

# Repeated Doses of UVR Cause Minor Alteration in Cytokine Serum Levels in Humans

Joanna Narbutt,<sup>1</sup> Aleksandra Lesiak,<sup>1</sup> Malgorzata Skibinska,<sup>2</sup> Anna Wozniacka,<sup>1</sup>  
Anna Sysa-Jedrzejowska,<sup>1</sup> Joalanta Lukamowicz,<sup>3</sup> and Henk van Loveren<sup>4</sup>

<sup>1</sup>Department of Dermatology, Medical University of Lodz, Krzemieniecka 5, 94-017 Lodz, Poland

<sup>2</sup>Department of Dermatology, Basildon University Hospital, SS165NL Essex, England

<sup>3</sup>Laboratory of Immunochemical Research, Polish Mother's Memorial Hospital-Research Institute, 93-338 Lodz, Poland

<sup>4</sup>National Public Health Institute, Bilthoven, 3720 BA, The Netherlands

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The aim of our study was to compare serum concentration of IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$  in 105 healthy volunteers before and after exposure to UVR: 25 subjects (10 days of UVB), 55 (10 days of UVB or solar-simulated radiation, followed by acute UVB dose), and 25 (local high dose of UVB). In all the individuals blood samples were analyzed before and after final irradiation by chemiluminescence assay. After 10 days of UVB irradiation a statistically significant increase in serum concentration only in IL-8 ( $P < .05$ ) and strong tendency in TNF- $\alpha$  ( $P = .05$ ) were observed. The applied schedules of irradiation have minor impact on serum cytokine level and still a threshold dose of UVR causing systemic immune impairment is unknown.

## INTRODUCTION

Ultraviolet radiation (UVR), an important environmental factor, exerts both beneficial and harmful effects on human beings. Positive health effects of UV are not only connected with general well-being and vitamin D production but are also used in the treatment of certain autoimmune and allergic diseases. UV exposure initiates a complex cascade of responses, which finally results in down-regulation of the immune system. Deleterious effects of UV exposure include erythema, burns, photoaging, DNA damage, carcinogenesis, and impaired resistance to bacterial, viral, parasitic and fungal infections [1, 2, 3, 4]. As various immune mediators (eg, IL-1, IL-6, IL-10, IL-12, TNF- $\alpha$ , prostaglandin  $E_2$ ) were found to be secreted by UV-irradiated keratinocytes and some were also found in the serum of UV-irradiated mice [5, 6]; therefore it is likely that any alteration in the skin immune system may have an impact on the systemic immunological response.

Under normal or pathological conditions, cytokines, circulating and released under various stimuli, can modulate cells' activities. They also take part in various biological phenomena such as inflammatory response, acute-phase reaction, and wound healing. One of the most

important cytokines is TNF- $\alpha$ , secreted under various stimuli such as infections and acute ultraviolet irradiation. This cytokine shows many biological activities, for example, enhances phagocytosis and cytotoxicity and modulates expression of proteins such as IL-1 and IL-6 [7]. Two isoforms of IL-1 (IL-1 $\alpha$  and IL-1 $\beta$ ) are important mediators of inflammatory reactions, promoting adhesion of neutrophils, monocytes, and T and B cells. This cytokine also influences Langerhans cells function and is involved in the cutaneous response to UV radiation [8]. IL-6 is a potent cytokine with various biologic activities including induction of fever and acute-phase response [9]. It is also the most important mediator of systemic sunburn reaction. Its synthesis is enhanced by inflammatory stimuli such as microbes [9]. Interleukin-8 is one of the proinflammatory chemokines which mobilizes and activates neutrophils, lymphocytes T, NK cells, and basophils. It presents chemotactic abilities for migratory immune cells and is a mitogen for epidermal cells. IL-10 is known to be important for systemic and local photoimmunosuppression. Some studies revealed its increased concentration in serum of UV-irradiated mice, while treatment with anti-IL-10 antibodies protected mice against UV-mediated immunosuppression [10, 11, 12].

