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Effective Treatment of Psoriasis with Narrow-Band UVB Phototherapy Is Linked to Suppression of the IFN and Th17 Pathways

Emőke Rácz1,2, Errol P. Prens1,2, Dorota Kurek3, Marius Kant1,2, Dick de Ridder4, Sabine Mourits1,2, Ewout M. Baerveldt1,2, Zeliha Ozgur5, Wilfried F.J. van IJcken5, Jon D. Laman2, Frank J. Staal2 and Leslie van der Fits1,2

Narrow-band ultraviolet-B (NB-UVB) phototherapy is an effective treatment for psoriasis. The molecular mechanisms underlying its efficacy are incompletely understood. To identify NB-UVB-induced molecular pathways that may account for its anti-inflammatory efficacy, gene expression profiling was performed using epidermal RNA from lesional and nonlesional skin from patients with psoriasis undergoing NB-UVB therapy. Downregulation of Th17 signaling pathway was observed during NB-UVB therapy in psoriatic epidermis. Strong inhibition of the Th17 pathway by UVB was confirmed in an ex vivo organ culture system by demonstrating reduced signal transducer and activator of transcription 3 (STAT3) phosphorylation and β-defensin-2 production. These results were further substantiated by demonstrating that NB-UVB inhibited the Th17-dependent psoriasis-like dermatitis in mice. Other pathways affected by NB-UVB therapy include the IFN signaling pathway, epidermal differentiation, and other well-known therapeutic targets in psoriasis, such as the glucocorticoid, vitamin D, peroxisome proliferator-activated receptor, and IL-4 signaling pathways. In conclusion, clinical improvement of psoriasis by NB-UVB is linked to suppression of Th17 and type I and type II IFN signaling pathways, which are critical in the pathogenesis of the disease. Our results show that clinically effective NB-UVB therapy is based on suppression of a broad range of important molecular pathways in psoriatic skin.

Journal of Investigative Dermatology (2011) 131, 1547–1558; doi:10.1038/jid.2011.53; published online 17 March 2011

INTRODUCTION

Narrow-band ultraviolet-B (NB-UVB) phototherapy is a standard treatment for psoriasis. Its mechanism of action is incompletely understood and has mainly been studied in vitro and in mice.

Locally, NB-UVB therapy reverses several pathologic alterations in psoriasis: the number of epidermal T lymphocytes and dendritic cells (DCs) decrease during phototherapy (Ozawa et al., 1999; Walters et al., 2003; Carrascosa et al., 2007; Erkin et al., 2007). T cells exhibit a functional shift toward less IFN-γ and more IL-4-production after UVB treatment (Piskin et al., 2003; Piskin et al., 2004), and NB-UVB therapy reduces keratinocyte proliferation (Erkin et al., 2007). The molecular mechanisms of these NB-UVB effects are unknown. UVB radiation is mostly absorbed by the epidermis, which is considered the primary target of UVB action.

We used gene expression profiling in order to identify pathways and mechanisms that are involved in the anti-inflammatory effect of NB-UVB. In addition, the effects of NB-UVB were investigated in the recently described and, on the molecular level, well-characterized, imiquimod-induced psoriasis-like dermatitis mouse model (van der Fits et al., 2009).

To date, the transcriptomic effects of NB-UVB have only been examined in nonlesional skin samples of three patients with psoriasis (Hochberg et al., 2007). NB-UVB-induced differentially expressed genes included S100A proteins, DC markers, tumor necrosis factor-α target genes, matrix metalloproteinases, and NF-kB target genes. In addition, IGF-binding protein-7 was identified as an antiproliferative molecule with low expression in nonlesional psoriatic skin.
Th1 and Th17 Pathways Are Suppressed during UVB Therapy

which was induced by NB-UVB phototherapy, possibly counting for its antipsoriatic effect (Hochberg et al., 2007). We analyzed epidermal gene expression profiles in lesional and nonlesional skin of 11 patients with psoriasis taken before and after standard NB-UVB treatment. Additionally, in the same patients, the short-term molecular effects of NB-UVB were investigated. Specific pathways affected by NB-UBV in psoriasis patients were further examined in vitro and validated in a psoriasis-like dermatitis mouse model.

