleukin 1-like molecules and tumor necrosis factor in human epidermis before and after UVB-irradiation *in vivo*. *Br J Dermatol* 1998; **118**:369–76.
28 Chung JH, Youn SH, Koh WS *et al.* Ultraviolet B irradiation-enhanced interleukin (IL)-6 production and mRNA expression are mediated by IL-1alpha in cultured human keratinocytes. *J Invest Dermatol* 1996; **106**:715–20.
29 Barr RM, Walker SL, Tsang W *et al.* Suppressed alloantigen presentation, increased TNF-alpha, IL-1, IL-1Ra, IL-10, and modulation of TNF-R in UV-irradiated human skin. *J Invest Dermatol* 1999; **112**:692–8.
30 Enk AH, Katz SI. Identification and induction of keratinocyte-derived IL-10. *J Immunol* 1992; **149**:92–5.
31 Kang K, Hammerberg C, Meunier L, Cooper KD. 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**:5256–64.
32 Piskin G, Bos JD, Teunissen MB. Neutrophils infiltrating ultraviolet B-irradiated normal human skin display high IL-10 expression. *Arch Dermatol Res* 2005; **269**:339–42.
33 Strickland I, Rhodes LE, Flanagan BF, Friedmann PS. TNF-α and IL-8 are upregulated in the epidermis of normal human skin after

UVB exposure: correlation with neutrophil accumulation and E-selectin expression. *J Invest Dermatol* 1997; **108**:763–8.
34 Pentland AP, Mahoney M, Jacobs SC, Holtzman MJ. Enhanced prostaglandin synthesis after ultraviolet injury is mediated by endogenous histamine stimulation. *J Clin Invest* 1990; **86**:566–74.
35 Mahns A, Wolber R, Stab F *et al.* Contribution of UVB and UVA to UV-dependent stimulation of cyclooxygenase-2 expression in artificial epidermis. *Photochem Photobiol Sci* 2004; **3**:257–62.
36 Muller-Decker K, Kopp-Schneider A, Marks F *et al.* Localization of prostaglandin H synthase isozymes in murine epidermal tumors: suppression of skin tumor promotion by inhibition of prostaglandin H synthase-2. *Mol Carcinog* 1998; **23**:36–44.
37 Pentland AP, Schoggins JW, Scott GA *et al.* Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis* 1999; **20**:1939–44.
38 Pentland AP, Scott G, VanBuskirk J *et al.* Cyclooxygenase-1 deletion enhances apoptosis but does not protect against ultraviolet light-induced tumors. *Cancer Res* 2004; **64**:5587–91.
39 Athar M, An KP, Morel KD *et al.* Ultraviolet B (UVB)-induced COX-2 expression in murine skin: an immunohistochemical study. *Biochem Biophys Res Commun* 2001; **280**:1042–7.
40 Kripke ML, Cox PA, Alas LG, Yarosh DB. Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. *Proc Natl Acad Sci U S A* 1992; **89**:7516–20.
41 Muller G, Saloga J, Germann T *et al.* IL-12 as mediator and adjuvant for the induction of contact sensitivity *in vivo*. *J Immunol* 1995; **155**:4661–8.
42 Schwarz A, Grabbe S, Aragane Y *et al.* Interleukin-12 prevents UVB-induced local immunosuppression and overcomes UVB-induced tolerance. *J Invest Dermatol* 1996; **106**:1187–91.
43 Schwarz A, Stander S, Bernburg M *et al.* Interleukin-12 suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair. *Nat Cell Biol* 2002; **4**:26–31.
44 Schwarz A, Maeda A, Kerneback K *et al.* Prevention of UV radiation-induced immunosuppression by IL-12 is dependent on DNA repair. *J Exp Med* 2005; **201**:173–9.
45 Young AR, Chadwick CA, Harrison GI *et al.* The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *J Invest Dermatol* 1998; **111**:982–8.
46 Stege H, Roza L, Vink AA *et al.* Enzyme plus light therapy to repair DNA damage in ultraviolet-B-irradiated human skin. *Proc Natl Acad Sci U S A* 2000; **97**:1790–5.
47 Eller MS, Ostrom K, Gilchrest BA. DNA damage enhances melanogenesis. *Proc Natl Acad Sci U S A* 1996; **93**:1087–92.
48 Sheehan JM, Cragg N, Chadwick CA *et al.* Repeated ultraviolet exposure affords the same protection against DNA photodamage and erythema in human skin types II and IV but is associated with faster DNA repair in skin type IV. *J Invest Dermatol* 2002; **118**:825–9.

