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Inflammation Research

Comparative quantification of IL-1β, IL-10, IL-10r, TNFα and IL-7 mRNA levels in UV-irradiated human skin in vivo

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Abstract. *Objective and Design:* Ultraviolet (UV) exposure induces local immunosuppression and inflammation in human skin. Cytokines are, in part, responsible for these responses. To investigate the effects of UV-induced gene expression at the molecular level we established a sensitive in vivo/ex vivo method for a comparative quantification of cytokines and receptors involved in the local skin immune reactions.
Material and Methods: Specific mRNA levels of human UV-irradiated skin were determined by real time quantification (TaqMan™ RT-PCR). Highly efficient PCR-reaction conditions were obtained by designing very short PCR-templates (72–87 bp). The most sensitive PCR-conditions were obtained by optimisation of primer and $Mn(OAc)_2$-concentrations, which led to significant PCR signals (C_T-value) of less than 36 cycles. A strong correlation between PCR efficiency of the internal control (GAPDH) compared to targets (IL-1β, IL-10, IL-10r, TNFα, IL-7) allowed the use of $\Delta\Delta C_T$-method to quantify comparable mRNA levels.
Results: Interleukin-1β (IL-1β), Interleukin-10 (IL-10), and tumour necrosis factor alpha (TNFα) mRNA levels were increased in a time- and dose-dependent manner. Interleukin-1β induction reached a maximum (approx. 44-fold) 6 h after a UV-dose equivalent to 3 times the minimal erythemal doses just perceptible (MED_{jp}). Maximal TNFα mRNA expression (approx. 14-fold) was also detected 6 h after UV exposure. Interleukin-10 mRNA induction reached a maximum of approximately 14-fold 24 h after UV-irradiation of 3 MED_{jp}. Time- and dose-dependent changes in Interleukin-7 and Interleukin-10 receptor mRNA levels did not occur after UV-irradiation.
Conclusions: Time-distinct gene induction of IL-1β, TNFα and IL-10 is involved in UV-induced immune reactions, but no considerable changes were found for IL-10r or IL-7.

Key words: TaqMan™ PCR – Cytokine – Receptor – UV-irradiation – Skin

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Introduction

Exposure of the skin to ultraviolet (UV) radiation initiates infiltration, local immune suppression and may also include both nonmelanoma skin cancer and malignant melanoma [1]. In this study, we demonstrated the comparative transcript levels of different cytokines involved in the local skin immune reactions following UV exposure using newly developed and highly sensitive quantitative reverse transcription polymerase chain reaction (RT-PCR) in small amounts of in vivo irradiated skin.

The small amounts of mRNA obtained from human skin punch biopsies, together with the low levels of specific cytokine mRNAs, made it necessary to develop a very sensitive PCR technique. The use of this sensitive TaqMan™ RT-PCR technique is based on the cleavage of sequence-specific fluorochrome labelled internal DNA oligonucleotide probes by the 5′-3′endonuclease activity of the r*Tth*-polymerase during polymerase chain reaction and online measurement of fluorescent intensity [2, 3]. PCR conditions were obtained by determining the optimal concentration of PCR primers, and $Mn(OAc)_2$-concentrations for each RNA of interest. Because the intent of the study was to analyse the different transcription levels in a comparative manner, various requirements had to be fulfilled. Comparative calculation of transcription levels using the $\Delta\Delta C_T$-method requires that the amplification efficiency be the same for both house keeping gene GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) and the target, for a given range of RNA concentration, as demonstrated in this study. Furthermore, PCR amplification fragments smaller than 100 base pairs had to be designed to achieve high PCR efficiency.

Cytokines are key molecules for controlling and modulating cell functions and are of great importance for maintaining cutaneous homeostasis. Furthermore, increased dermal and epidermal cytokine production or altered cytokine profiles have been implicated in the pathophysiology of various skin disorders, and the sunburn reaction [4]. Treatment of mouse and human skin with low doses of UV led to function-

al changes of different epidermal and dermal cells, in part, mediated by cytokines. TNFα and IL-1β are potent cytokines involved in UV-induced morphological and functional changes of epidermal Langerhans cells, the major antigen presenting cells in the epidermis, and also in UV-induced depletion of epidermal Langerhans cells [5]. Macrophages infiltrating the skin following UV irradiation produce large amounts of IL-10, a potent immunoregulatory cytokine that may account for local delayed immunosuppressive environment [6, 7]. Keratinocyte-derived IL-7 is involved in survival and proliferation of murine dendritic epidermal T-lymphocyte subsets, and its gene expression is affected by UV-radiation [8, 9].