Most studies regarding the influence of UVR on levels of inflammatory cytokines were conducted mainly in cultures of human keratinocytes or in laboratory animals. Few studies were also performed in human subjects; however the groups were small and in most cases only single, high doses of UVR were used.

Correspondence and reprint requests to Joanna Narbutt, Department of Dermatology, Medical University of Lodz, Krzemieniecka 5, 94-017 Lodz, Poland; joanna.narbutt@onet.pl

TABLE 1. Clinical characteristics of studied volunteers. F denotes female; M denotes men; and MED denotes minima erythema dose.

Group	Number of subjects	Gender F/M	Mean age	Phototype II/III	Mean MED (J/cm <sup>2</sup> )
A	25	12/13	31	12/13	0.16
B	30	16/14	27.7	8/22	0.15
C	25	13/12	25	14/11	0.14
D	25	13/12	29.5	16/9	0.14

Thus, the aim of our study was to compare serum concentration of IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$  in human healthy volunteers before and after exposure to different schedules and sources of UV irradiation, mainly low, chronic doses.

## MATERIAL AND METHODS

### Human subjects

The study included 105 healthy volunteers, aged between 18 and 55 years with either skin phototype II or III, according to Fitzpatrick's scoring system [13]. A clinical characteristic of all the volunteers is presented in Table 1. Minimal erythema dose values in all examined subjects groups were comparable and ranged from 0.14 to 0.16 J/cm<sup>2</sup>.

General health of the volunteers was good; they did not have any skin conditions nor were they receiving any medications. People exposed to sunlight or sunlamps less than two months prior to the study were excluded. To decrease the influence of natural solar radiation, all the procedures were performed between November 2003 and February 2004. Each volunteer gave written informed consent before entering the study and all the experiments were approved by the local Ethics Committee. The investigations were carried out in accordance with the Declaration of Helsinki. Before entering the study the subjects underwent thorough physical examination, blood cell count, and urinalysis. All the subjects in each group were sex and age matched (Table 1). The volunteers were divided into 4 groups (A–D) exposed to irradiation in the following way:

- (i) Group A: irradiation with UVB lamps with a dose of 0.7 personal UVB MED (whole body for 10 consecutive days),
- (ii) Group B: irradiation with UVB lamps with a dose of 0.7 personal UVB MED (minimal erythema dose) (whole body for 10 consecutive days) followed by a single UVB exposure with a dose of 3 MED (buttock skin, 10  $\times$  10 cm),
- (iii) Group C: irradiation with Cleo Natural lamps with a dose of 1.2 SED (standard erythema dose when 1 SED is equivalent to an erythema radial exposure of 100 J/m<sup>2</sup>) of SSR (solar-simulated radiation) for 10 consecutive days followed by a single UVB exposure with a dose of 3 MED (buttock skin, 10  $\times$  10 cm) [14],

- (iv) Group D: irradiation with a single UVB exposure with a dose of 3 MED (buttock skin, 10  $\times$  10 cm).

### Sources of irradiation

#### Ultraviolet B

UVB was generated by 100 W B12 lamps (Philips, Eindhoven, The Netherlands) giving an even field of irradiance (UVB 285–340 nm) of about 3.85 mW/cm<sup>2</sup> on the skin surface at 20 cm from the source. Measurement of the intensity of the B12 lamps was performed with the UV meter (type I) (Waldmann Medizintechnik, Villingen-Schwenningen, Germany).

Local UVB irradiation was performed using a Waldmann Medizintechnik UV 109 device (Waldmann Medizintechnik) with a dose of 3 MED applied on right buttock skin (10  $\times$  10 cm).

#### Solar-simulated radiation

The SSR was generated by 100 W Cleo Natural lamps (Philips) giving an even field of irradiance (4% UVB 280–315 nm; 96% UVA 315–400 nm) of about 4.95 mW/cm<sup>2</sup> (280–400 nm) on the skin surface at 20 cm from the source. Measurement of the intensity of the Cleo lamps was performed with the Solar Light 3D UV meter (Solar Light Co, Philadelphia, Pa).

