Effects of therapies on cytokine patterns in psoriasis
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Ultraviolet-B irradiation decreases IFN-γ and increases IL-4 expression in psoriatic lesional skin \( \textit{in situ} \) and in cultured dermal T cells derived from these lesions

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Abstract
Type 1 cytokine producing T cells play an important role in the pathogenesis of psoriasis. Ultraviolet-B (UVB) irradiation is effective in the treatment of this disease. In normal skin, UVB causes a change in dermal microenvironment, leading to a decrease of IFN-γ expressing type 1 T cells and a concurrent increase of IL-4 expressing type 2 T cells. The aim of this study was to show whether UVB irradiation causes a like-wise shift of type 1 and type 2 responses in psoriatic skin. For this purpose, biopsies were obtained from the lesional skin of psoriatic patients before, 2 days and 14 days after a single exposure to 4 MED UVB. Sections from these biopsies were immunostained (CD3, IFN-γ and IL-4) or RNA was extracted and analyzed for the expressions of IFN-γ and IL-4 by PCR. In addition, primary cultures of T cells from dermal cell suspensions were stained intracellularly for IFN-γ and IL-4 expression and CD4+ and CD8+ T subsets were analyzed by flow cytometry. IFN-γ was abundantly expressed in situ before irradiation and decreased in all patients after UVB irradiation, whereas, IL-4 expression was variably expressed before irradiation and increased in different degrees after irradiation. Cytokine mRNA expressions determined by PCR showed a clear decrease of IFN-γ and increase of IL-4 following UVB irradiation. Both CD4+ and CD8+ dermal T cells were found to produce less IFN-γ and more IL-4 following UVB irradiation as determined by flow cytometry. Decrease in IFN-γ expression and increase in IL-4 expression of dermal T cells in psoriatic lesions after UVB irradiation may lead to decrease in local immunoreactivity. These changes could be part of the therapeutic effects of UVB on psoriasis.
Introduction

Psoriasis is a chronic inflammatory skin disease. Its most common form is plaque psoriasis, which is characterized by sharply demarcated, erythematous and desquamating plaques of various shapes and sizes in different locations. The clinical course of the disease is accompanied by unpredictable relapses and remissions. The etiopathogenesis of the disease is still unknown despite extensive efforts\(^1\). Because of the enormous epidermal thickening, dominating the histological picture and contributing to infiltrated skin appearance, many early studies were dedicated to the changes in keratinocytes\(^2\)\(^-\)\(^4\). Later on, polymorphonuclear cells infiltrating dermis and forming Munro's microabscesses in the epidermis were thought to be responsible for the epidermal changes\(^5\)\(^-\)\(^7\). Lately, more attention has been paid to mononuclear cells found in the psoriatic inflammatory cell infiltrate. Most of these cells were found to be T cells infiltrating both epidermis and dermis\(^8\)\(^-\)\(^10\).

T cells are likely to be important in the pathogenesis of psoriasis, illustrated by the observations that specific suppression of T cell function is successful in antipsoriatic therapy\(^11\)\(^-\)\(^12\) and that T cells appear in lesions before hyperproliferation of keratinocytes\(^13\)\(^-\)\(^14\). The finding that lesional T cells can induce keratinocyte proliferation\(^15\)\(^-\)\(^16\) also suggest that T cells play a prominent role in lesion formation.

Many studies have been performed to characterize the nature of T cells from psoriatic lesions. The results of these studies are variable concerning both the phenotype and the cytokine expression of these cells, however most investigators agree on the predominance of type 1 cytokine expression in psoriatic lesions\(^17\)\(^-\)\(^21\). Recently, Austin et al. showed that both cytotoxic (CD8\(^+\)) and helper (CD4\(^+\)) T cells from psoriatic lesions express mainly type 1 cytokines i.e. IFN-\(\gamma\), IL-2 and TNF-\(\alpha\)\(^22\).