In view of the known effects of different cytokines involved in UV-induced effects in human skin, the purpose of the present study was to examine gene expression of IL-1β, IL-10, TNFα, IL-10r and IL-7. These cytokines were selected due to their potential role in the induction and establishment of UV-induced local skin immune reactions.

Material and methods

Volunteers

Five healthy volunteers with either skin type I/II, II or type II/III were recruited at St. John's Institute of Dermatology, Guy's, King's, and St Thomas' School of Medicine, King's College London, St Thomas' Hospital, London, UK. Skin type was assessed by detailed interview. Ages were in the range 20–44 y, median 25.8 y, 2 were female. Each volunteer was fully informed of the procedures and gave written consent prior to taking part in the study, which were approved by the Ethics Committee of St Thomas' Hospital, London, UK.

Skin irradiation and punch biopsies

Solar-simulated radiation (SSR) was generated by a 1 kW solar sun simulator (Oriel, Letherhlead, UK) giving an even field of irradiance (290–400 nm) of approximately 15 mW/cm^2 on the skin surface, at 11 cm from the source. Irradiance was determined by a double-monochromator spectroradiometer (Bentham instruments, Reading, UK) and was routinely monitored with a wide band thermopile radiometer (Medical Physics, Dryburn Hospital, Durham, UK). The minimal erythema dose of SSR required to cause a 'just perceptible' erythema (MED_{jp}) at 24 h on skin not previously UVR or sunlight exposed was determined for all subjects. Areas (1 cm^2) of upper buttock skin were irradiated with a geometric series of $\sqrt{2}$ incremental doses of SSR and the resulting erythema visually scored 24 h later. The median MED_{jp} was 3.24 J/cm^2, range 1.8 to 3.6 J/cm^2 of SSR-irradiation. Punch biopsies (4 mm diameter) of epidermal/dermal skin tissue were taken at the indicated time points (6 and 24 h after irradiation) and snap frozen in liquid nitrogen.

RNA-Isolation from skin biopsies

Total RNA of snap frozen skin biopsies was isolated using an RNA-isolation kit (Quiagen, Hilden, Germany). In detail, frozen biopsies were transferred to 400 µl lysis buffer containing 1% β-mercaptoethanol (Sigma, Deisenhofen, Germany). Skin biopsies were desegregated using a homogenizer (Ultra-Turrax T8, IKA, Staufe, Germany). After adding 200 µl 70% (v/v) ethanol (Merck, Darmstadt, Germany) total RNA was immobilised on spin columns and eluted in diethylpyrocarbonat-treated water (Fluka, Buchs, Germany). Concentration and purity of RNA was determined by measuring the absorbance at 260 nm and 280 nm in a spectrophotometer (Pharmacia, GeneQuant, LKB Biochrom, UK). Ribosomal RNAs (28S rRNA, 5.0 kb and 18S rRNA, 1.9 kb) were visualised on a 1.2% agarose gel following electrophoresis to demonstrate the integrity of the RNAs.

TaqMan™-RT-PCR

Reverse transcription of RNA and amplification of specific cDNAs were performed in a single-tube single-enzyme system using the TaqMan™-EZ RT-PCR kit (PE Applied Biosystems, Foster City, CA, USA). For the polymerase chain reaction (PCR) optical reaction tubes (PE Applied Biosystems, Foster City, CA, USA) were used which focus the fluorescent light to the detection unit of the ABI PRISM 7700 Sequence detection system (PE Applied Biosystems, Foster City, CA, USA). To obtain PCR conditions with reduced variability, PCR master mixes containing all reagents except primer/probe/ $Mn(OAc)_2$-premix were prepared. Potential PCR contamination products were digested by uracil-N-glycosylase (UNG), since dTTP is substituted by dUTP during PCR reaction [10]. All PCR assays were prepared in triplicates in 50 µl or 25 µl PCR reactions.

