Effects of therapies on cytokine patterns in psoriasis
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Clinical improvement in chronic plaque-type psoriasis lesions after narrow band-UVB therapy is accompanied by a decrease in the expression of interferon-γ inducers, IL-12, IL-18 and IL-23

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**Abstract**

Type 1 cytokine producing T cells are important in the pathogenesis of psoriasis vulgaris, efficient therapy for which is provided by narrow band ultraviolet B (NB-UVB). The expression of the type 1 cytokine interferon (IFN)-γ is regulated by interleukin (IL)-12, IL-15, IL-18, and IL-23, however not much is known about the effect of this therapy on the levels of these cytokines in lesional psoriatic skin in situ. In this study, we investigated the effects of NB-UVB therapy on the expression of IFN-γ-inducing cytokines. Ten patients with chronic plaque type psoriasis selected to be treated with NB-UVB therapy were recruited for these experiments and the expression of cytokines IL-12, IL-15, IL-18, IL-23 and IFN-γ in lesional psoriatic skin before, during and after therapy was determined by immunohistochemistry. Double staining was performed to determine the cell types expressing these cytokines. The decrease in the psoriasis area and severity index (PASI) was accompanied by a significant decrease in the expression of IFN-γ, and concomitantly, significant reduction of IFN-γ-inducers, IL-12, IL-18 and IL-23. Thus, we concluded that the decrease of IFN-γ expression in psoriasis lesions after NB-UVB therapy could be a result of diminished expression of IL-12, IL-18 and IL-23 in lesional skin. Therapies targeting these three cytokines should therefore be considered in the treatment of psoriasis.
Introduction

Psoriasis is histologically characterized by hyperproliferation of keratinocytes accompanied by a dermal and epidermal inflammatory cell infiltration. Today, it is widely accepted that the inflammatory cell infiltrate in psoriatic lesional skin influences hyperproliferation of keratinocytes. The importance of the interaction between keratinocyte and inflammatory cells in the pathogenesis of psoriasis via soluble factors, i.e. cytokines, has been shown by different groups: the majority of T cells in psoriatic lesions produce interferon (IFN)-γ, rather than IL-4, showing the predominance of type 1 effector T cells, and subcutaneous injection of IFN-γ was reported to cause formation of psoriatic skin lesions at the site of injection. Cytokines (IL-10, IL-11 and IL-4) and cytokine inhibitors (anti-IL-2, anti-TNF-α) that suppress type 1 cytokine responses were also found to have a high efficacy for the treatment of psoriasis. Collectively, these findings demonstrate that psoriasis is a type 1 cytokine-related disease.

UVB therapy is one of the most commonly applied treatments for psoriasis. UVB is known to affect cytokine expression and we and others have shown that broad band-UVB irradiation and narrow band (NB)-UVB therapy suppress type 1 cytokine responses in normal skin and psoriatic lesional skin. Acute exposure to high doses of UVB seems to suppress type 1 (i.e. IFN-γ) and concomitantly provoke type 2 (i.e. IL-4) cytokine expression, while chronic exposure to low doses of NB-UVB results predominantly in suppression of IFN-γ expression. Interestingly, in vitro stimulation experiments revealed that dermal T cells obtained after therapy with NB-UVB radiation displayed a reduced capacity to express IFN-γ compared to dermal T cells isolated before therapy, thus indicating a change in phenotype of T cells remaining in the skin after exposure to UVB. As IFN-γ predominates and is thought to be important in psoriasis disease activity, suppression of the expression of this type 1 cytokine might play a role in the healing of skin lesions after UVB therapy.

