Chapter 5

Time dependent mRNA expression in UV-induced inflammation of skin after a single dose of UVB. A comparison between healthy controls and patients with polymorphous light eruption

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ABSTRACT

Ultraviolet (UV) radiation suppresses immune responses in human skin, leading to changes in cell-composition and cytokine levels. Patients with polymorphous light eruption (PLE) develop a pruritic rash within a few hours after UV exposure. Repeated challenges with UVB induce a pathological skin reaction. In PLE patients significantly less neutrophils infiltrate the skin at 18 h after a single UVB exposure. This suggests a dysregulation during the early phase of the UVB-induced immune response in patients with PLE.

The aim of the study was to determine *in vivo* the sequential changes in mRNA regulating a broad spectrum of proteins involved in UV-induced inflammation of the skin within the first hours after UVB exposure. Furthermore, we compared these expression patterns between healthy controls and PLE patients, in order to unravel possible mechanisms underlying the early phase of the pathological response to UVB in PLE.

Skin biopsies were collected from 16 healthy controls and 7 PLE patients at 0, 3, 6 and 18 hours after a single UV exposure of 3 MED UVB together with skin biopsies from lesional PLE skin (n=6). mRNA was extracted and gene regulation of IL-1β, TNF-α, IL-20, IL-8, Gro-all, E-selectin, ICAM-1, MMP-1, Desmoplakin-1, IL-24, IL-1RA, IL-4, IL-10, IL-12, IFN-γ, CD25 and FOXP3 was quantified by RT-PCR.

Gene specific expression time-dependency was observed after UVB exposure in healthy controls. At 3 and 6 h after UVB irradiation, no distinctive mRNA expression pattern between PLE patients and healthy controls was shown. However, at 18 h after UVB exposure, PLE patients showed significantly increased mRNA expression of ICAM-1, CD25 and FOXP3 compared with healthy controls. Furthermore, in unirradiated skin of PLE patients the expression of IL-1β was decreased.

UVB exposure of human skin leads to early sequential changes in mRNA regulating a broad spectrum of proteins involved in UV-induced inflammation of the skin. Our results suggest that there is no difference in the early inflammatory response to a single exposure of 3 MED UVB between patients and healthy controls. Eighteen hours after UVB exposure an increased mRNA expression of ICAM-1, CD25 and FOXP3 was observed in PLE patients in contrast to healthy controls. However, these results do not explain the differences found in neutrophil skin infiltration between PLE patients and healthy controls during the first hours after UVB exposure.

INTRODUCTION

Ultraviolet (UV) radiation, in particular the UVB range (280-315 nm), suppresses immune responses in animals and humans (1,2). Exposure of human skin to ultraviolet UVB leads to changes in the cell composition and cytokine levels in the skin (3,4). UV exposed keratinocytes produce ICAM-1, IL-1, IL-6, IL-8, IL-10,TNF-α and PGE_2, which can result in the onset of UV-induced erythema and attraction of leukocytes to the skin (5-7). Within a few hours after UV exposure neutrophils migrate into the skin, followed by an influx of macrophages. Upon UV-exposure, neutrophils contain IL-4 and IL-10 protein, whereas macrophages contain IL-10 (8,9). Furthermore, UV exposure results in an initial depletion of epidermal T-cells followed by a selective influx of CD4+ T-cells after 2 days (10). These CD4+ cells express CD25 and may release IL-10 upon stimulation in the skin (11).

In contrast to the influx of leukocytes, Langerhans cells (LC), which are the major antigen-presenting cells of the epidermis, migrate out of the skin to the draining lymph nodes after UV exposure (12,13). In the lymph nodes, the UV-irradiated LC can selectively present antigen to Th2 type cells, without stimulating Th1 type cells (14). The modified cellular composition and concomitant cytokine production in the skin after UV exposure promotes a Th2-skewed response, which benefits in UV-induced immunosuppression.