Oligonucleotide sequences and target specific fluorescence-labelled DNA-probes were chosen using Primer Express™ software version 1.0 (PE Applied Biosystems, Foster City, CA, USA) to span exon junctions of GAPDH, IL-1β, IL-10, TNFα or IL-7 to produce one expected PCR-fragment (Table 1). In preliminary experiments, PCR conditions were optimised for oligonucleotides (forward primer and reverse primer) in a 3×3 matrix of 50, 300 and 900 nM (Table 2). RNA was obtained from isolated peripheral blood mononuclear cells (PBMC) obtained from heparinized venous blood. To maximize PCR sensitivity, optimal $Mn(OAc)_2$ concentrations were determined in the range of 2.0 to 5.0 mM (Table 2). Sequence specific probes were covalently labelled with 6-carboxyfluorescein (FAM) respectively with VIC fluorescence reporter dyes at the 5′-end. Each of the reporter dyes is quenched by TAMRA (6-carboxy-N,N,N′,N′-tetramethylrhodamine) attached via a linker arm located at the 3′-end. The RT-reaction conditions were 2 min at 50°C as initial step to activate uracil N-glycosylase (UNG), followed by 30 min 60°C as reverse transcription and completed by a UNG-deactivation at 95°C for 5 min. The 40 cycles of a two-step PCR-reaction conditions were 20 s at 94°C and 1 min at 59°C.

Quantification of mRNA expression

PCR reactions were performed in a 96 well microtiter plate format on a ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA) equipped with a Sequence Detection System software version 1.6 (PE Applied Biosystems, Foster City, CA, USA) [2]. Using the internal fluorogenic oligonucleotide probes equipped with a 5′-reporter dye and the 3′-quencher dye fluorescence signals were generated during each PCR cycle via the 5′-3′endonuclease activity of r*Tth* DNA polymerase [3]. Each well was monitored for fluorescent dyes and signals were regarded as significant if the fluorescence intensity exceeded statistically (10-fold) the standard deviation of the baseline fluorescence, defined as threshold cycle (C_T). C_T is defined as the cycle at which a significant increase in ΔR_n is first detected. Whereby ΔR_n is the difference between R_{n+} and R_{n-}:

$$\Delta R_n = (R_{n+}) - (R_{n-}),$$

where $R_{n\,(normalised\ reporter)}$ is obtained by dividing the emission intensity of the reporter dye by the emission intensity of the passive reference for a given reaction tube:

$$R_{n+} = \frac{\text{Emission Intensity of Reporter}}{\text{Emission Intensity of Passive Reference}} \text{ PCR with template}$$

Table 1. Composition of PCR assays.

	GAPDH	IL-1β	IL-7	IL-10	IL-10r	TNFα
TaqMan™ EZ buffer[a] (µl)	10	10	10	10	10	10
$Mn(OAc)_2$ (mM)	4	2.5	3.5	4.5	3.5	3.0
dATP (µM)	300	300	300	300	300	300
dCTP (µM)	300	300	300	300	300	300
dGTP (µM)	300	300	300	300	300	300
dUTP (µM)	600	600	600	600	600	600
Forward primer (nM)	300	50	300	300	300	300
Reverse primer (nM)	900	900	900	900	900	900
TaqMan™-probe (nM)	100	100	100	100	100	100
r*Tth* DNA polym.[b] (U)	5	5	5	5	5	5
AmpErase UNG[c] (U)	0.5	0.5	0.5	0.5	0.5	0.5
RNA-template (ng)	50	50	50	50	50	50
H_2O (µl)	ad 50	ad 50	ad 50	ad 50	ad 50	ad 50

[a] 5× TaqMan EZ-buffer contains: 250 mM bicine, 575 mM potassium acetate, 0.05 mM EDTA, 300 nM ROX (passive reference), 40% (w/v) glycerol, pH 8.2
[b] 2.5 U/µl r*Tth* polymerase-mix contains: 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% (w/v) Tween 20.
[c] 1 U/µl UNG contains: 30 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, 1 mM DTT, 5% glycerol (v/v), 0.5% (w/v) Tween 20.

Table 2. Sequence of PCR primers and sequence specific probes for different targets.