The production of IFN-γ is strictly controlled by the expression of other cytokines. The major inducers of IFN-γ secretion are IL-12, IL-18, and the recently identified IL-23 and IL-27. IL-12 and IL-23 are both heterodimeric molecules, sharing a common p40 subunit which is covalently bound to a unique p35 subunit or p19 subunit to form the biologically active IL-12 and IL-23, respectively. In addition, IL-15 was found to induce type 1 cytokine responses. In this study, we investigated the modulation of the expression of IL-12, IL-15, IL-18 and IL-23 in psoriatic skin lesions after NB-UVB therapy.
**Materials and methods**

**Patients and narrow band-UVB therapy.** Ten patients (7 women and 3 men; mean age 49 ± 16) with chronic plaque type psoriasis who were referred to the Phototherapy Unit of the Department of Dermatology at the Academic Medical Center in Amsterdam for NB-UVB therapy were included in this study. The study design was approved by the local ethics committee. The patients stopped systemic therapies 4 weeks and topical treatments, other than emollients, 2 weeks before their inclusion to the study. Irradiation was given in a TL-01 lamp (311 nm, Philips, The Netherlands) equipped cabinet three times per week. The initial UVB dose was determined according to the skin type of patients. Although this was not an inclusion criterion, all patients in this study were determined to have skin type 3 according to the Fitzpatrick classification which is representative for our patient population. These patients typically started to the therapy with a NB-UVB dose of 0.08 J/cm². The dose was increased by $3^{1/2}$ times at each subsequent exposure. In this group of patients, the maximum dose applied was up to 2.25 J/cm². In case of UVB-induced skin irritation, previous dose was applied or the treatment was discontinued according to the severity of the reaction. The NB-UVB therapy was continued for 10.6 ± 1.9 weeks. Clinical condition at the time of biopsy-taking was determined by psoriasis area and severity index (PASI). Punch biopsies of 5 mm were taken before, at the 3rd week and at the end of the NB-UVB therapy from the plaques located on the lower back of patients. A template was used to mark the plaque where the first biopsy was taken. This was done to obtain the later biopsies from the same plaque. Because we kept a distance of at least 2 cm between each biopsy on the same plaque, it was not always possible to get all biopsies from the same plaque. Therefore, we also include the nearby plaques with similar clinical appearance on the template to take biopsies at later time points. Skin sections of 5 mm from frozen skin biopsies were used for immunostaining. Three normal control biopsies of 5 mm were taken from skin specimens obtained from plastic surgical operations of the breast or abdomen.

**Antibodies.** Primary mouse anti-human antibodies against cytokines used for immunohistochemistry were as follows: Biotin-conjugated anti-IL-12/IL-23 p40 (clone C8.6, BD Pharmingen, San Jose, CA), anti-IL-12 p70 (clone 24945.11, R&D Systems, Minneapolis, MN), anti-IL-18 (clone 25-2G, R&D Systems), anti-IL-15 (clone 34505.11, R&D Systems) and anti-IFN-γ (clone 25723, R&D Systems). Polyclonal rabbit anti-human IL-23 p19 subunit was a kind gift of Dr. J. Pirhonen (Dept. of Microbiology, National Public Health Institute, Helsinki, Finland). To identify the cell types expressing IL-12, IL-18, and IL-23 p19 subunit, the following FITC-labelled antibodies were used in double staining: anti-
CD3 (clone 5K7, T cells; BD Pharmingen, San Jose, CA), anti-CD36 (clone FA6.152, macrophages; Beckman Coulter, Fullerton, CA), anti-CD15 (clone C-3D1, polymorphonuclear leukocytes; Dako, Glostrup, Denmark), anti-CD83 (clone HB15, activated/mature dendritic cells; BD Pharmingen), anti-HLA-DR (clone L-243, BD Pharmingen). Staining with isotype controls was performed to confirm the specificity of the staining with monoclonal antibodies (data not shown). As control for the polyclonal rabbit anti-IL-23 p19 antibody we used the polyclonal rabbit antibody against factor Xllla (Biogenex, San Ramon, CA), which stained only dermal dendritic cells and did not show any staining of epidermal cells (data not shown).