Polymorphous light eruption (PLE) is an idiopathic photosensitivity disorder of which the pathogenesis is not fully understood. PLE patients develop pruritic erythematous papular, vesicular or plaque-like lesions on sunlight exposed areas of the skin several hours to days after UV exposure. In UV-induced PLE lesions, a dense perivascular upper dermal infiltrate of predominantly T-lymphocytes is visible in the upper part of the dermis already within 5 h after irradiation which reaches a peak at 72 h after irradiation. In the first 72 h a predominance of $CD4^+$ cells is present. Thereafter, $CD8^+$ cells dominate the infiltrate (15,16). The histological features, together with the delayed onset of pathological PLE skin lesions after UVB, gave rise to the hypothesis of PLE being a DTH-like response to UV-induced neoantigens (15-19). However, since PLE lesions can be elicited already within a few hours after UV exposure, an earlier pathogenic mechanism might be responsible for the initiation of PLE (20,21). Previously, it was shown that the influx of neutrophils, which contain IL-4 and TNF-α, was significantly decreased in 3 and 6 MED UVB-exposed skin of PLE patients compared with healthy controls at 18 and 14 h after UVB exposure (21,22). Furthermore, after 6 MED exposure to UVB an increased number of LC persisted in the skin of PLE patients compared with healthy controls (23).

The aim of our study was to determine *in vivo* the sequential changes in mRNA regulating a broad spectrum of proteins involved in UV-induced inflammation of

the skin in the first 18 hours after UVB exposure. Furthermore, we compared the expression patterns of healthy controls with those of PLE patients.

MATERIAL AND METHODS

Subjects

In total, 16 healthy controls (12 males and 4 females, ages between 19 and 52 y, mean age 28 y) and 7 moderate to severe PLE patients (4 males and 3 females, ages between 28 and 44 y, mean age of 36 y) with a normal minimal erythema dose (MED) were studied. PLE patients with a positive skin reaction to UVB irradiation, irrespective of a reaction to UVA irradiation were included. A positive skin reaction was defined as elicitation of PLE skin lesions after a maximum of 4 daily increasing exposures with UVB as described previously (24). Patients who received UV hardening therapy or used oral immunosuppressive medication were excluded. The buttock skin of patients and healthy controls needed to be restricted from sunlight or tanningbed exposure for at least 6 weeks. The study was approved by the local medical ethical committee and the participants gave their written consent.

Phototesting procedure

The MED of buttock skin was determined using a Philips (Philips, Eindhoven, The Netherlands) TL12 lamp (57.7% of UV output in UVB (280-315nm)) as described by Boonstra *et al.* (25). Subsequently, the unaffected buttock skin was exposed to a single dose of 3 MED UVB. Four-millimeter punch biopsies were obtained under local anaesthetics, using 2% xylocaine with adrenalin. Biopsies were taken at 3 and 18 hours after UVB irradiation, together with a control biopsy of the unirradiated buttock skin. In 7 healthy controls and 5 PLE patients an additional biopsy was taken 6 hours after UVB irradiation. Due to technical difficulties in obtaining good quality mRNA, not all consecutive biopsy time-points in all subjects could be included in the study. An overview of subjects included in the different analyses is shown in Table I. Furthermore, we obtained biopsies of lesional PLE skin in 6 patients, together with a control biopsy of unirradiated non-lesional skin. Lesional PLE skin (papular type in all patients) was elicited upon repetitive irradiation with UVB. Number of irradiations and total UVB dose to elicit PLE differed between patients.

Table I. Included subjects

	controls	PLE patients
total number	n = 16	n = 7
RT-PCR	total studied#	total studied##
unexposed skin	n = 16	n = 7
3h after UVB	n = 15	n = 7
6h	n = 7	n = 5
18h	n = 13	n = 7

exept IL-4, IL-10, IL-12: 0h=7, 3h=7, 6h=7, 18h=5

exept IL-4, IL-10, IL12: all timepoints n=5

Biopsies	n=6
L- and NL-PLE skin	IL-4, IFN-γ

L= lesional; NL= non-lesional

RNA isolation and cDNA synthesis

Frozen tissue sections (80-100 of 20 μm) were homogenized in ice-cold RNeasy Lysis Buffer (RLT) containing β-mercaptoethanol (Sigma, St Louis, MO). RNA was isolated with the RNeasy micro kit (Qiagen, Hamburg, Germany) and treated with DNAse, according to the manufacturer's protocol. Reverse transcription was performed with 200 ng total RNA using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA), according to the manufacturer's protocol.