Target sequence	Primer	Sequence (5′-3′)	Position	Amplicon lenght (bp)
GAPDH	GAPDH forward	TGGGTGTGAACCATGAGAAG	1610	
	GAPDH probe[a]	CCTCAAGATCATCAGCAATGCCTCC	1641	76
	GAPDH reverse	GCTAAGCAGTTGGTGGTGC	1685	
IL-1β	IL-1β forward	CTGATGGCCCTAAACAGATGAAG	1564	
	IL-1β probe[b]	TTCCAGGACCTGGACCTCTGCCCTC	1590	87
	IL-1β reverse	GGTCGGAGATTCGTAGCAGCTGGAT	1625	
IL-7	IL-7 forward	GCTTCACCCAGGGCAGCT	810	
	IL-7 probe[a]	TTTCTTCCTTTAACCTGGCCAGTGCAGTTC	831	83
	IL-7 reverse	TTAAAAGTTTCAGAAGGCACAACAATA	867	
IL-10	IL-10 forward	GGGAGAACCTGAAGACCCTCA	368	
	IL-10 probe[a]	CTGAGGCTACGGCGCTGTCATCG	391	72
	IL-10 reverse	TGCTCTTGTTTTCACAGGGAAG	418	
IL-10r	IL-10r forward	GCGAATGACACATATGAAAGCATC	518	
	IL-10r probe[a]	TCCGAGAGTATGAGATTGCCATTCGCA	552	83
	IL-10r reverse	GAACGTGAAGTTTCCCGGC	584	
TNFα	TNFα forward	ATCTTCTCGAACCCCGAGTGA	322	
	TNFα probe[a]	CCCATGTTGTAGCAAACCCTCAAGCTGA	354	83
	TNFα reverse	CGGTTCAGCCACTGGAGCT	387	

[a] VIC-labelled DNA-probe.
[b] FAM-labelled DNA-probe.

$$R_{n^-} = \frac{\text{Emission Intensity of Reporter}}{\text{Emission Intensity of Passive Reference}} \text{ PCR without template}$$

Relative mRNA quantitation was performed using the comparative $\Delta\Delta C_T$-method. The relative quantification of target, normalised to an endogenous reference (GAPDH) and a relevant unirradiated control is given by:

$$\text{relative quantitation} = 2^{-\Delta\Delta C_T},$$

whereas $\Delta\Delta C_T$ is defined as the difference of mean $\Delta C_{T\,(\text{irradiated sample})}$ and the mean $\Delta C_{T\,(\text{unirradiated control})}$,

$$\Delta\Delta C_T = \Delta C_{T\,(\text{irradiated sample})} - \Delta C_{T\,(\text{unirradiated control})}$$

and the ΔC_T is defined as difference in mean $C_{T\,(\text{IL-1}\beta,\,\text{IL-10, TNF}\alpha,\,\text{IL-7 or IL-10r})}$ and mean $C_{T\,(\text{GAPDH})}$ as endogenous control,

$$\Delta C_T = C_{T\,(\text{IL-1}\beta,\,\text{IL-10, TNF}\alpha,\,\text{IL-7 or IL-10r})} - C_{T\,(\text{GAPDH})}.$$

Statistics

All results comparing SSR irradiated skin versus control skin or 1 MED_{jp} sample versus 3 MED_{jp} sample were analysed using the paired Student's *t*-test. These data are presented as mean ± SEM, and were considered significant if $p < 0.05$.

Results

Establishment of combined gene expression assay comparing IL-1β, IL-10, IL-10r, TNFα and IL-7 gene mRNA levels

To determine the relationship of PCR efficiency of target PCR (IL-1β, IL-10, IL-10r, TNFα, IL-7) compared to GAPDH PCR various PCR reactions with different RNA concentrations obtained from PBMC were performed. Efficiencies of target and reference (GAPDH) are approximately equal with 0.1 to 200 ng total RNA (Fig. 1). Application of $\Delta\Delta C_T$-method requires a slope-value (plotted log input RNA amount versus ΔC_T) of approximately 0.1 or less to demonstrate equal PCR efficiency of target and GAPDH respectively. Utilisation of the established gene expression detection assays were confirmed by reasonable mean C_T values ($C_T < 36$) for unirradiated and untreated skin samples (Fig. 2).