**Immunohistochemical staining.** Immunohistochemical staining of cryostat sections was performed as described before. Briefly, following fixation at 4 °C for 10 min in acetone, sections were incubated sequentially with 10% normal goat serum (Dako) at room temperature for 15 min, primary antibody overnight at 4 °C, biotin-conjugated goat anti-mouse (Dako) at room temperature for 30 min and avidine peroxidase (Dako) at room temperature for 30 min (Dako). For p19 staining, the second step was performed with biotin-conjugated goat anti-rabbit (Dako) instead of biotin-conjugated goat anti-mouse. After each incubation step, except for the incubation with normal goat serum, the sections were washed 3 times with Tris-buffered saline. Peroxidase activity was detected as red color using the chromogen 3-amino-9-ethylcarbazole (AEC; Sigma-Aldrich, St Louise, MO). Hematoxilin was used to perform nuclear staining.

For double staining, the alkaline phosphatase-anti-alkaline phosphatase technique (Dako) was used to intensify the signal of the primary cytokine antibodies. After that, the sections were incubated sequentially with 10% normal mouse serum (Dako), second primary antibody, rabbit anti-FITC (Dako) and goat anti-rabbit peroxidase (Dako). The color development was achieved by using naphtol-AS-MX-phosphate (Sigma) for blue and AEC for red.

**Microscopy.** The sections were counted and scored at a 200x magnification by two investigators who were blinded for the sections. Epidermal and dermal positive cells were counted separately in at least 3 sections of each biopsy. The cell numbers were corrected to 1 mm² of epidermis and dermis. Ten, the mean cell numbers from 3 sections were calculated for each biopsy from each of the 8 patients. The mean cell numbers from the biopsies of the different patients are presented in the results. In case of epidermal stainings of IL-12 / IL-23 p40, IL-23 p19, IL-18 and IL-15, we used a scoring to determine the intensity of the staining as these cytokines were stained diffusely in the epidermis and not
in single cells. The staining intensity of the total length of the section was arbitrary scored as follows: 0, no staining; 1, very weak staining; 2, weak staining; 3, moderate staining; 4, strong staining; 5, very strong staining. Basal and suprabasal stainings were scored separately, as the staining intensity was different in these two compartments (see epidermal staining pattern of IL-15 in Fig. 3P).

Statistics. Mean and standard deviation were calculated for each variable. The p value was determined by the student’s t test (paired samples) to compare the results from the variables before, at the 3rd week and after the therapy.

Results
Decrease in the expression of IFN-γ-inducing cytokines after NB-UVB therapy.
Nine out of 10 patients showed a good clinical response to the NB-UVB therapy as determined by PASI (Fig. 1). In one of the 9 patients, the plaque chosen as biopsy site did not show significant clinical and histological changes, while other lesions of this patient improved causing a marked decrease of the PASI after the therapy (PASI before therapy 9.2 and after therapy 3.7).

Staining patterns of different cytokines are summarized in Table 1. Comparison of mean scores of the immunohistochemical staining in the next experiments was based on the results of the eight responder patients as our main goal was to determine whether the clinical improvement was related to a decrease in the expression of IFN-γ-inducing cytokines. Results of the nonresponders will be discussed at the end of this section. Confirming our earlier observation17, NB-UVB treatment caused a significant decrease in IFN-γ expression in lesional skin (p = 0.04 in dermis; p = 0.02 in epidermis) (Fig. 2A, 2G and 3A-C).