Real-time PCR

Real-time PCR was performed using a MyiQ real-time PCR detection system (Biorad, Hercules, CA, USA) using SYBR Green I (Biorad). Primers (Sigma-Genosys, The Woodlands, TX, USA) were designed using Primer 3 software of the Whitehead Institute/ MIT centre for genome Research (26) (Table II). Amplification was performed using cDNA, SYBR Green I mastermix and 300 nM of each primer, according to the following conditions: 3 minutes at 95°C to activate DNA polymerase, 45 cycles of 10 seconds at 95°C, 20 seconds at 61°C and 25 seconds at 72°C. PCR was checked for a-specific products by performing dissociation curves after each PCR run. The signal of SYBR Green I was measured against the passive signal of the reference dye, fluorescein, to normalize for non-PCR-related fluorescence fluctuations occurring from well to well. Each assay included a standard curve of four serial concentrations of a pool of all cDNA samples to determine linearity and PCR efficiency. Results were normalized for PCR efficiency and for the reference genes β-actin, GAPDH and HPRT1 using ΔΔCT method (27).

Table II. Sequences of primers used for real time PCR

Name	Forward primer sequence 5′-3′	Reverse primer sequence 5′-3′
IL-1ß	GCTTATTACAGTGGCAATGAGGAT	GGTGGTCGGAGATTCGTAGC
IL-1RA	GAAGATGTGCCTGTCCTGTGTC	CGCTTGTCCTGCTTTCTGTTC
CD25	GCGGAGACAGAGGAAGAGTAGAA	CGACCATTTAGCACCTTTGATT
IL-4	AGCAGTTCCACAGGCACAAG	ACTCTGGTTGGCTTCCTTCAC
IL-8	AGCTCTGTGTGAAGGTGCAGTT	GGGTGGAAAGGTTTGGAGTATG
IL-10	GAGAACCAAGACCCAGACATCA	GTGGAGCAGGTGAAGAATGC
IL-12	ATGCCGTTCACAAGCTCAAGT	GGTGGGTCAGGTTTGATGATG
IL-20	GCCAATTCCTTTCTTACCATCAA	GCTGTATTTCTTCATTGCTTCCTC
IL-24	CAACCCAGTCAAGAAAATGAGATG	GCATCCAGGTCAGAAGAATGTC
TNF-a	CTCCAGGCGGTGCTTGTT	GCCAGAGGGCTGATTAGAGAGA
IFN-g	TTTGGGTTCTCTTGGCTGTTACT	GTTCCATTATCCGCTACATCTGAAT
Gro-all	CACCCCAAGAACATCCAAAG	TGTGGCTATGACTTCGGTTTG
E-selectin	ACATCTCAGGGACAATGGACAG	CTTCAGGACAGGCGAACTTG
ICAM-1	GCCAACCAATGTGCTATTCAAA	GCCAGTTCCACCCGTTCT
Desmoplakin-1	CCGCTGGCAAAGGATAGA	CGGCGTTTAGCATCATAGAGC
MMP-1	CCAGATGCTGAAACCCTGAA	TGAGGACAAACTGAGCCACATC
FOXP3	AAACAGCACATTCCCAGAGTTC	GCGTGGCGTAGGTGAAAG
β—Actin	AGCCTCGCCTTTGCCGA	CTGGTGCCTGGGGCG
GAPDH	AGAAGGCTGGGGCTCATTT	GAGGCATTGCTGATGATCTTG
HPRT1	TGGTCAGGCAGTATAATCCAAAGA	TCAAGGGCATATCCTACAACAAA

Quantification of data and statistical analysis

PCR-data were expressed as fold-regulation compared with the mean expression of unirradiated healthy control skin, which was arbitrarily set at 1. A minimum of 1.5 fold-change mRNA expression was determined suitable for statistical analysis. All PCR data are expressed as the mean ± 95% Confidence interval (CI) and differences between values were compared by the Wilcoxon Rank test (paired in lesional versus not lesional skin). Differences between study groups were tested using Mann-Whitney *U* test. Statistical difference was defined as $p<0.05$.

RESULTS

Phototesting

The MED's of all participants were in normal range and did not differ between controls and patients. The mean MED in healthy controls was 93.4 ± 35.2 mJ/cm^2 and in PLE 80.6 ± 24.5 mJ/cm^2. After a single exposure with 3 MED UVB, none of the patients developed a pathological skin reaction.