RESULTS

Clinical efficacy of NB-UVB therapy
The mean psoriasis area and severity index (PASI) reduction after standard NB-UVB therapy in all patients included in this study (n = 11) was 84.1% (range 58– 100%) (Supplementary Table S4 online). The mean PASI score decreased from 14.4 (range 10.0– 31.0) to 2.4 (range 0– 8.4). For microarray analysis, patient samples were pooled in two groups, each containing four patients (pool A and pool B, Supplementary Table S5 online). The mean PASI reduction was similar in these two groups of patients.

Correlation of gene expression profiles
The degree of similarity between global gene expression profiles was assessed using the OmniViz package (OmniViz, Maynard, MA). Expression profiles of the two pools of patient samples were similar for all conditions, demonstrating valid duplicate measurements.

Two major clusters of samples were identified on the basis of strong similarities in gene expression (Figure 1). Lesional skin biopsies taken before therapy formed the first cluster. The second larger cluster could be divided into three smaller subclusters (Figure 1) that are formed by combinations of lesional skin biopsies taken at 50% PASI reduction, or at the end of the treatment, together with nonlesional biopsies (Figure 1). These second three subclusters were quite similar to each other, indicating that gene expression in lesional skin taken at 50% PASI reduction and at the end of treatment was highly similar to that of nonlesional skin. Thus, NB-UVB therapy induced a shift in gene expression in lesional skin toward that of nonlesional skin.

Differences between untreated lesional and nonlesional epidermis
Gene expression profiles of untreated psoriasis lesions were compared with untreated nonlesional skin. In lesional skin, 251 genes were upregulated and 383 genes downregulated compared with nonlesional skin, similarly to previously reported profiles in untreated lesional and nonlesional psoriatic skin (Bowcock et al., 2001; Oestreicher et al., 2001; Zhou et al., 2003; Kulski et al., 2005; Quekenborn-Trinquet et al., 2005; Mee et al., 2007; Reischl et al., 2007; Yao et al., 2008; Gudjonsson et al., 2010a, b), although these studies extracted RNA from whole skin biopsies whereas we exclusively analyzed epidermal RNA. The genes upregulated in lesional skin included members of the type I and type II IFN signaling pathway, Th17 pathway, innate cytokine genes, and other immune response genes; epidermal differentiation-associated genes, and proteinases such as kallikreins and cathepsin C. Genes expressed higher in nonlesional skin were involved in cell proliferation, cell cycle progression, and epidermal differentiation.

Genes affected by NB-UVB phototherapy in lesional and nonlesional epidermis
The genes up- or down-regulated in lesional and nonlesional skin after 12 weeks of NB-UVB therapy are shown in Supplementary Tables S1– S3 online. There was only a marginal overlap between genes regulated in lesional and nonlesional skin by NB-UVB. Five of these were downregulated (S100A9, keratin 6, z-actinin, STEAP4, and ets homologous factor), and one, betacellulin, upregulated.

Transcriptome analysis of psoriatic lesions showed that NB-UVB strongly suppressed genes of the Th17 and IFN signaling pathways and members of the epidermal differentiation complex (Table 1). The functional annotation of the differentially expressed genes in lesional skin before versus after NB-UVB therapy is shown in Supplementary Figure S1 online. The expression of immune response genes and of genes involved in antiviral responses and stress responses was suppressed by NB-UVB therapy, whereas genes involved in epidermis development, extracellular matrix components, and negative regulators of biological processes were induced.

NB-UVB therapy affects recognized therapeutic targets in psoriasis
Ingenuity Pathway Analysis software (Ingenuity Systems, www.ingenuity.com) was additionally used to identify

Figure 1. Correlation view of expression profiles of the different epidermal RNA samples. The degree of similarity between global gene expression profiles was assessed using the OmniViz package. The red squares indicate positive pairwise correlations (equality in gene expression between clusters) and blue squares indicate negative pairwise correlations. A, B, patient pools; D, sample taken at 50% psoriasis area and severity index (PASI) reduction; E, sample taken after completion of narrow-band ultraviolet-B (NB-UVB) therapy; L, lesional samples; NL, nonlesional samples; T0, sample taken before the first irradiation.
over-represented signaling pathways that were affected by NB-UVB therapy in psoriasis lesions. As shown in Table 2, we found that NB-UVB regulated pathways that are:

1. recognized therapeutic targets in psoriasis, such as glucocorticoid receptor and vitamin D receptor signaling.
2. previously identified as potential therapeutic targets in psoriasis, such as peroxisome proliferator-activated receptor and IL-4 and IL-10 signaling (Asadullah et al., 2003; Ghoreschi et al., 2003; Martin, 2003; Robertshaw and Friedmann, 2005; Sertznig et al., 2008; Docke et al., 2009).
3. described as targets of UV radiation in normal skin and that are simultaneously involved in the pathogenesis of psoriasis, such as NF-κB, mitogen-activated protein kinase, p53, and IGF-1 signaling.