The expression of the common p40 subunit shared by IL-12 and IL-23 was abundant in

![Figure 1. Decrease in the psoriasis area and severity index (PASI) after the NB-UVB therapy (n = 10, * p < 0.05).](image-url)
psoriatic lesional skin before therapy (Fig. 3D). The overall expression in epidermis and dermis of affected skin was approximately twice that than in nonlesional and in normal skin (Fig. 2B). Epidermal expression was strong but diffuse and confined to the basal and suprabasal keratinocytes while dermal expression was mostly seen in the perivascular area (Table 1, Fig. 3D). After NB-UVB therapy, the expression of the IL-12/IL-23 p40 subunit in the lesions decreased approximately 60% in the dermal and epidermal compartments (p = 0.04 in dermis and p = 0.003 in epidermis; Fig. 2B, 2H and 3D-F). The expression of the IL-12 p70 heterodimer, was generally confined to the cell infiltrate in the papillary dermis (Table 1, Fig. 3G). No positive cells were found in the epidermis. The expression of the biologically active p70 heterodimer of IL-12 diminished significantly upon NB-UVB therapy in the dermis as illustrated in Fig. 2C and Fig. 3G-I (p = 0.001).

**Figure 2.** Change in the expression of cytokines before, during and after therapy with NB-UVB in lesions with clinical improvement (n = 8). Bars represent either absolute cell counts or scores in the dermis (a-f) and in the epidermis (g-k) as explained in the Materials and Methods. Standard deviations were calculated from the mean total cell numbers or scores (* p < 0.05, ** p ≤ 0.001).
Figure 3. Determination of cytokine expression in psoriatic lesional skin sections before (right column), after 3 weeks (middle column) and at the end (left column) of the NB-UVB therapy. (a-c), IFN-γ; (d-f), p40; (g-i), IL-12 p70; (j-l), IL-23 p19; (m-o), IL-18; (p-r), IL-15. Presence of cytokine was detected as red color and blue nuclear staining was performed with hematoxilin. Original magnification x 25 (see page 194).
The expression of the IL-23 p19 subunit was increased in all skin compartments in psoriatic lesional skin as compared to normal skin (Fig. 2D and Fig. 3J). The staining with p19 antibody was strikingly diffuse and strong in epidermis suggesting that keratinocytes could express this subunit of IL-23 (Table 1). Upon treatment with NB-UVB, this expression decreased significantly both in epidermis and dermis (p < 0.001 in dermis and p = 0.03 in epidermis; Fig. 2D, 2I and Fig. 3J-L).

Another important inducer of IFN-γ expression, IL-18, was found to be located in similar cutaneous sites in the psoriatic lesion as the IL-12 / IL-23 p40 and IL-23 p19 subunits (Table 1, Fig. 3M). The expression of IL-18 was diminished predominantly in epidermis after NB-UVB therapy (p = 0.01 in dermis, p = 0.001 in epidermis; Fig. 2E, 2J and Fig. 3M-O). IL-15 has been suggested to play an important role in the psoriatic lesional activity being both an activator of T cells, inducer of Th1 responses and suppressor of apoptosis. Expression of IL-15 was mainly confined to basal epidermal cells and dermal perivascular cells in normal and psoriatic lesional skin. However, in the psoriatic lesional skin, IL-15 was expressed more strongly in epidermal cells and by higher numbers of dermal cells than in nonlesional and normal skin (Table 1, Fig. 2F and 2K). IL-15 expression decreased after the NB-UVB therapy, but this decrease did not show any significance due to the high variation of expression between the patients (Fig. 2F, 2K and Fig. 3P-R).

Concerning the two nonresponders, a minor decrease or even an increase (IFN-γ and IL-18) in the expression of cytokines was found in the lesional skin upon UVB therapy, which is in sharp contrast to the marked decrease of IFN-γ and IFN-γ- inducing cytokines observed in the eight responders (Fig. 4). The results of the nonresponders further stressed that clinical improvement of psoriasis lesions is related to a diminished expression of type 1 cytokines.