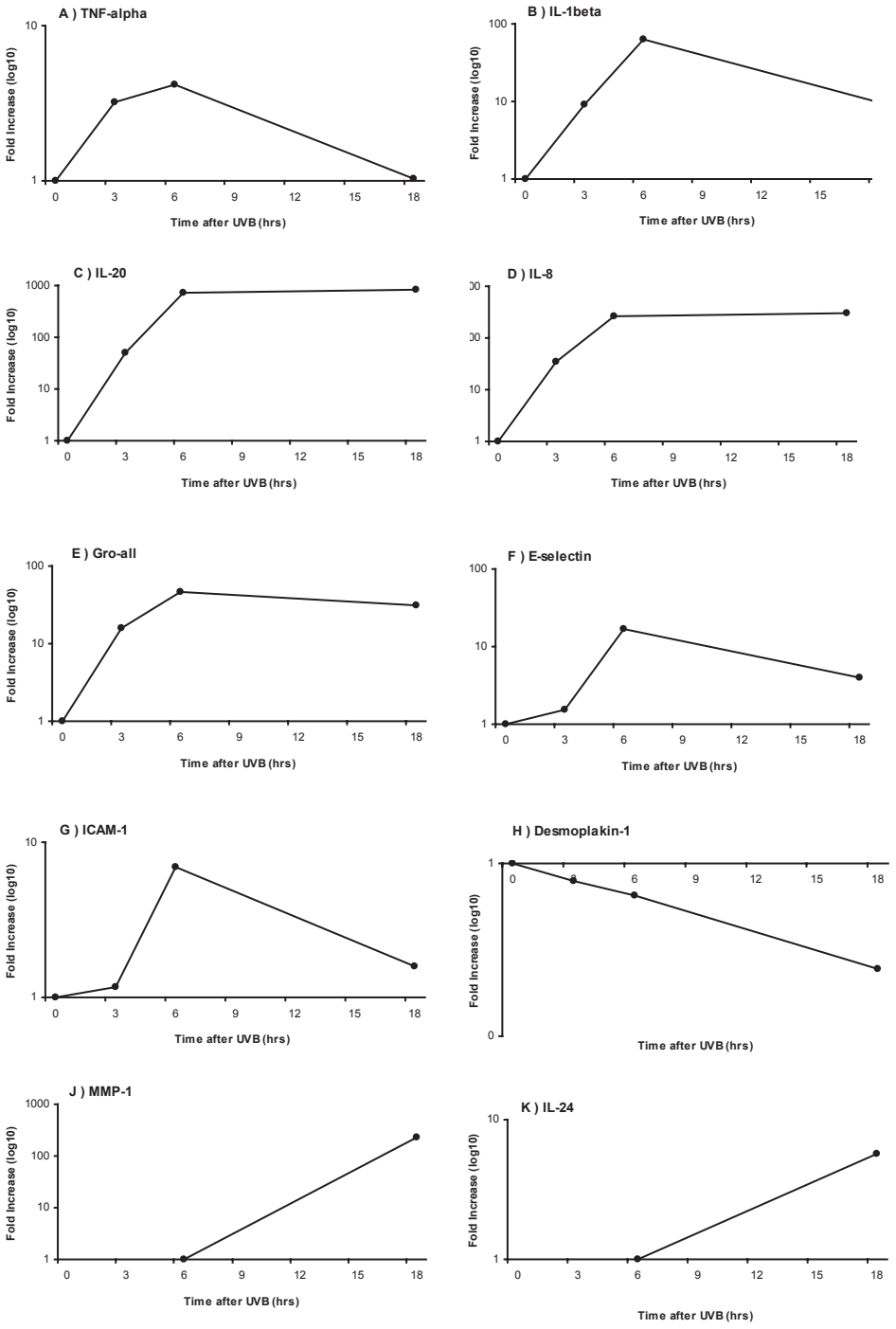

Figure 1. mRNA expression depicted in (log10)fold regulation in time (hrs) after 3 MED UVB irradiation in healthy control skin. A: TNF-α, B: IL-1β, C: IL-20, D: IL-8, E: Gro-all, F: E-selectin, G: ICAM-1, H: Desmoplakin-1, J: MMP-1, K: IL-24.