Successful NB-UVB treatment of psoriasis is associated with downregulation of the Th17 pathway in lesional skin

Among the genes most downregulated during NB-UVB phototherapy in lesional skin were many known members of the Th17 pathway (Figure 2a). From these, S100A9 and β-defensin-2 were selected to validate the microarray data. Using mRNA of individual patients, we confirmed the

<table>
<thead>
<tr>
<th>Th17 pathway</th>
<th>Name</th>
<th>Symbol</th>
<th>Fold change at PASI 50%</th>
<th>Fold change at the end of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>Average</td>
<td>Pool A</td>
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<tr>
<td>S100 calcium binding protein A7</td>
<td>S100A7</td>
<td>30.2</td>
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<td>1.7</td>
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<tr>
<td>Defensin, β-2</td>
<td>DEFB4</td>
<td>28.2</td>
<td>1.8</td>
<td>1.7</td>
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<td>Peptidase inhibitor 3, skin-derived (SKALP)</td>
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<td>26.3</td>
<td>4.1</td>
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<td>Keratin 16</td>
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<td>37.0</td>
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<td>3.1</td>
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<tr>
<td>IL 8</td>
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<td>6.0</td>
<td>12.4</td>
<td>3.4</td>
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IFN signaling

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<th>Fold change at the end of treatment</th>
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<tr>
<td></td>
<td></td>
<td>Average</td>
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<td>S100A7</td>
<td>30.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Defensin, β-2</td>
<td>DEFB4</td>
<td>28.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Peptidase inhibitor 3, skin-derived (SKALP)</td>
<td>SKALP</td>
<td>8.9</td>
<td>26.3</td>
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<td>S100 calcium binding protein A9</td>
<td>S100A9</td>
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<td>17.2</td>
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<td>S100 calcium binding protein A12</td>
<td>S100A12</td>
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<td>Keratin 16</td>
<td>KRT16</td>
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<td>Lipocalin 2 (oncogene 24p3)</td>
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<td>37.0</td>
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<td>Serpin peptidase inhibitor, clade B (ovalbumin), member 3</td>
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<tr>
<td>IL 8</td>
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<td>12.4</td>
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Epidermal differentiation

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</thead>
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<td>Keratin 15</td>
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<td>3.1</td>
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<tr>
<td>Transglutaminase K</td>
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<td>1.9</td>
<td>1.7</td>
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</table>

Abbreviations: NB-UVB, narrow-band ultraviolet-B; NS, statistically not significant; PASI, psoriasis area and severity index. ▼ indicates downregulation; ▲ indicates upregulation.
downregulation of S100A9 by quantitative real-time PCR (RT-PCR; Figure 2b). No clearcut correlation between the S100A9 mRNA levels and the individual PASI scores was observed (Pearson’s correlation test, data not shown). Downregulation of β-defensin-2 protein expression during NB-UVB phototherapy in lesional psoriatic skin was confirmed by immunofluorescent staining (Figure 2c).

To examine whether UVB has an early local effect (not requiring systemic involvement or an associated immunologic network) on the Th17 pathway, or whether its downregulation is a consequence of the improvement of psoriasis, in vitro experiments were performed. Skin explants were stimulated with the Th17-associated cytokine IL-22, which is known to induce a psoriasis-like phenotype in reconstituted human epidermis, including induction of acanthosis, antimicrobial peptides such as human β-defensins, and activation of signal transducer and activator of transcription 3 (STAT3; Sa et al., 2007; Eyerich et al.,

<table>
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<tr>
<th>Pathway</th>
<th>P-value</th>
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<th>Downregulated</th>
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<td>Glucocorticoid receptor signaling</td>
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<td>IL8, STAT3, STAT1</td>
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<td>Proposed as therapeutic target in psoriasis</td>
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<td>IL-4 signaling</td>
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<td>Other</td>
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<td>GM-CSF signaling</td>
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<td>ALDH1A3, UGT1A9, HS3ST3A1</td>
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<td>FcγRI signaling</td>
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2009). NB-UVB irradiation of IL-22-stimulated skin biopsies blocked IL-22-induced STAT3 phosphorylation (Figure 2d and e). In addition, the IL-22-induced expression of human β-defensin-2 in the epidermis (Liang et al., 2006; Sa et al., 2007) was also drastically inhibited by NB-UVB (Figure 2f).