Table 1. Staining pattern of cytokines in psoriatic lesional skin

<table>
<thead>
<tr>
<th>Cytokine/ Cytokine subunit</th>
<th>IFN-γ</th>
<th>IL-12 / IL-23 p40</th>
<th>IL-12 p70</th>
<th>IL-23 p19</th>
<th>IL-18</th>
<th>IL-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staining pattern</td>
<td>Epidermal scattered, dermal perivascular</td>
<td>Diffuse strong epidermal, dermal perivascular and dermal scattered</td>
<td>Dermal perivascular and dermal scattered</td>
<td>Diffuse strong epidermal, dermal perivascular and dermal scattered</td>
<td>Diffuse strong epidermal, dermal perivascular and dermal scattered</td>
<td>Diffuse strong basal epidermal, dermal perivascular and dermal scattered</td>
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</tbody>
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**Figure 4.** Effect of NB-UVB therapy on IFN-γ and IFN-γ-inducing cytokines. The mean cell numbers and staining-intensity scores after therapy subtracted from the mean before therapy. A positive value indicates that NB-UVB therapy reduced the cytokine expression. The responders (n = 8, white bars) show a clear decrease of IFN-γ and IL-18 expression whereas the opposite was true for the nonresponders (n = 2, black bars). Although the patient groups were too small to draw firm conclusions, the reduction of the common p40 and the specific IL-12 p70 and IL-23 p19 subunits in the responder group seems to be more pronounced than in the nonresponders.

**In situ determination of cells expressing IFN-γ inducers.** Double staining revealed that those cells which expressed IL-12 p70, IL-23 p19 and IL-18, were located in close proximity to CD3+ T cells as seen in Fig. 5A, 5B and 5C, respectively. IL-12 p70+, IL-23 p19+ and IL-18+ cells co-expressed HLA-DR indicating a possible role for antigen-presenting cells in the high IFN-γ expression of the activated T cells (Fig. 5D-F). Most of the CD36+ macrophages located in the inflamed psoriatic lesional dermis costained with IL-12 p70, IL-23 p19 and IL-18 (Fig. 5G-I). Approximately a quarter of the CD83+ dendritic cells

**Figure 5.** Double immunoenzymatic staining of IL-12 p70, IL-23 p19 and IL-18 with various cell surface markers. Single and double stained cells are marked by thin and thick arrows, respectively. (a), IL-12 p70 (blue) and CD3 (red); (b), IL-23 p19 (red) and CD3 (blue); (c), IL-18 (blue) and CD3 (red); (d), IL-12 p70 (blue) and HLA-DR (red); (e), IL-23 p19 (red) and HLA-DR (blue); (f), IL-18 (blue) and HLA-DR (red); (g), IL-12 p70 (blue) and CD36 (red); (h), IL-23 p19 (red) and CD36 (blue); (i), IL-18 (blue) and CD36 (red); (j), IL-12 p70 (blue) and CD83 (red); (k), IL-23 p19 (red) and CD83 (blue); (l), IL-12 p70 (blue) and CD15 (red). Original magnification x200 (page 195).
coexpressed these cytokines as illustrated in Fig. 5J and 5K. As it is known that the human polymorphonuclear leukocytes can express IL-12 p70 heterodimer\(^{21,22}\), we also investigated the CD15\(^+\) polymorphonuclear leukocytes as a possible source of this cytokine. Interestingly, IL-12 p70 was expressed by numerous CD15\(^+\) polymorphonuclear leukocytes (Fig. 5I).