#### Phototesting

Phototesting (ie, assessment of sensitivity to UVB radiation) of each volunteer was undertaken using a Waldmann Medizintechnik UV 109 device with incremental UV doses on six squares (1 cm  $\times$  1 cm) on the inner surface of the forearm. The MED was defined as the dose of UVB that induced a perceptible erythema observed 24 hours later.

#### Blood collection

In the subjects from Groups A and D blood samples were taken on two occasions (before phototesting, 24 hours after the 10th exposure in Group A, and 24 hours after local 3 MED UVB irradiation in Group D). In all the individuals from Groups B and C blood samples were taken three times (before the phototesting, 24 hours after the 10th exposure to UVB or SSR, and 24 hours after 3 MED irradiation). After centrifugation all serum samples were stored at  $-70^{\circ}$  C until analyzed.

### Cytokine measurement

Serum samples were analyzed for IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$  serum concentration with chemiluminescence assay (Diagnostic Products Corporation, Los Angeles, Calif) according to the manufacturer's instructions. Barcode-labeled units contained one bead with a murine-specific monoclonal antibody in which alkaline phosphatase (conjugated to a specific rabbit polyclonal antibody) was an enzyme for chemiluminescence reaction. These IMMULITE kit components were required for immunochemiluminescence assay measurements by the IMMULITE 1000 analyzer. After 30 or 60 minutes of incubation of antigen with a specific antibody, chemiluminescence substrate was hydrolyzed by alkaline phosphatase and then a beam of photons was released. Intensity of chemiluminescence reaction was measured by a luminometer and it was in line with the concentration of determined cytokine. Moreover for each cytokine measurement IMMULITE cytokine control module was performed.

Sensitivity and reference ranges for IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$  were 5.0 pg/mL, 0–5 pg/mL; 2.0 pg/mL, 0–9.7 pg/mL; 5.0 pg/mL, 0–62 pg/mL; 1.5 pg/mL, 0–9.1 pg/mL; and 4.0 pg/mL, 0–8.1 pg/mL, respectively.

### Statistical analysis

For the statistical analysis, the medians and the range of values (min-max) were calculated. Nonparametric Wilcoxon pair test and nonparametric chi [2] test ( $df = 1$ ) after transformation of the variables into binominal ones (below and above detection limit) were used for comparison of the results before and after irradiation. Comparisons and correlations were considered significant when  $P < .05$ .

### RESULTS

All the results of the analyses of cytokine concentrations are presented in Table 2. Cytokine levels measured before and after irradiations under various protocol conditions in the majority of examined subjects were within normal ranges.

IL-1 $\beta$  concentrations were below detectable levels in all (105/105) volunteers, both before and after UVB or SSR irradiation.

IL-6 was detectable in 17 out of 105 individuals before irradiation and its concentration increased slightly after UV exposure (in each assessed group). However, no statistical correlation between UV exposure and IL-6 level was found.

IL-8 levels were detectable before irradiation in 29 out of 105 volunteers. We found a statistically significant increase ( $P < .05$ ) in IL-8 serum level after 10 days of UVB irradiation in Groups A and B (mean values raised from 5.34 to 6.04 pg/mL and from 5.65 to 6.22 pg/mL, resp). Local acute UVB irradiation which was additionally applied in Group B did not cause any significant changes in this cytokine level.

No changes in IL-8 level were observed in Group D in which only local acute dose of UVB was applied. In contrast to chronic low doses of UVB, repeated for 10 days, irradiation with SSR (Group C) did not lead to any alterations in IL-8 serum concentration.

In Group B IL-10 was over reference range (0–9.1 pg/mL) before UVB irradiation (15.5 pg/mL) only in one volunteer but its level was much higher after 10-day UVB exposure (29.8 pg/mL) and undetectable after the additional 3 MED irradiation. The same tendency was observed in Group A. Also in one individual before irradiation the level was 12.0 pg/mL and, after 10 days of repeated exposure, –14.5 pg/mL. In Group C two subjects had elevated IL-10 levels before irradiation; however 10-day SSR exposure did not cause any increase in the interleukin level. In Group D no effect of an acute single dose of UVB on serum level of this cytokine was observed.