Time-related mRNA expression, the early induced genes after UVB exposure in skin of healthy controls

TNF-α, IL-1β and IL-20 were the first cytokines upregulated 3h after UVB exposure (Figs 1*A,B,C*). The maximum expression was demonstrated at 6 h after UVB exposure and expression of TNF-α and IL-1β diminished thereafter, whereas IL-20 expression was sustained. UV-exposure stimulated cells to produce a chemotactic milieu for infiltrating leukocytes. Already 3 h after UVB, upregulation of chemokines IL-8 and growth-related oncogene α, β, γ (Gro-all) was observed (figure 1*D,E*). Comparable to the gene expression of IL-20, IL-8 and Gro-all did not decline after reaching its peak at 6 h, but persisted during the test period.

The second wave of UV-induced mRNA expression in skin of healthy controls: adhesion molecules

The early upregulation of TNF-α and IL-1β was followed by that of E-selectin and ICAM-1 starting at 6 h post UVB (figure 1*F,G*). E-selectin and ICAM-1 expression declined again at 18 h after UV-exposure.

The third wave of expression in skin of healthy controls: cell-coherence and immunoregulatory genes

Kinetics of matrix-metallo proteinase (MMP)-1 and desmoplakin-1, involved in dermal (MMP-1) and epidermal (Desmoplakin-1) cell-coherence was determined. MMP-1 expression was not detectable until 6 h after UVB exposure, but at 18 h post UVB a clearly visible upregulation of MMP-1 was detected (figure 1*H*). Desmoplakin-1 expression was slowly downregulated after UVB exposure by more than 1.5 fold decrease at 18 h after UVB exposure (figure 1*J*). In addition, IL-24 expression was determined. Its UV-induced upregulation in time mimicked that of MMP-1 expression (figure 1*K*)

After UVB exposure, expression of anti-inflammatory (IL-10 and IL-1RA) and immunoregulatory (CD25 and FOXP3) genes could be shown, however the level after UVB exposure did not differ from unirradiated skin. IL-10 expression was only detected at 18 h after UVB exposure. Th1 (IL-12 and IFN-γ) and Th2 (IL-4) skewing cytokines were not detectable in the first 18 h after UVB exposure. Table III shows the fold-increase regulation of all tested genes.

Decreased IL-1β expression in unirradiated PLE skin, but early and second wave UV-induced mRNA expression does not differ in PLE patients

In general, UVB-induced effects on expression levels were comparable between healthy controls and PLE patients during the first 6 h after UVB irradiation (Table III). However, it was shown that PLE patients have highly significant down-regulation of IL-1β mRNA ($p=0.0008$) in unirradiated buttock skin compared with healthy controls (Table III).

PLE patients showed increased regulation of ICAM-1, CD25 and FOXP3 mRNA at 18h after UVB exposure (third wave)

At 18 h after UVB expsoure, ICAM-1, CD25 and FOXP3 mRNA expression was significantly more upregulated in PLE patients compared with healthy controls (ICAM-1, CD25, FOXP3: $p<0.05$). However, in the first 6 h after UVB expsoure, the concentration and kinetics of these genes was similar in PLE patients and healthy controls.

Lesional PLE skin shows Th1 skewing

In the first 18 h after UVB exposure, no Th1 or Th2 skewing cytokines were expressed above detection level. To detect UV-induced IL-4 and IFN-γ expression in skin of PLE patients, lesional PLE skin was analyzed and compared with non-lesional PLE skin (using paired Wilcoxon Rank test). IL-4 could not be detected in either non-lesional or lesional skin. IFN-γ, however, was significantly upregulated in lesional PLE skin compared with non-lesional skin ($p=0.03$), indicating a Th1 skewed milieu (data not shown).

DISCUSSION

UV-induced immunosuppression is a result of an altered microenvironment in the skin. UV-induced mRNA expression of cytokines and chemokines has been described before (4,28). However, we tested simultaneously a broad panel of genes involved in skin inflammation upon UVB exposure *in vivo* using PCR technique. mRNA expression of genes was tested at 3, 6 and 18 h after a single exposure to 3 MED UVB, resulting in 3 time-dependent gene expression waves.