**Discussion**

UVB radiation modulates cytokine expression in the effector cells in human skin resulting in local and systemic immunosuppression\(^{23}\). This immunomodulatory effect is simultaneously advantageous and disadvantageous as it suppresses reactivity to self-antigens produced as a result of UV exposure, but on the other hand, may promote induction of skin cancer. Recent studies showed that the efficacy of UVB therapy in psoriasis could also be explained by this immunosuppressive effect. These studies demonstrated that UVB therapy leads not only to a decrease in the number of T cells invading the psoriatic skin, but also diminishes the predominance of the type 1 cytokine IFN-\(\gamma\) in the lesions\(^{15-17, 24}\). The presence of proinflammatory and type 1 cytokines depends on the availability of factors controlling their secretion and activity. The major inducers of IFN-\(\gamma\) are IL-12, IL-15, IL-18, IL-23 and IL-27, therefore it was not surprising to observe in this study that IL-12, IL-18 and IL-23 were abundantly expressed in psoriatic lesional skin. Unfortunately the expression of IL-27 could not be measured in these experiments due to a lack of available antibodies. The biologically active IL12 p70 heterodimer is formed by covalent binding of p40 and p35 subunits. In support of our results our findings on the expression of the common p40 subunit and the IL-12 p70 heterodimer, Yawalkar et al have also reported that expression of the p40 and IL-12 p70 in psoriatic lesional skin was higher compared to the expression in nonlesional and normal skin\(^{25}\). Moreover, they also demonstrated that the expression of IL-12 p70 heterodimer was mainly confined to dermal mononuclear cells, but not to keratinocytes. Our double staining experiments showed that the increased expression of IL-12 p70 in the psoriatic lesions takes place in dendritic cells, macrophages, and neutrophils. It has recently been shown that the p40 subunit of IL-12 and a p19 subunit form the heterodimeric cytokine IL-23, which has several functions in common with IL-12\(^{26}\). In the present study, we showed that both p40 and p19 subunits were abundantly expressed in psoriatic lesional skin which may give rise to the expression of biologically active IL-23. It was interesting to observe that the expression of these two subunits was confined to the epidermal cells and increased in lesional skin as compared to normal skin, suggesting that keratinocytes may have the ability to produce IL-23. Kopp et al showed
that p40 transgenic mice constitutively produce IL-23 (p19 / p40) in basal keratinocytes by cosecretion of transgenic p40 with endogenous p19, which could suggest that IL-23 may be important in the cutaneous inflammatory responses. Support for this suggestion comes from a recent report, in which an upregulation of p19 and p40 subunits in lesional dermis compared to nonlesional skin was demonstrated at the mRNA level, while the p35 subunit of IL-12 was not found to be increased. IL-23 has been shown to promote type 1 cytokine responses predominantly in memory T cells; because the majority of T cells found in psoriatic lesions are memory cells, it can be speculated that the increased expression of IL-23 in psoriatic lesions may be responsible, at least in part, for the overexpression of IFN-γ by memory T cells in these lesions. However, it is important to consider that, besides keratinocytes, T cells and neutrophils located in the lesional epidermis may contribute to the epidermal expression of cytokines. Because of the diffuse staining pattern we observed in the epidermis, except for IFN-γ staining, it is difficult to determine the contribution of each of these cell types to the epidermal cytokine expression.

Suppression of IL-12 expression by an anti-IL-12 antibody was shown to diminish the disease activity in a murine psoriasis-like skin disorder. Interestingly, IL-12 was found to be downregulated in UVB-irradiated normal skin, while its immunosuppressive effect was reversed by administration of IL-12. This suggests that the immunomodulatory effects of NB-UVB therapy could, at least partially, be achieved by a decrease in functional IL-12. In line with this, our results demonstrated a decrease of both the common IL-12 / IL-23 p40 subunit and the IL-12 p70 heterodimer in psoriatic lesions after treatment with NB-UVB.

Moreover, the p19 subunit of IL-23 significantly decreased in lesional skin after therapy. We can not exclude, however, that decreased numbers of inflammatory cells (such as T cells, dendritic cells and macrophages) in epidermis and dermis may have contributed to the decreased expression of IL-12 and IL-23 in the lesional skin after NB-UVB therapy. IL-18 is a cytokine resembling IL-1 and principally produced by macrophages, but also by keratinocytes in the skin. It has similar functions to IL-12, being an inducer of IFN-γ expression from T cells and natural killer cells. IL-18 was found to be increased in skin lesions and the peripheral blood of psoriasis patients. In our study, the upregulated expression of IL-18 in psoriatic lesional skin was found to be suppressed after NB-UVB therapy, predominantly in the epidermis. As this cytokine promotes type 1 cytokine responses, its UVB-induced suppression could be important in the decreased disease activity in psoriatic lesions. Previous reports have demonstrated that UVB irradiation in vitro does not alter IL-18 expression in Langerhans cells, but that it increases IL-18 production by
keratinocytes\textsuperscript{39}. However, these in vitro studies were performed on cells from normal skin which is not comparable to our in situ study on psoriatic lesions.