Taken together, we demonstrate that improvement of psoriasis by NB-UVB is accompanied by suppression of the Th17 pathway in the psoriatic epidermis, and that in vitro already a single NB-UVB dose leads to suppression of the epidermal effects of the Th17 cytokine IL-22.

Successful NB-UVB therapy of psoriasis is accompanied by downregulation of the IFN signaling pathway in the epidermis. In psoriasis lesions, members of the IFN signaling pathway and several IFN-inducible genes were downregulated during NB-UVB therapy (Figure 3a). We selected the type I IFN-inducible cytoplasmic RNA receptor IFIH1/MDA5 (IFN induced with helicase C domain 1/melanoma differentiation associated gene 5) to validate the microarray data using mRNA of individual patients, including skin biopsies taken at 50% PASI score reduction. Error bars indicate SEM, n = 4 patients.

Normal skin biopsies were obtained from healthy volunteers. Biopsies were cultured with or without recombinant human IL-22 (50 ng ml⁻¹) in the culture medium. After 16 hours in culture, biopsies were irradiated with a single NB-UVB dose of 600 mJ cm⁻². The cultured biopsies were collected 6 hours after UV irradiation. Phosphorylated signal transducer and activator of transcription 3 (STAT3) staining of the collected biopsies was performed using a monoclonal phospho-STAT3 antibody. Phospho-STAT3-positive cells in the epidermis were counted, and represented as mean ± SEM (n = 4) (e) and a representative example is shown (d). Scale bar for panel d = 20 µm. (f) Epidermal β-defensin-2 mRNA expression was measured by real-time PCR (RT-PCR). ABL1 was used as an internal control gene. Error bars indicate SEM, n = 4 subjects.

Figure 2. Successful narrow-band ultraviolet-B (NB-UVB) therapy of psoriasis is associated with suppression of the Th17 pathway. (a) Th17 pathway. Gray indicates gene downregulation. (b) Epidermal S100A9 (S100 calcium binding protein A9) mRNA expression was measured by real-time PCR (RT-PCR). ABL1 (Abelson murine leukemia viral (v-abl) oncogene homolog 1) was used as an internal control gene. Black bars show lesional and gray bars show nonlesional psoriatic samples; psoriasis area and severity index (PASI) 50% is sample taken at 50% PASI score reduction. Error bars indicate SEM, n = 4 patients. (c) β-Defensin-2 in psoriasis during NB-UVB therapy, as determined by immunofluorescent staining with a monoclonal anti-β-defensin-2 antibody in lesional skin biopsies taken before, during, and after NB-UVB therapy. Scale bar = 200 µm. (d–f) A single NB-UVB irradiation counteracts the effects of IL-22 in vitro. Normal skin biopsies were obtained from healthy volunteers. Biopsies were cultured with or without recombinant human IL-22 (50 ng ml⁻¹) in the culture medium. After 16 hours in culture, biopsies were irradiated with a single NB-UVB dose of 600 mJ cm⁻². The cultured biopsies were collected 6 hours after UV irradiation. Phosphorylated signal transducer and activator of transcription 3 (STAT3) staining of the collected biopsies was performed using a monoclonal phospho-STAT3 antibody. Phospho-STAT3-positive cells in the epidermis were counted, and represented as mean ± SEM (n = 4) (e) and a representative example is shown (d). Scale bar for panel d = 20 µm. (f) Epidermal β-defensin-2 mRNA expression was measured by real-time PCR (RT-PCR). ABL1 was used as an internal control gene. Error bars indicate SEM, n = 4 patients.
During NB-UVB therapy induction of normal epidermal differentiation was observed

Many genes that are linked to terminal epidermal differentiation were affected during NB-UVB therapy according to our microarray (Table 1). Corneodesmosin gene was selected to validate the microarray results by RT-PCR using mRNA from individual patients. Corneodesmosin mRNA expression was significantly decreased during NB-UVB therapy (Figure 3d), whereas no clearcut correlation between PASI score and CDSN mRNA levels could be observed in the individual patients (Pearson’s correlation test, data not shown). Microarray data were also validated by demonstrating a decreased protein expression of keratinocyte transglutaminase, an enzyme important in epidermal differentiation, after NB-UVB therapy (Figure 3e).