UVB is able to penetrate the epidermis, but only a small fraction will reach the upper dermal compartment directly (29). Therefore, it is most likely that the earliest wave in gene expression (TNF-α, IL-1β, IL-20, IL-8 and Gro-all; within 3 h after UVB exposure) will be mainly produced by epidermal cells such as KC and LC. TNF-α and IL-1β are expressed by KC and LC; both cytokines are involved in LC emigration and upregulation of IL-6, prostaglandin, complement and selectins, thereby facilitating leukocyte infiltration upon UV exposure (28,30). Next to KC, also EC, macrophages and neutrophils can produce IL-8 and Gro-all chemokines. This may explain the sustained expression levels of IL-8 and Gro-all at 18 h after UVB exposure, when influx of inflammatory cells (neutrophils, macrophages and T-cells) in UV-irradiated skin has been initiated (31-33).

IL-20 is a pro-inflammatory cytokine, produced by KC and skin macrophages upon many different stimuli, including UV-exposure (34,35). Furthermore, in response to IL-20, KC express other pro-inflammatory genes including TNF-α, which leads to

Table III. mRNA fold-regulation after 3 MED UVB exposure in healthy controls and PLE patients

	Healthy controls (time after UVB)				PLE patients (time after UVB)			
	0 hrs	**3 hrs**	**6 hrs**	**18 hrs**	**0 hrs**	**3 hrs**	**6 hrs**	**18 hrs**
Chemokines								
IL-8	1	35.4 [16-77]	258.1 [116-575]	308.5 [148-643]	1.3 [0-4]	25.9 [7-98]	165.8 [68-406]	157.2 [65-381]
Gro-all	1	16.0 [10-27]	46.0 [26-82]	30.9 [21-47]	1.4 [1-3]	12.9 [5.8-29]	36.7 [17-80]	28.8 [15-54]
Pro-inflammatory								
IL-1ß	1	9.0 [5-15]	62.3 [32-120]	9.7 [6-17]	**0.2 [0.1-0.4]***	7.1 [7-98]	37.9 [68-406]	10.2 [65-381]
IL-12	nds	nds	nds	nds	nds	nds	nds	nds
IL-20	1	49.8 [26-95]	733.3 [0-3x108]	831 [471-1472]	0.5 [0.2-1.5]	32.9 [10-112]	504.7 [121-2103]	754.6 [248-2300]
IL-24	nds	nds	1	5.7 [3-11]	nds	nds	0.4 [0.1-3]	8.6 [5-16]
TNF-a	1	3.2 [3-4]	4.2 [3-6]	1.0 [1-1]	1.0 [1-1]	4.4 [3-6]	3.8 [3-5]	1.0 [1-1]
IFN-γ	nds	nds	nds	nds	nds	nds	nds	nds
Adhesion molecules								
E-selectin	1	1.5 [1-3]	16.7 [8-34]	4.0 [2-8]	0.9 [0.2-3.2]	2.1 [1-6]	12.9 [4-46]	7.2 [5-12]
ICAM-1	1	1.2 [1-1]	6.9 [4-11]	1.6 [1-2]	1.63 [1-2]	1.7 [1-2]	6.5 [4-10]	**2.5 [2-3]****
Cell-coherence								
Desmoplakin-1	1	0.8 [0.6-1]	0.7 [0.6-1]	0.3 [0.2-0.4]	1.2 [1-2]	1.3 [1-2]	0.9 [0.6-1]	0.3 [0.3-0.4]
MMP-1	nds	nds	1	226.8 [115-449]	nds	nds	1.1 [0.3-5]	288.0 [157-529]
Immunosuppressive								
IL-1RA	1	0.7 [0.5-0.8]	0.6 [0.5-0.8]	0.6 [0.4-0.7]	0.8 [0.6-1]	0.9 [0.7-1]	0.7 [0.4-0.7]	0.2 [0-1.2]
IL-4	nds	nds	nds	nds	nds	nds	nds	nds
IL-10	nds	nds	1	1.4 [0.5-4]	nds	nds	1.7 [0.7-4]	0.7 [0.3-2]
Immunomodulatory								
CD25	1	0.8 [0.6-1]	1.4 [1-3]	1.5 [1-2]	1.3 [1-2]	1.2 [1-2]	2.1 [1-4]	**2.8 [2-5]****
foxp3	1	0.9 [0.6-2]	1.2 [1-2]	1.0 [0.7-1]	0.6 [0.2-2]	1.3 [1-2]	1.7 [1-3]	**1.9 [1-3]****

Numbers are presented as fold-regulation ± 95% CI compared with the mean regulation of healthy control skin at 0 hrs, which is arbitrarily set at 1 Gene upregulation: numbers larger than 1 ; gene downregulation: numbers smaller than 1 nds= no detectable signal; *p<0.001; **p<0.05; significance between PLE patients and healthy controls

activation of NF-κB (36). In our study, IL-20 expression was found at 3 h after UVB exposure and levels remained high during the first 18 h (figure 1C).