IL-15 has structural and functional similarities to IL-2. However, unlike IL-2, it is produced by numerous cell types\textsuperscript{20}. Expression of IL-15 in keratinocytes is enhanced by IFN-\(\gamma\)\textsuperscript{40} and, vice versa, leading to the classification of IL-15 as a proinflammatory type 1 cytokine\textsuperscript{41-43}. Since psoriatic lesions are dominated by type 1 cytokines, it is not surprising that the expression of IL-15 was reported to be higher in psoriatic skin lesions than in normal skin\textsuperscript{44}; the high expression of IL-15 and the IL-15 receptor in psoriatic lesional keratinocytes was suggested to give rise to juxtacrine signaling that results in decreased apoptosis, eventually leading to thickening of epidermis. More recently it was reported that IL-15 also has a direct stimulatory effect on keratinocytes proliferation\textsuperscript{45} and that blockage of IL-15 may be used as a therapy for psoriasis\textsuperscript{46}. Earlier studies concerning the effects of UV-radiation in IL-15 expression in normal skin revealed that the constitutive expression of IL-15 mRNA and protein in normal skin is upregulated after in vitro UVB and PUVA exposure\textsuperscript{47,48}. However, Blauvelt et al demonstrated that the IL-15 expression decreases in in vitro UVB-irradiated keratinocytes\textsuperscript{49}. Our study revealed that the therapeutic use of NB-UVB resulted in only a slight suppression of IL-15 expression in psoriatic lesions, suggesting that inhibition of IL-15 has a less prominent role in diminishing disease activity.

The biological impact of UVB-irradiation is mediated by several changes in the micromilieu of the human skin. In addition to the production of soluble factors, such as cytokines, the resident cells in the skin also show considerable changes: Langerhans cells and T cells are vulnerable to the apoptotic effects of UVB depending on the dose and type of radiation; in addition, Langerhans cells are known to exhibit different cell surface markers after UVB exposure as compared to the unexposed Langerhans cells resulting in a loss of activation of type 1, but not type 2, T cells\textsuperscript{50}. Moreover, immunocompetent cells such as neutrophils and macrophages invade the skin after UVB exposure and contribute to the immunosuppressive environment by production of cytokines such as IL-4 and IL-10\textsuperscript{14,23}. Although our present study focused on the suppression of type 1 cytokine responses in the UVB-treated skin, other UVB-induced changes in the cutaneous micromilieu can play roles in the therapeutic effect of UVB.

Altogether, our study clarifies some aspects of the mechanisms of decreased type 1 cytokine responses after NB-UVB therapy in psoriatic lesional skin. Suppression of the expression of IL-12, IL-18 and IL-23, which are all inducers of proinflammatory type 1 cytokine responses, could explain the decreased expression of IFN-\(\gamma\), an important cytokine mediating the
pathophysiological changes seen in psoriatic lesions. Future studies are needed to investigate whether the decreased expression of these cytokines is a direct effect of UVB radiation on the production capacity of the different cell types or just due to the decreased number of inflammatory cells producing these cytokines. Our results provide support to justify the use of newly developed cytokines and cytokine inhibitors in the treatment of psoriasis.

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References


37. Gangemi S, Merendino RA, Guarneri F, et al. Serum levels of interleukin-18...


41. Seder RA. High-dose IL-2 and IL-15 enhance the in vitro priming of naive CD4+ T cells for IFN-gamma but have differential effects on priming for IL-4. *J. Immunol.* 1996;156:2413-2422.