Before irradiation TNF- $\alpha$  serum level was detected in 90 out of 105 volunteers (86%). In 76 out of 90 its concentration was within reference ranges and only in 14 cases it was over the expected values (8.2–24.3 pg/mL). In Groups A and B a slight, statistically significant, increase in serum level was observed after 10 days of irradiation (for both comparisons  $P = .05$ , resp).

Local acute UVB doses and repeated SSR irradiation did not change the concentration of this cytokine further.

### DISCUSSION

To our knowledge, our study is the first one in which serum concentrations of IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$  were examined in human healthy volunteers exposed to repeated suberythral UVB and/or SSR doses. We investigated also SSR, as human beings are usually exposed to chronic and low solar UVR not only UVB. We also wanted to find out whether chronic irradiation might result in an adaptive effect. For this purpose in two groups of volunteers (B and C) chronic irradiation was followed by a single, acute local UVB exposure. Groups A and D were the controls for this hypothesis.

In our study IL-1 $\beta$  serum levels were undetectable in all volunteers both before and after UVB and/or SSR irradiation. Literature data showed that both whole-body and local UV irradiation of human subjects increase IL-1 and IL-6 levels [8, 15, 16]. Granstein and Sauder [8] showed that IL-1 serum level increased after whole-body 1 MED UV irradiation. The highest concentration of IL-1 was observed between 1 to 4 hours after exposure and it returned to baseline within 8 hours after exposure. In our study significantly lower doses of UV were used and blood samples were taken 24 hours after UV exposure. This may explain why we observed no alteration in IL-1 $\beta$  serum level in the volunteers and shows that such effects may be rather transient.

In our volunteers IL-6 was detectable only in 17 out of 105 individuals before irradiation. After irradiation we observed a slight increase in its level (see Table 2); however it was within normal ranges and the differences were not

TABLE 2. Serum concentrations of selected cytokines in all examined groups. N is the number of volunteers, D detectable level, MED minimal erythema dose, UVB ultraviolet radiation B, and SSR solar-simulated radiation.

Group		Cytokines (pg/mL)			
		IL-6 D (range)	IL-8 D (range)	IL-10 D (range)	TNF- $\alpha$ D (range)
A N = 25	Before UVB	N = 5 (2.14–6.86)	N = 4 (5.02–9.46)	N = 1 (12.0)	N = 23 (4.04–10.5)
	After 10 days of 0.7 MED UVB	N = 9 (2.01–7.45)	N = 9 (5.09–8.78)	N = 1 (14.5)	N = 20 (4.04–10.5)
B N = 30	Before UVB	N = 2 (2.43; 6.84)	N = 9 (5.0–9.51)	N = 1 (15.5)	N = 21 (4.12–9.79)
	After 10 days of 0.7 MED UVB	N = 3 (2.89–6.9)	N = 16 (5.12–14.0)	N = 1 (29.8)	N = 23 (4.03–10.1)
	After 3 MED UVB	N = 3 (2.88–6.9)	N = 10 (5.2–25.6)	N = 0	N = 23 (4.04–8.56)
C N = 25	Before SSR	N = 6 (2.03–3.59)	N = 9 (5.01–7.9)	N = 1 (11.7)	N = 23 (4.79–16.9)
	After 10 days of 1.2 SED SSR	N = 3 (2.47–9.1)	N = 3 (6.59–7.3)	N = 2 (10.4; 15.5)	N = 18 (4.14–24.3)
	After 3 MED UVB	N = 4 (2.06–7.9)	N = 6 (5.31–8.78)	N = 2 (8.38; 12.2)	N = 19 (4.14–19.0)
D N = 25	Before UVB	N = 4 (2.31–3.6)	N = 7 (5.01–7.58)	N = 2 (7.11; 9.35)	N = 23 (4.47–9.66)
	After 3 MED UVB	N = 2 (2.88; 4.58)	N = 7 (5.02–8.02)	N = 3 (5.6–8.67)	N = 19 (4.22–8.88)

statistically significant. No correlation between UV exposure and IL-6 level could be found. Urbanski et al [15] examined the serum level of IL-6 in human subjects irradiated with UV dose causing severe sunburn reaction (4 MED, single whole-body irradiation). They showed a significant increase in this cytokine level 12 hours after UV irradiation. We used low UV doses and in all cases serum concentration was measured 24 hours after irradiation which probably resulted in the obtained results, again supporting the notice of effects being transient.