The second wave of gene expression (between 3 and 6 h post-UVB) was formed by E-selectin and ICAM-1. As expected, E-selectin and ICAM-1 were upregulated at a later timepoint than TNF-α and IL-1β, starting at 6 h after UVB exposure and, as shown before, E-selectin expression mimicked the kinetics of neutrophil influx in the skin (21). The third wave of gene expression (18 h after UVB exposure) included

genes coding for proteins involved in cell-coherence and immunoregulation. Expression of matrix metallo proteinase (MMP)-1 and desmoplakin-1 was determined since these genes are involved in KC cell-coherence and degradation of extracellular matrix (ECM) proteins. Loss of cell-coherence will facilitate cell movement (37). Next to degradation of the ECM, MMPs can indirectly regulate chemokine and cytokine activity, thereby providing further evidence that MMPs play an active role in immunomodulation (reviewed by Parks *et al.* (38)). We could not detect MMP-1 expression at 3 and 6 h after UVB exposure, however there was a strong expression at 18 h after UVB exposure. MMP-1 can be expressed by KC, fibroblasts and infiltrating leukocytes upon a variety of stimuli including IL-1, which might explain the time delay in expression after UVB exposure (39,40). Desmoplakin reduction is supposed to play a role in metastases of tumour cells (41). To our knowledge, this is the first report of UV-induced down-regulation of desmoplakin-1. We hypothesize that this down-regulation might facilitate migration of cells into and out of the epidermis after UV-exposure.

Furthermore, during the third wave also immunoregulatory genes were expressed, however, at low levels (IL-1RA, IL-10, FOXP3, CD25) or not detectable at all (IL-4, IL-12, IFN-γ). Recently, Averbeck *et al.* (42) showed that after 3 MED UVB exposure in the first 8 h both Th1 and Th2 cytokines at protein level were detectable (IL-2, IL-12, and IL-4). At 24 h post-UVB, the Th1 cytokine protein amount decreased in contrast to Th2 cytokines, indicating a switch from a mixed Th1/Th2 milieu to a Th2 milieu upon UVB exposure. IFN-γ, however, was not detected at all timepoints. In their study, protein levels were measured from extracellular microdialysate samples collected over periods of 8 h. Since we determined gene expression at specific timepoints in stead of collectively sampling different timepoints, this might explain why we not able to detect any of the Th1 and Th2 cytokines. However, their findings support the idea that UV-exposure skews the skin milieu to Th2.

The kinetics of IL-10 expression in our study might be explained by skin infiltrating neutrophils, macrophages and T-cells several hours post-UVB (3,43,44). Furthermore, we observed that IL-24 gene expression was detectable at 6 h after UVB exposure and upregulated at 18 h. Macrophages and Th2 type cells, which start to infiltrate the skin at 18 h after UVB are the most likely source of IL-24 expression. The precise biological function of IL-24 is still elusive, but it appears to involve pro-inflammatory effects on KC by activating Stat-1 and Stat-3 transcription factors (45). Summarizing, we tested mRNA regulation of a broad spectrum of proteins involved in UV-induced inflammation of skin and this regulation showed gene specific time-dependency.

PLE patients were shown to have a significant downregulation of IL-1β mRNA in unirradiated buttock skin compared with healthy controls (Table III). IL-1β can be produced by KC, monocytes, macrophages, LC and dendritic cells (46,47). IL-1β

is involved in UV-induced migration of LC, which seems to be impaired in PLE patients (23,48). Furthermore, IL-1β can upregulate E-selectin expression, leading to neutrophil skin infiltration, which seems also to be impaired in PLE patients (21,49). However, IL-1β expression in PLE patients directly after UVB exposure holds an equal trend with healthy controls, indicating that there is no defect in IL-1β regulation upon UVB. It has to be further investigated whether a decreased amount of IL-1β gene expression in unirradiated skin of PLE patients might be partly responsible for an impaired LC and neutrophil migration in PLE patients. Therefore, efficiency of IL-1β mRNA translation to protein should be quantified.