Th17 and epidermal differentiation are important target pathways of NB-UVB in the psoriasiform dermatitis model in mice

Imiquimod-induced psoriasiform dermatitis in mice is mediated via the Th17 pathway (van der Fits et al., 2009). We investigated whether NB-UVB affected this type of skin inflammation. In imiquimod-induced psoriasiform dermatitis erythema, scaling and thickness were clearly reduced after NB-UVB irradiation (Figure 4d). Epidermal thickness, the number of CD11c+ DCs, the number of epidermal neutrophils, and angiogenesis were all reduced in imiquimod plus NB-UVB-treated mouse skin when compared with those treated with imiquimod alone (Figure 4a–c). Thus, as in human psoriasis, NB-UVB therapy resulted in a clear improvement of the psoriasiform skin inflammation in mice.
as assayed by clinical, histological, and immunohistochemical parameters.

To determine whether NB-UVB affects the Th17 pathway and epidermal differentiation in this murine psoriasiform model, we performed immunofluorescent staining for STAT3 activation, and for keratin 1/10. NB-UVB treatment induced a clear reduction in phosphorylated STAT3 and induced the expression of keratin 1/10 (epidermal orthodifferentiation; Figure 4e-g).

Figure 4. Improvement of the imiquimod-induced psoriasiform dermatitis in mice by narrow-band ultraviolet-B (NB-UVB) is accompanied by a reduction of epidermal phospho-signal transducer and activator of transcription 3 (STAT3) staining and by induction of normal differentiation. BALB/c mice were treated daily with IMQ cream or control cream on the shaved back skin, and irradiated or sham-irradiated every other day with NB-UVB, starting on the first day of imiquimod treatment. (a–c) Mice were killed on day 6. Imiquimod-induced inflammation was studied on sections made from the back skin of the mice. Immunohistochemical staining of myeloid dendritic cells (CD11c, a), granulocytes (GR1, b), and endothelial cells (MECA-20, c) is shown. Scale bars = 100 μm. (d) Erythema, scaling, and thickness of the back skin were scored daily on a scale from 0 to 4. The cumulative score (erythema plus scaling plus thickness) is shown. Symbols indicate mean score ± SEM of three mice per group. (e) The number of phospho-STAT3-positive cells were counted in three photos made of different sections by two independent researchers, and are shown relative to the total number of epidermal cells per section. Error bars indicate the SEM. (f) Immunofluorescent staining for phosphorylated STAT3 of the back skin of the mice. Scale bar = 20 μm. (g) Keratin 1/10 immunofluorescent staining of the back skin is shown. Scale bar = 100 μm.
Thus, as observed in patients with psoriasis, NB-UVB suppresses the psoriasiform dermatitis in mice, which is accompanied by downmodulation of the Th17 pathway and by induction of epidermal orthodifferentiation.

The first NB-UVB dose has minor effects in lesional skin after 6 hours, whereas in nonlesional skin marked gene expression changes are observed

To study the short-term effects of NB-UVB on gene expression in psoriatic skin, skin biopsies were taken before and 6 hours after the first irradiation. In lesional skin, only three genes (CYP1B1 (cytochrome P450, subfamily 1, polypeptide 1), periostin, and cryptochrome 1) were significantly (>2-fold) upregulated 6 hours after the first irradiation. Of these, the upregulation of periostin and cryptochrome persisted in lesional skin after 3 months of treatment. Assessment of cryptochrome 1 expression by RT-PCR using RNA samples from individual patients confirmed the microarray results (Figure 5a).

In contrast, in nonlesional skin, the same NB-UVB dose modulated the expression of 272 genes >2-fold, 6 hours after the first irradiation, whereas none of these were affected in lesional skin.

To investigate whether the limited response to NB-UVB in lesional epidermis was because of incomplete penetration of NB-UVB into the thickened psoriatic epidermis, immunostaining for cyclobutane pyrimidine dimers (CPDs) as marker of UV damage was performed on lesional and nonlesional biopsies taken before and 15 minutes after the first UV irradiation (70% minimal erythema dose). Robust CPD positivity was observed in almost all epidermal layers of the psoriatic plaque (Figure 5b and c). Thus, the relatively small number of genes affected by a single NB-UVB dose in lesional skin is not because of the lack of penetration of NB-UVB in lesional epidermis.

The short-term response to NB-UVB was also evaluated in lesional and nonlesional skin at 50% clinical improvement. At this time point, NB-UVB modulated the expression of 65 genes in lesional and 63 genes in nonlesional skin. There was, again, a limited overlap (11 genes).