Literature data revealed that irradiation of cultured human corneal stroma cells and whole human corneas with acute UVR doses induced a significant increase of IL-1, IL-6, IL-8, and TNF- $\alpha$  in both corneal stroma cells and whole human corneas [17]. We detected IL-8 before irradiation in 29 out of 105 volunteers. Only in volunteers exposed to 10-day irradiation with 0.7 MED of UVB (Groups A and B) we found statistically significant

( $P < .05$ ) differences in IL-8 level before and after irradiation. In Group B, IL-8 level decreased after additional irradiation with a dose of 3 MED which could suggest that exposure to repeated UVB irradiation causes adaptation to the effects of higher UV doses. However, in Group D, an acute dose of UVB, 3 MED regarded as an immunosuppressive one [18], applied locally on a small area, caused no alteration in IL-8 level. Thus, we conclude that acute local doses of UVB do not influence IL-8 serum concentrations. It was revealed that UVB radiation induced an increase in IL-8 mRNA expression in normal cultured human epidermal keratinocytes [19]. Kondo et al [20] also showed a significant elevation in IL-8 level 24 hours after exposure to UVB (100–300 J/m<sup>2</sup>) in supernatants of cultured keratinocytes.

No significant alterations in IL-10 were found in our study, and its cytokine level was detected before irradiation only in single subjects. After UVB or SSR irradiation,

an increase in those cases was observed; however in none it exceeded normal ranges. A small number of cases observed do not allow us to draw any conclusion and we may suppose that this tendency was coincidental. It was revealed that UVB also increased the production of IL-10 in the epidermis, mainly due to influx of macrophages [21]. However, in supernatants of UVB-irradiated human keratinocytes, IL-10 was not detected when ELISA assay was used [2].

Barr et al [22] observed a slight increase in IL-10 level in suction blisters after exposure to 3 MED of SSR and concluded that this dose is the threshold one for local induction rather than systemic alteration of this cytokine.

TNF- $\alpha$  serum level was detected in most volunteers (90 out of 105) before irradiation. Similarly to the results obtained on IL-8 level, a modest increase after 10-day irradiation was observed, but additional UV exposure failed to influence the concentration any further. Neither local acute UVB doses nor repeated SSR irradiation for 10 days changed this cytokine serum concentration. It is worth mentioning that TNF- $\alpha$ , as a proinflammatory cytokine, was detected in 86% of the examined volunteers; however in the majority of them its concentration was within reference ranges. Köck et al [7] revealed that exposure of human carcinoma cell line cells and normal human keratinocytes to UVB at a dose of 100 J/m<sup>2</sup> caused an increase in the expression of TNF- $\alpha$  mRNA with a maximum production 12 hours after irradiation. Exposure of human skin to solar-simulated radiation (SSR) in a dose of 3 MED resulted in 8-fold TNF- $\alpha$  increase in suction blisters 15 hours after irradiation then rapidly declined to control values. The same authors [7] revealed that single whole-body exposure to UVB (4 MED) caused an increase in serum TNF- $\alpha$  level 12 and 24 hours after irradiation. Exposure of human skin to solar-simulated radiation (SSR) in a dose of 3 MED resulted in TNF- $\alpha$  increase in suction blisters [22].

In our study no alterations in cytokine levels in serum, apart from IL-8 and TNF- $\alpha$ , were found. These results are in line with those obtained by McLoone et al [10]. In other studies, release of cytokines into circulation was caused by higher, strongly erythemogenic doses of UVR [7, 15, 16, 20, 23]. Until now not much was known about the influence of suberythemal doses of UVB and/or SSR irradiation on serum profile of selected cytokines, nor had we any information whether local irradiation with acute UVB doses could influence these parameters. Our results indicate the necessity of further investigation to determine the threshold for different sources and different outputs of UV which could cause impairment of systemic immunity.

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