In psoriasis patients with extensive or persisting lesions, one of the best treatment options is ultraviolet B (UVB) irradiation. Studies by Krueger et al. and Ozawa et al. showed that lesional T cell numbers decrease after UVB treatment, caused by apoptosis of T cells\(^23\)\(^-\)\(^24\). The decrease of lesional T cell numbers was thought to be responsible for healing of skin lesions after UVB treatment. We demonstrated that T cells in the epidermis of healthy individuals also decrease after a single UVB irradiation. In addition, we showed that 2 days later a new group of T cells infiltrate the dermis of normal UVB-exposed skin\(^25\)\(^-\)\(^26\). Characterization of the cytokine production profile of these dermal T cells from UVB-irradiated skin revealed a shift towards type 2 T cell responses, i.e. more IL-4 expression and/or less IFN-\(\gamma\) expression as compared to control dermal T cells from unirradiated skin\(^27\). This preferential type 2 T cell response, probably promoted by the dermal microenvironment, counteracts the proinflammatory effects of the type 1 cytokines.
In this study, we investigated whether or not in lesional psoriatic skin a switch of cytokine expression from type 1 to type 2 cytokines occurs after single UVB irradiation similar to the switch as observed in normal skin. We found that a single UVB irradiation caused a reduction of IFN-γ expression while increasing IL-4 expression in psoriatic skin. This change in cytokine balance may contribute to the therapeutic effect of UVB in psoriasis.

**Materials and methods**

**Patients.** Patients with moderate to severe plaque type psoriasis, who registered in the Department of Dermatology at Academic Medical Center in Amsterdam were included. Exclusion criteria were photo-aggravated disease, cutaneous malignancy and use of phototoxic medications. Systemic treatments for a minimum of 4 weeks and topical treatments for a minimum of 2 weeks were discontinued. Informed consent was obtained from each patient.

**UVB irradiation and biopsies.** A 1000 W xenon-arc lamp (Oriel, Stratford, CT) equipped with a 303-nm interference filter (Jenaer Glaswerke, Schott & Gen., Germany) was used for local UVB irradiation. The minimal erythema dose (MED) was determined for each patient. Multiple single doses of 4 MED of UVB were locally applied to different clinically similar lesions on gluteal area. Under local anesthesia, two 5-mm. punch biopsies per time point were obtained before irradiation, and 2 and 14 days afterwards. One of the biopsies from each time point was transferred to sterile phosphate buffered saline (PBS) containing 100 μg/ml gentamycin for immediate isolation of single dermal cells and to set-up cell cultures. The other biopsy was embedded in cryomatrix (Shandon, England), snap frozen and, stored at −80°C until use for immunohistochemistry and PCR analysis.

**Immunohistochemical staining of T cells and cytokines.** The frozen biopsies were cut with a cryostat (5 mm) and sections were placed on organosilan-coated glass slides. After air drying, the sections were fixed with acetone for 10 min at 4°C. Subsequently, endogenous peroxidase activity was blocked by incubation with 0.1% sodium azide and 0.3 % H₂O₂ in PBS for 20 min at room temperature (RT). After that, the sections were washed in tris- buffered saline (TBS) and incubated 15 min with 10 % normal goat serum (Dako, Denmark). For CD3 staining the sections were incubated with FITC-labeled anti-CD3 (Becton Dickinson, Mountain View, CA) for 1 h at RT, with rabbit anti- fluorescent isothiocyanate (FITC; Dako) for 30 min at RT and finally with goat-anti-rabbit- peroxidase (Dako) for 30 min at RT. For cytokine staining the skin sections were incubated overnight
at 4°C with anti-IL-4 (Genzyme, Cambridge, MA) or anti-IFN-γ (R&D Systems, Minneapolis, MN) followed by incubation with goat-anti- mouse-biotin (Dako) for 30 min at RT and incubation with streptavidin-peroxidase (Dako) for 30 min at RT. To double stain for CD3 and IFN-γ above mentioned CD3 and IFN-γ staining protocols were sequentially performed, but in between the protocols the sections were incubated with 10 % normal mouse serum (Dako) and instead of goat-anti-rabbit-peroxidase, goat-anti-rabbit-alkaline phosphatase (Dako) was used. After each incubation step, sections were washed with TBS. The peroxidase activity was detected as red by 3-amino 9-ethyl carbazole (Sigma, Missouri) reaction. The alkaline phosphatase activity was determined as blue by using naphtol-AS-MX-phosphate (Sigma). Hematoxilin was used as counterstaining. Epidermal thickness was microscopically measured at the thickest part of each section.