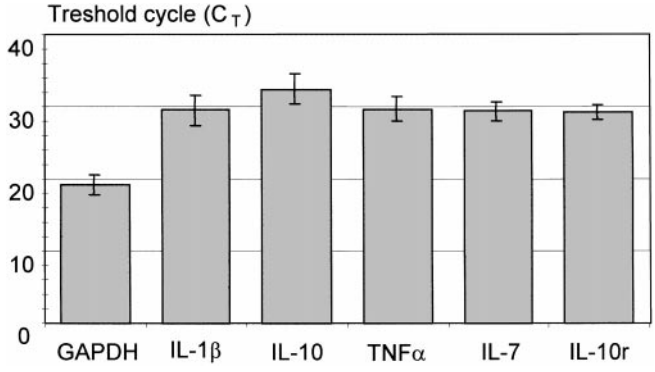

Fig. 2. Threshold amplification levels of unirradiated skin samples (controls). Significant PCR-signals were analysed as C_T-values. 50 ng total RNA was analysed in triplicate determinations. The means of different volunteers (n = 5) and standard deviation β are shown.

Upregulation of IL-1β gene expression caused by single SSR

Because IL-1β has been shown to be involved in different immunological disorders in human skin, we determined comparative and quantitative IL-1β mRNA levels in UV-irradiated human skin in relation to time- and dose-dependency.

Single SSR UV-irradiation with both 1 and 3 MED_{jp} causes significant IL-1β gene induction 6 h (4.7-fold, 43.9-fold resp.) as well as 24 h (4.6-fold, 16.4-fold resp.) after irradiation compared to the unirradiated control (Fig. 3a).

Significant dose-dependent IL-1β mRNA induction was detected 6 h after irradiation (9.3-fold) and also 24 h after irradiation (3.6-fold). 1 MED_{jp} causes equal IL-1β induction 6 and 24 h post irradiation. 24 h after irradiation a decrease (2.7-fold) in IL-1β mRNA levels was measured after application of 3 MED_{jp} compared to 6 h.

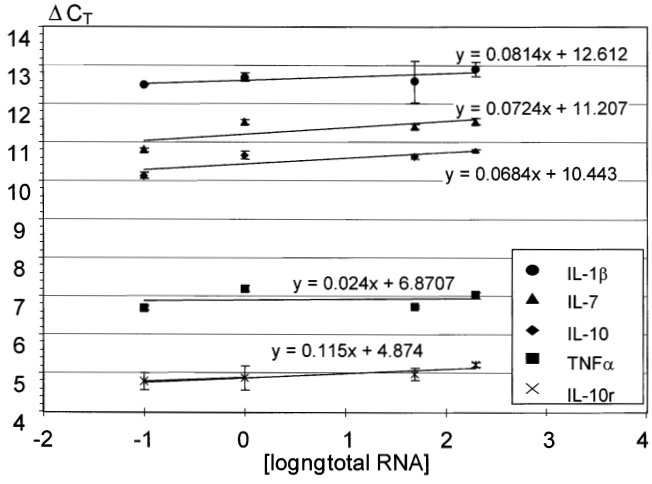

Fig. 1. TaqMan™ RT-PCR efficiency: GAPDH versus IL-1β, IL-7, IL-10, TNFα and IL-10r. Determination of relationship of TaqMan™ RT-PCR efficiency of internal control (GAPDH) versus the different mRNA targets (IL-1β, IL-7, IL-10, TNFα, IL-10r) in a concentration range of 0.1 to 200 ng total RNA. Total RNA obtained from peripheral blood mononuclear cells (PBMC) was used to determine C_T values in a comparable TaqMan™-RT polymerase chain reaction. ΔC_T (mean C_T of GAPDH subtracted from mean C_T of IL-1β, IL-7, IL-10, TNFα, IL-10r, respectively) was calculated for a given RNA-concentration and plotted versus RNA concentration [log ng total RNA]. C_T values were determined in triplicates, standard deviation (SD) for ΔC_T were calculated using following equation: $SD_{(\Delta C_T)} = \sqrt{(SD^2_{GAPDH} + SD^2_{target})}$, curve parameters are given for each (efficiency-plot y = slope x + y-axis intersection).

Induction of different gene expression of IL-10 and TNFα caused by single SSR

TNFα and IL-10 are involved in local and acute UV-induced immune mechanisms of human skin. TNFα and IL-10 mRNA levels were analysed using a comparative and quantitative method to analyse time- and dose-dependency of mRNA induction followed by single SSR-radiation.