After UVB exposure, also in PLE patients three waves of mRNA expression kinetics could be shown. Strikingly, the first and second waves of gene expression were similar to those observed in healthy controls, indicating no general defect in UV-induced gene regulation in the first 6 h after UVB exposure in PLE patients. The similarity in mRNA regulation of E-selectin, IL-8 and Gro-all in PLE patients compared with healthy controls after UVB exposure, does not explain the previously shown decreased number of skin infiltrating neutrophils in PLE (21). Therefore, another immunological or even non-immunological factors must be involved, such as urocanic acid, membrane lipids and DNA or members of the innate immune system (complement, Toll-like receptors) (50,51).

In contrast to the expression levels during the first 6 h after UVB exposure, after 18 h (third wave) differences could be observed in mRNA expression levels between PLE patients and healthy controls. Adhesion molecule ICAM-1 expression was increased in both healthy controls and PLE patients at 6 h after UV exposure. The expression declined at 18 h post-UVB, but to a significantly lesser extent in PLE patients compared with healthy controls ($p<0.05$). We were not able, using whole biopsy mRNA, to determine the responsible cells for the increased ICAM-1 expression in PLE patients, but most likely at this timepoint, endothelial cells and infiltrating leukocytes are the main source (52). In contrast, Norris *et al.* (17), found increased ICAM-1 protein expression in basal KC of pathological PLE lesions, particularly the epidermal sites overlying areas of dermal leukocyte infiltrates. They suggested that the ICAM-1 expression in KC was induced by IFN-γ released by immunologically activated T-cells in lesional PLE skin. However, since we did not induce pathological PLE lesions during the first 18h after UVB exposure in our test model and T-cell influx has yet to start at this timepoint, we do not expect that KC are responsible for the increased ICAM-1 expression. In addition to the results found by Norris and co-workers (17), we determined T-cell activation in skin of PLE patients and observed a significantly increased mRNA expression of CD25 and FOXP3 in comparison with healthy controls at 18 h after UV-exposure (both $p<0.05$). We also stained FOXP3 (eBioscience, San Diego, CA) and CD3 (BD Biosciences, San Jose, CA) protein in skin biopsies of PLE

patients and healthy controls. CD3 numbers increased equally in PLE patients and healthy controls at 18 h after UVB exposure, (data not shown). Furthermore, we found that the percentage of FOXP3 positive T-cells varied randomly between 1-6% of the population CD3 positive cells in both PLE patients and healthy controls and this percentage was not influenced by UVB exposure (data not shown). Therefore, we believe that T-cells expressing a regulatory phenotype (Tregs) make up a fixed proportion of the residual and skin infiltrating T-cell population and are not overexpressed in the total CD3 population upon UVB exposure. We did test the functional potency of the skin infiltrating Tregs in UV-exposed skin of PLE patients.

Th1 and Th2 cytokine gene expression could not be detected in the first 18 h after UVB exposure in PLE patients. However, in biopsies of 6 PLE patients with induced lesional PLE skin by repetitive UVB exposures, a Th1 response could be detected since IFN-γ expression was significantly upregulated in contrast to IL-4 expression being undetectable (IFN-γ $p<0.05$ using paired Wilcoxon rank test; data not shown). The fact that IL-4 mRNA expression could not be detected after UV-irradiation might be due to the fact that the number of infiltrating neutrophils in both healthy and PLE skin was too low to find a IL-4 mRNA signal. Furthermore, it might also be possible that neutrophils are not able to upregulate IL-4 mRNA once inside the skin and that the observed IL-4 protein expression reflects IL-4 protein storage in infiltrating neutrophils.

In conclusion, we showed a time-dependent expression of mRNA regulating a broad spectrum of proteins involved in UV-induced inflammation of skin after a single dose of 3 MED UVB. In general, no distinctive early mRNA expression pattern between PLE patients and healthy controls was found, except for a decreased IL-1β mRNA expression in unirradiated PLE skin. We did find an increased mRNA expression of ICAM-1, CD25 and FOXP3 in skin of PLE patients compared with healthy controls at 18 h after UVB exposure, indicating differential regulation of genes. However, these results do not explain the differences found in neutrophil skin infiltration between PLE patients and healthy controls during the first hours after UVB exposure.

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