Cell counts of immunostainings. CD3+ cells in whole epidermis were counted in each section and cell numbers were corrected to 1 mm² epidermis. In dermis, CD3+ T cells were counted 1 mm deep subepidermally through the horizontal length of each section, then the positive cell numbers were corrected to 1 mm² dermis. The cell counts from 2 sections of each stained biopsy were averaged. Positive cells in the cytokine stainings were counted in the same way, averaging 3 sections from each biopsy. All the counts were performed blind by two investigators.

Isolation of dermal cells and dermal T cell cultures. The biopsies were washed in PBS and incubated in 0.3 % dispase (Boehringer Mannheim, Germany) overnight at 4°C to enable separation of epidermis and dermis. The dermis was cut into little pieces and incubated in PBS containing 0.2% collagenase D (Boehringer Mannheim), 40 U/ml DNase I (Boehringer Mannheim) and 2 % fetal calf serum (FCS) for 2 h in a shaking bath at 37°C. Then, the mixture was filtered through a cell strainer to remove tissue debris. The cell suspension was washed twice in Iscove’s modified Dulbecco’s medium (IMDM; Bio Whittaker, Belgium) with 2 % FCS. Afterwards, cells were transferred to 96-well round bottom plates in 200 ml IMDM containing 10 % pooled normal human serum, 1 ml/ml phytohemagglutinin (PHA; Difco Laboratories, Detroit, Michigan) and 50 U/ml recombinant human IL-2 (Cetus Corp., Emmeryville, CA) to stimulate T cell growth.

Intracellular cytokine staining and flow cytometric analysis of dermal T cells. T cell cultures were stimulated with 25 ng/ml phorbol 12-myristate 13-acetate (Sigma) and 1 mg/ml ionomycin (Sigma) in the presence of 3mg/ml Brefeldin A (Sigma) for 4 h at 37°C in
an incubator containing 5 % CO₂. The cells were washed with PBS containing 2 % FCS and 0.1 % natrium azide after each step. They were divided over Falcon tubes (R&D), (each tube containing 1x 10⁶ cells) and first incubated for 15 min with allophycocyanin (APC)-labeled anti-CD4 (Becton Dickinson, California) or APC-labeled anti-CD8 (Becton Dickinson) for surface staining at RT, followed by incubation with permeabilizing solution for 10 min and finally, incubation with FITC-labeled anti-interferon-γ (Becton Dickinson) with phycoerythrin (PE)-labeled anti-IL-4 (Becton Dickinson) for 30 min for cytokine staining. Surface antibodies were replaced by APC-labeled anti-IgG1 (Becton Dickinson) and cytokine antibodies were replaced by FITC-labeled anti-IgG2b and PE-labeled anti-IgG1 as isotype controls of antibodies. After staining procedure, the cells were fixed with PBS containing 1 % paraformaldehyde. Flow cytometric analysis of the cells was performed with FACScalibur equipped with Cell Quest software (Becton Dickinson).