A single exposure of skin to 1 and 3 MED_{jp} induces 6 h (1.9-fold, 4.8-fold resp.) and 24 h (1.8-fold, 14.3-fold resp.) post irradiation increases in IL-10 gene expression (Fig. 3b), and also causes induction of TNFα mRNA expression at 6 h (4.7-fold, 14.1-fold resp.) and 24 h (2.9-fold, 4.8-fold resp.) after irradiation (Fig. 3c).

SSR doses of 1 MED_{jp} and 3 MED_{jp} causes significant dose-dependent IL-10 mRNA induction 6 h (2.5-fold) and 24 h (8.0-fold) post irradiation (Fig. 3b). As determined for IL-1β, and IL-10 dose-dependent TNFα mRNA induction were determined 6 h (3.0-fold resp.) and 24 h (1.7-fold) after irradiation with SSR-doses of 1 and 3 MED_{jp} (Fig. 3c). Equal IL-10 mRNA levels are detected for 1 MED_{jp} 6 and 24 h after SSR irradiation, whereas 3 MED_{jp} causes an 3.0-fold increased gene induction 24 h after irradiation compared to 6 h (Fig. 3b). TNFα mRNA expression decreased 3.0-fold 24 h after 3 MED_{jp} irradiation compared to 6 h after irradiation as well as 1.7-fold after SSR irradiation of 1 MED_{jp} (Fig. 3c).

To obtain more detailed information not just about IL-10 but also about the receptor of IL-10 (IL-10r) in human skin, we asked whether IL-10r mRNA levels are acutely modulated in human skin followed by single UV-radiation. IL-10r