Detection of IL-4 and IFN-γ mRNA in skin sections and dermal T cells by reverse transcriptase- PCR. Twenty cryostat sections of 10 mm thickness were treated in 500 ml of TRIzol Reagent (Gibco, England) to homogenise the tissue and the cells. After that, the total RNA was isolated according to the manufacturer's protocol. Five mg of total cellular RNA was reverse transcribed in a reaction volume of 20 ml, and 1 ml of the resulting cDNA was amplified by cytokine specific PCR. The PCR conditions and electrophoresis of the PCR products are described in detail elsewhere (28). The following specific primer sets were provided by Isogen Bioscience BV, The Netherlands: GAPDH forward primer 5'-CGA GAT CCC TCC AAA ATC AA-3' and reverse primer GAPDH 5'-AGG TCA GGT CCA CCA CTG AC-3'; IL-4 forward primer 5'-TGC CTC CAA GAA CAC AAC TG-3' and reverse primer 5'-AAC GTA CTC TGG TTG GCT TC-3'; IFN-γ forward primer 5'-GCA GAG CCA AAT TGT CTC CT-3' and reverse primer 5'-ATG CTC TTC GAC CTC GAA AC-3'. The PCR products were of the expected size and the specificity was confirmed by sequence analysis. The ethidium bromide-stained PCR products were scanned by the EAGLE EYE R® II video system (Stratagene, La Jolla, CA), and the signal strength was integrated to obtain the densitometric value for each PCR product. To allow semiquantitative analysis, the densitometric values of IL-4 and IFN-γ in each sample were related to the signal of housekeeping gene GAPDH, which is assumed to be produced at a constant rate.

Statistical analysis. Unpaired student t test is used to compare the groups. Two-tailed p value< 0.05 was considered as significant.
Results
Single local UVB irradiation results in clinical and histologic improvement of psoriatic skin lesions. Five adult patients (4 male and 1 female, age range 40-78 yrs, average $53.6 \pm 15.1$ yrs) participated in the study. The MEDs of these patients were ranging between 343-871 J/m$^2$ and correlated with the skin types of the patients (skin type II-IV). Dermal T cell cultures were obtained from all 5 patients, while in 3 patients additional biopsies were taken to perform immunohistochemistry. Locally irradiated skin lesions healed clinically 14 days after 4 MED UVB irradiation in all patients regarding desquamation, erythema and skin infiltration (Figure 1). This change was also observed as significantly decreased epidermal thickness (from $0.8 \pm 0.4$ mm to $0.3 \pm 0.03$ mm) in frozen sections 14 days following irradiation (62 % decrease $p < 0.05$) (Figure 2). Epidermal T cell numbers determined by CD3 staining were progressively decreased on day 2 and day 14 following UVB irradiation. Mean epidermal T cell numbers of 3 patients were $52.8 \pm 10.5$ per mm$^2$

Figure 1. Single 4 MED UVB irradiation of psoriatic skin results in local clinical healing. The psoriatic plaque of patient was irradiated locally at the circled area. After 14 days following single UVB irradiation, the lesion healed only at the irradiated area observed as diminished erythema, infiltration and desquamation. This change was observed in all included patients ($n=5$) (page 188).

Figure 2. CD3 expression in psoriatic skin sections before and after single 4 MED UVB irradiation. Immunoperoxidase staining of skin sections with CD3 (red) showed that T cells were mainly located in epidermis and in dermis forming clusters before irradiation. Epidermal T cells progressively decreased at day 2 and day 14 following irradiation, on the other hand, dermal T cells slightly increased at day 2 and decreased at day 14. Epidermal thickness was markedly decreased after 14 days following irradiation (page 188).
epidermis before irradiation which decreased to 37.5 ± 29.7 per mm² at day 2 (29 %
reduction, p>0.05) and 8.5 ± 1.5 per mm² at day 14 (83.9 % reduction, p < 0.05). On the
other hand, dermal T cell numbers (416.2 ± 205.8 per mm² dermis) increased slightly after
2 days (447.2 ± 232.6 per mm²), followed by a decrease 14 days after irradiation (155±
82.8 per mm²). The changes in dermal T cell numbers following UVB irradiation were,
however, not statistically significant.

**IFN-γ expression decreases in psoriatic skin following single UVB irradiation,**
**whereas IL-4 expression shows a variable pattern.** Expressions of IFN-γ and IL-4 in
skin were determined immunohistochemically as red staining using AEC as chromogen.
IFN-γ expression was observed mainly in papillary dermis forming clusters in un-irradiated
skin (Figure 3A). IFN-γ staining co-localized with T cells as defined by CD3 staining (Figure
3C). In all patients, untreated psoriatic lesions displayed high IFN-γ expression (54.1 ±
19.4 per mm²) which was significantly decreased after UVB at day 2 (18.3 ± 9.2 per mm²,
p< 0.01) and/or day 14 post-irradiation (9.8 ± 2.2 per mm², p<0.01) as determined in 9

![Figure 3. IFN-γ expression in psoriatic skin sections decreased after single 4 MED UVB irradiation. IFN-γ
staining (red) of psoriatic skin sections showed a prominent expression especially in papillary dermis (A). This
expression was suppressed markedly 14 days following UVB irradiation (B). Double staining with IFN-γ (red)
and CD3 (blue) showed the colocalization of this cytokine with T cell infiltration in psoriatic skin (C)
(☞ page 188).](image)

![Figure 4. IL-4 expression of psoriatic skin is variable before and after single UVB irradiation. IL-4 (red) positive
cells were absent or present in low numbers before irradiation in psoriatic skin (A). It was increased 2 days after
UVB irradiation (p<0.001) (B). This expression was not accompanied by T cell staining (data not shown)
(☞ page 188).](image)
sections in total (Figure 3B). IL-4 staining was absent (Figure 4A) in one patient and present in low numbers in two patients before the irradiation (4.2 ± 5.2 per mm²). The expression of IL-4 significantly increased especially in upper dermis and also in epidermis as single cells forming no groups at day 2 after UVB irradiation (21.4 ± 5.5 per mm² p<0.01) (Figure 4B). Double staining with CD3 and IL-4 yielded only an occasional double positive cell (data not shown). At day 14, the IL-4 expression completely disappeared.

Single UVB irradiation decreased IFN-γ and increased IL-4 expression in T cells isolated from dermis from psoriatic lesions. To show whether or not there is a change in type 1/ type 2 cytokine ratios following single 4 MED UVB irradiation, IFN-γ and IL-4 expressions of CD4+ and CD8+ dermal T cells were intracellularly determined by flow cytometry. After staining, the dermal T cells were first electronically gated in forward scatter (cell size) and side scatter (granularity) dot plots, and then CD4+ and CD8+ cells were identified by APC staining. IFN-γ expression was ranging between 83.7-2.6 % (28.8 ± 33.3 %) in CD4+ T cells and 84.5-51.2 % (51.2 ± 31.3 %) in CD8+ T cells. On the other hand, expression of IL-4 was much lower in both types of T cells (in CD4+ cells between 1-18.8 %; 5.9 ± 7.3 % and in CD8+ cells between 1.3-19.2 %; 5.6 ± 7.6 %) as compared to IFN-γ expression before treatment. Although the IFN-γ/IL-4 ratios in the 5 psoriasis patients were variable (Table 1), UVB irradiation resulted in an overall decreased IFN-γ and increased IL-4 expression in CD4+ or CD8+ dermal T cells at day 2 and/or day 14 (Figure 5). IFN-γ expression decreased to 10.5 ± 7.2 % and 9.1 ± 2.9 % 14 days after UVB exposure in CD4+ cells. Similar decrease in IFN-γ expressions also occurred in CD8+ cells (29.9 ± 34.4 % at day 2 and 18.9 ± 18.3 % at day 14 following UVB exposure). IL-4 expression was progressively increased in both CD4+ cells (11.8 ± 13.2 % at day 2 and 16 ± 13.8 % at day 14) and CD8+ cells (9.8 ± 11.6 % at day 2 and 15.8 ± 7.8 % at day 14). Decreased IFN-γ

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<td>Day 0</td>
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<td>Patient 1</td>
<td>35.8</td>
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<td>Patient 2</td>
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<td>Patient 4</td>
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<td>Mean± SD</td>
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and increased IL-4 expression led to markedly reduced IFN-γ/IL-4 ratios of both CD4+ and CD8+ cells (Table 1), but due to the high individual differences in expressions of cytokines before and after UVB irradiation the strong reduction of this ratio was not significant in our group of 5 psoriasis patients. Similar changes were also observed with intracellular IFN-γ/IL-13 ratios in dermal T cells (data not shown).