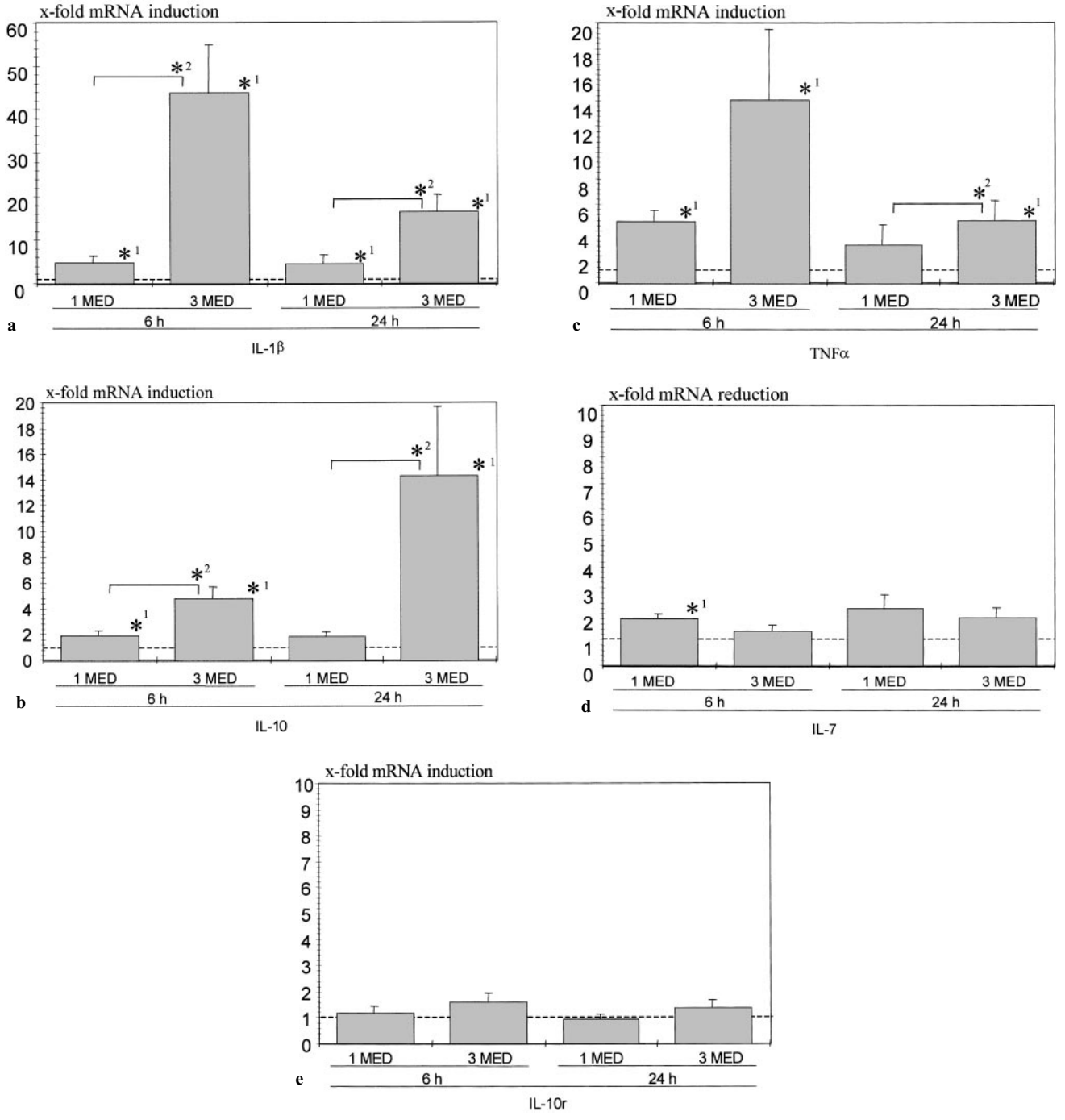

Fig. 3. Quantitative mRNA modulation of IL-1β, IL-10, IL-10r, TNFα and IL-7 in UV-irradiated human skin. Relative quantification of different cytokines on transcription level were detected by TaqMan™ RT-PCR followed by $\Delta\Delta C_T$-analysis. Human volunteers (n = 5) were irradiated with either 1 or 3 MED_{jp} SSR. Punch biopsies were taken at indicated time points and total RNA was isolated. Optimised reverse transcription of RNA and amplification of specific cDNA were performed using the ABI PRISM 7700 Sequence detection system. RT-PCRs were set out in triplicates. Significant PCR fluorescent signals (C_T) for IL-1β, IL-10, IL-10r, TNFα, IL-7 respectively were normalised to a PCR fluorescent signal obtained from an endogenous reference (GAPDH). Comparative and relative quantification of gene products, normalised to GAPDH and an unirradiated control were calculated by: $2^{-\Delta\Delta C_T}$, whereas $\Delta\Delta C_T$ is defined as difference of mean $\Delta C_{T(sample)}$ and $\Delta C_{T(unirradiated\ control)}$, and ΔC_T is defined as difference of mean $C_{T(IL\text{-}1\beta,\ IL\text{-}10,\ TNF\alpha,\ IL\text{-}7\ respectively)}$ and mean $C_{T(GAPDH)}$.

*1 Significant difference compared to unirradiated control skin (n = 5, $p < 0.05$).

*2 Significant difference comparing 1 MED_{jp} to 3 MED_{jp} (n = 5, $p < 0.05$).

mRNA was detected easily in human skin biopsies. IL-10r gene expression was not significantly modulated in human skin 6 h and 24 h by 1 MED_{jp} respectively 3 MED_{jp} SSR irradiation (Fig. 3e).

Single SSR of skin with 1 or 3 MED_{jp} causes no time- or dose-dependent changes in IL-7 mRNA levels 6 and 24 h post irradiation

We analysed IL-7 mRNA levels, because IL-7, produced by human keratinocytes, plays an important role in the survival and proliferation of T-lymphocytes. In order to investigate if cell-derived IL-7 is modulated in UV-irradiated human skin, we analysed IL-7 mRNA levels in SSR-irradiated skin using a quantitative and comparative RT-PCR method. Single SSR exposure to skin with either 1 MED_{jp} or 3 MED_{jp} did not give rise to dose-dependent changes of IL-7 mRNA levels 6 or 24 h after irradiation (Fig. 3d). A small but significant decrease of IL-7 mRNA level of 1.8-fold 6 h after a SSR dose of 1 MED_{jp} compared to the unirradiated control was detected.

Discussion

Numerous studies have demonstrated that UV-radiation has profound effects on the skin's immune system [11, 12]. However, the precise mechanism by which UV-radiation induces immunosuppression is still unclear and is an area of intensive research. Using a highly sensitive and reproducible method, as demonstrated in this work, it was possible to analyse mRNA levels of cytokines and receptors potentially involved in UV-induced immunomodulation.