![Figure 5](image)

**Figure 5.** Intracellular cytokine staining of CD4+ and CD8+ dermal T cells of psoriasis patients before and after single UVB irradiation. T cells cultured from psoriatic dermis showed high expression of IFN-γ and low expression of IL-4 both in CD4+ and in CD8+ cells determined by flow cytometry. Each row represents the dot plots of one patient at day 0, 2 and 14 following UVB irradiation. The simultaneous expression of IFN-γ (x axis) and IL-4 (y axis) in CD4+ T cells (A and B) or CD8+ T cells (C and D) is depicted. After UVB irradiation both CD4+ and CD8+ T cells showed decreased IFN-γ and increased IL-4 expression in all patients, however, these changes in cytokine expressions were quite variable in degree and timing after UVB irradiation in different patients.
IFN-γ mRNA expression decreases and IL-4 mRNA expression increases in psoriatic skin after single UVB irradiation. Frozen sections from lesional skin before and after UVB irradiation were used for the determination of cytokine mRNA by RT-PCR (Figure 6). IFN-γ mRNA expression decreased from 0.96 ± 0.29 to 0.78 ± 0.61 at day 2 and 0.48 ± 0.24 at day 14 after UVB irradiation. Although the expression after 14 days was half of the pre-treatment value this change was not statistically significant. IL-4 mRNA was low-expressed (0.43 ± 0.02) before irradiation as compared to IFN-γ expression. Following irradiation, IL-4 mRNA expression increased to 0.69 ± 0.34 after 2 days and 1.83 ± 1.44 after 14 days. This marked increase in IL-4 expression following UVB irradiation was also not statistically significant due to high standard deviations between individual values.

Figure 6. The kinetics of IFN-γ and IL-4 mRNA expression in psoriatic skin. RNA was extracted from cryostat sections and subjected to RT-PCR. The PCR products were harvested at successive cycle stops (indicated as 1 through 4) which all are within the exponential phase of the amplification reaction. IFN-γ mRNA expression decreased and IL-4 expression increased compared to housekeeping gene expression (GAPDH) after single 4 MED UVB irradiation in frozen sections of psoriatic skin. Results of one representative is shown.

Discussion
In this study, we demonstrated that a single UVB irradiation of psoriatic lesional skin with 4 MED resulted in decreased IFN-γ and increased IL-4 expressions in situ and in primary cultures of dermal T cells derived from in vivo-UVB exposed skin. UVB irradiation causes local and systemic immunosuppression as demonstrated by suppressed delayed type and contact hypersensitivity 28,29. Many different cytokines are secreted upon UVB irradiation 30. Especially PGE2, IL-10 and IL-4, which increase after UVB irradiation in mice, were suggested to form an immunosuppressive cascade 31. We also observed increased IL-4 expression after UVB irradiation in situ in skin of healthy individuals and subsequent dermal
T cell cultures. Phototoxic effects on immunocompetent cells provide an alternative way to explain immunosuppressive effect of UVB. The reduction of T cell numbers was found to be 85 % in epidermis and 29 % in dermis after broad-band UVB therapy of psoriasis, and this T cell depletion was suggested to be the major therapeutic mechanism of UVB irradiation in T cell related inflammatory skin diseases. It seems justified to assume that epidermal T cells are important in maintaining psoriasis activity as compared to dermal T cells. However, one might think that the remaining dermal T cells could sustain or restart the inflammatory reactions in the psoriatic skin lesions soon after the completion of UVB therapy. This matter triggered us to study the IFN-γ and IL-4 expression profiles in skin and dermal T cell cultures following UVB irradiation, keeping in mind the increased type 2 cytokines after UVB exposure of healthy skin.