Development of very sensitive TaqMan™ RT-PCR (Tab. 2), giving significant PCR fluorescence signals within less than 36 cycles (Fig. 2), made it feasible to calculate the mRNA levels in a comparative and quantitative manner [2]. In addition, efficient PCR is achieved by amplification of very short templates of between 72 and 87 base pairs (Tab. 1). Several other quantitative mRNA detection methods have been developed [13, 14], but dynamic range and manipulation of PCR products as for example, gel electrophoresis, hybridisation and probe-detection is required [15, 16]. In addition, Northern blots require relatively large amounts of RNA which is a limiting factor when small tissue samples are the source material, although RT-PCR followed by Southern blotting overcomes this limitation if quantitative analysis of small amounts of specific RNA is required. Demonstration of linear PCR efficiency at template concentrations ranged from 0.1 to 200 ng RNA has been shown for the different RT-PCR assays (Fig. 1) and enabled the usage of the $\Delta\Delta C_T$-method to calculate relative induction/reduction factors (x-fold) compared to a given control.

SSR-doses from up to 3 MED_{jp} are environmentally relevant, readily feasible on exposure to summer sunlight in temperate latitudes, causing an intense erythema without blistering. On cellular level, 3 MED_{jp} causes a significant suppression of alloantigen presentation 15 to 24 h after irradiation [17, 18]. On the molecular level, 3 MED_{jp} of SSR induced a rapid and strong induction of IL-1β and also TNFα mRNA 6 h after irradiation and was down regulated 24 h after irradiation (Fig. 3a, c). These data, considering a time-shift between transcription-level and protein-level, correlate to IL-1β and TNFα increased protein levels caused by 3 MED_{jp} SSR found in suction blister exudates [17]. In contrast, Strickland et al. [19] reported increased epidermal TNFα mRNA and protein 24 h after UV-B irradiation of skin. However, intradermal administration of TNFα into murine skin has been shown to cause loss of epidermal Langerhans cells [20], supporting the importance of TNFα in UV-induced local immune suppression. Controversy exists if IL-1β, an essential mediator for contact sensitisation in mouse [21], is involved in Langerhans cell migration as demonstrated for TNFα. Nevertheless Cumberbach et al. [5] demonstrated IL-1β-induced Langerhans cell depletion in murine skin and accumulation in local lymph nodes by a mechanism different from that of TNFα.

UV-radiation of 3 MED_{jp} SSR induced higher IL-10 mRNA induction 24 h post-irradiation rather than 6 h compared to IL-1β and TNFα mRNA levels. Furthermore, 1 MED_{jp} SSR caused a weak induction of IL-10 transcription at 6 and 24 h (Fig. 3b). In correlation with these data based on mRNA levels, Barr et al. [17] reported that induction of IL-10 expression caused by 3 MED_{jp} is small and transient 15 to 24 h after SSR irradiation, but not at 4 and 8 h. In addition, Skov et al. [22] found maximal levels of IL-10 protein 24 h after irradiation, indicating later induction of higher IL-10 mRNA and protein levels respectively. Because the highest level of IL-10 transcription was found in skin biopsies 24 h after irradiation with the higher UV-dose, it is more likely that infiltrating IL-10-secreting macrophages, as reported in UV-B-irradiated skin [6], are responsible for IL-10-mediated local immune reactions. In addition, significant modulation of the IL-10 receptor (IL-10r) mRNA was not found in UV-irradiated skin (Fig. 3e). No dose- and time-dependent modulation of IL-7 mRNA levels was found in UV-irradiated human skin, but a very small decrease of IL-7 mRNA levels was observed 6 h after 1 MED_{jp} SSR (Fig. 3d). However, negative regulation of IL-7 could be conductive to the local and acute induction of UV-induced alloantigen suppression in human skin.

In this study, we have developed separate highly sensitive one-tube PCR assays for quantification of different mRNA levels. In contrast to other sensitive techniques detecting gene transcripts, the TaqMan™ RT-PCR using the $\Delta\Delta C_T$-method permits a comparative quantification of mRNA levels. Due to the technique's high sensitivity, time- and dose-dependent regulation of cytokine mRNA levels was observed, demonstrating the capability of comparative quantification of small amounts of in vivo material. In summary, following UV-irradiation maximal mRNA levels of the inflammatory cytokines IL-1β and TNFα were detected 6 h after irradiation, in contrast to the antiinflammatory cytokine IL-10 where maximal mRNA levels were determined 24 h after irradiation, but no considerable modulation of transcription was found for IL-7.

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