Similar to earlier studies on UVB treated patients, we found that the T cell numbers in the epidermis decreased after a single UVB irradiation with 4 MED, which was accompanied by a reduced thickness of the epidermis. Interestingly, this decrease (83.9 % reduction) upon a single high-dose UVB exposure was almost equal to the decrease in epidermal T cell numbers in UVB-treated patients receiving repetitive low-dose UVB irradiation during 4-6 weeks commonly applied in clinical practice. In contrast to the progressively decreasing numbers of epidermal T cells in lesional skin after a single UVB exposure, dermal T cells slightly increased in number after 2 days, but were strongly reduced 14 days after irradiation. This reduction at day 14 was even more than the dermal T cell numbers in psoriatic skin after regular UVB therapy (80 % in our study vs. 29 % in the study of Ozawa et al). The kinetics of dermal T cells in UVB-exposed psoriatic skin is similar to the results of Di Nuzzo et al. showing an influx of T cells to the dermis in healthy skin 2 days after UVB exposure. This increase in dermal T cells can not be due to the migration of epidermal T cells to the dermis, because most epidermal T cells are killed following UVB irradiation.

In line with earlier studies, we found prominent IFN-γ staining in lesional skin, especially in the papillary dermis. This IFN-γ expression decreased following UVB exposure, which could be related to decreased numbers of T cells, being the main source of this cytokine. In 2 patients this decrease was observed already after 2 days following irradiation, despite the presence of high T cell numbers, suggesting that IFN-γ expression was down-regulated in the T cells present in the irradiated psoriatic lesion. IL-4 expression in lesional skin before irradiation was found in 2 patients. This observation is in agreement with other studies that demonstrated the presence of type 2 cytokines in untreated lesional skin.
although type 1 cytokines are predominant\textsuperscript{18,22,33}. Considering the individual variability of lesion morphology, severity and disease course in psoriasis patients, it is clear that the inflammatory activity of the lesions may vary enormously, even in different lesions of the same individual. This dynamic inflammatory state of the disease results in great range of differences in observations. In response to the single UVB irradiation, IL-4 expression was increased in skin sections at day 2. This result shows similarity to our earlier results in which we observed a dose-dependent induction of IL-4 expression in the first few days after a single 4 MED UVB irradiation of healthy skin\textsuperscript{27}. The expression of IFN-\(\gamma\) at mRNA level determined in frozen sections decreased progressively just like the expression at protein level. However, IL-4 mRNA expression did not correlate with the expression at protein level, especially at day 14 after UVB exposure. The high IL-4 mRNA expression at day 14 could reflect the presence of potential IL-4 producing cells in psoriatic skin even after 2 weeks following the UVB irradiation. This suggestion is supported by our results on lesional dermal T cells analysed by flow cytometry for the expressions of IFN-\(\gamma\) and IL-4. Before the irradiation both CD4\(^+\) and CD8\(^+\) dermal T cells were producing high amounts of IFN-\(\gamma\) which supports the results of Austin et al. claiming that the lesional and blood T cells of CD4\(^+\) and CD8\(^+\) cells produce mainly type 1 cytokines\textsuperscript{22}. Szabo et al. also reported similarly high IFN-\(\gamma\) expression of isolated lesional T cells. Their results were very heterogeneous like our results, due to the intersubject variation in T cell numbers and intracellular IFN-\(\gamma\) expression\textsuperscript{14} and this interpatient variability of results was also observed after UVB irradiation. In all our patients intracellular cytokine staining revealed decreased IFN-\(\gamma\) and increased IL-4 expression, but the time point and degree of the change in ratios were very different from one patient to another. It should be noted that the IFN-\(\gamma\) / IL-4 ratio of each individual patient was markedly decreased after UVB exposure (Table 1), but taken as a group this change was not significant.

Overall, our results showed that a single 4 MED UVB irradiation results in decreased IFN-\(\gamma\) and increased IL-4 expression in psoriatic lesional skin in addition to an ultimate reduction in lesional T cell numbers. This cytokine shift from type 1 to type 2 in lesions could be important in the long lasting therapeutic effect of UVB on psoriatic skin by creating a skin microenvironment which does not allow the formation of new skin lesions. In connection to this, a protective role of type 2 cytokines promoted by different therapies has been described in psoriasis vulgaris\textsuperscript{35,36}. It is important to find out whether a similar mechanism is operational in patients treated with UVB and how the skin of psoriatic patients react to lesional dermal T cells which remain in dermis after UVB therapy.
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