**NB-UVB and ustekinumab induce different molecular changes in nonlesional psoriatic skin**

The importance of the Th1/Th17 pathways and the abnormal epidermal differentiation in the pathogenesis of psoriasis is well known. In order to compare the effects of two different systemic therapies on epidermal gene expression profiles, the expression of selected genes of the Th1, Th17, and epidermal differentiation pathways was analyzed in nonlesional epidermis of patients with psoriasis treated with the anti-IL-12/23 p40 (ustekinumab). Nonlesional skin biopsies were collected before treatment and at 50% PASI score reduction, whereby RNA was extracted from the separated epidermis. RT-PCR analysis of the expression of β-defensin-2, S100A7, IFIH1/MDA5, and GATA3 (GATA binding protein 3) showed that the molecular effects of ustekinumab in the epidermis were different from that of NB-UVB therapy (Figure 6).

**DISCUSSION**

Our results show that clinically effective NB-UVB therapy is associated with suppression of type I and type II IFN signaling, downmodulation of the Th17 pathway, and modulation of genes involved in epidermal differentiation in lesional psoriatic epidermis.

IL-23 and the Th17 cytokines IL-17, IL-22, and IL-21 are key cytokines in the pathogenesis of psoriasis (Blauvelt, 2008; Nograles et al., 2010). IL-23 is overproduced in psoriatic lesions, and this cytokine stimulates Th17 cells to produce Th17 cytokines (Lee et al., 2004; Piskin et al., 2006; Wilson et al., 2007). The prototypic Th17 cytokine IL-22 is a potent stimulator of keratinocyte proliferation and of the production of antimicrobial peptides, thereby representing a key effector cytokine in the pathogenesis of psoriasis (Wolk et al., 2006; Sa et al., 2007; Nograles et al., 2008; Eyerich et al., 2009). Polymorphisms in genes encoding IL-23 receptor and IL12/IL23p40 are associated with susceptibility to psoriasis (Cargill et al., 2007; Nair et al., 2008). Biologics targeting IL12/IL23p40 are highly effective in psoriasis (Krueger et al., 2007). The Th17 pathway is also rapidly inhibited in psoriatic skin during treatment with cyclosporine A and etanercept (Zaba et al., 2007). Cyclosporine A modulation of this pathway is observed within 2 weeks after the start of treatment and correlates well with the clinical improvement (Haider et al., 2008).

The suppressive effects of NB-UVB therapy on the IL-23/IL-17 axis in psoriasis have recently been shown by immunohistochemistry and RT-PCR analysis of lesional skin (Johnson-Huang et al., 2010). Suppression of the IL-23/IL-17
Th1 and Th17 Pathways Are Suppressed during UVB Therapy

axis was only observed in clinical responders (Johnson-Huang et al., 2010). Collectively, these results indicate that the in vivo downregulation of the Th1 and Th17 pathways is achievable via intervention in different specific molecular pathways (tumor necrosis factor, calcineurin, costimulatory adhesion molecules, and UV receptors) and that it is crucial for the clinical efficacy of antipsoriatic treatments.

In our study, expression of Th17 pathway genes was suppressed in both lesional and nonlesional skin by NB-UVB therapy. NB-UVB irradiation inhibited the Th17/IL-23-dependent psoriasiform dermatitis in mice, and this was accompanied by decreased STAT3 phosphorylation in the epidermis. Inhibition of the Th17 pathway was further confirmed in skin explants where NB-UVB inhibited the IL-22-induced phosphorylation of STAT3 and the induction of β-defensin-2, at 6 hours after irradiation. This demonstrates that NB-UVB irradiation exerts a rapid inhibitory effect on the Th17 pathway. The exact mechanism of STAT3 inhibition by NB-UVB has yet to be determined. In primary human keratinocytes, inhibition of STAT3 activation by UVB was reversed by vanadate, a general inhibitor of tyrosine phosphatases, indicating the involvement of at least a tyrosine phosphatase in UVB-induced STAT3 inhibition (Sano et al., 2005). Interestingly, STAT3 phosphorylation is also inhibited by the reactive oxygen species-inducer manumycin in cancer cells (Dixit et al., 2009). As reactive oxygen species are also generated during NB-UVB irradiation, they might also be involved in the inhibition of STAT3 phosphorylation by NB-UVB. Nevertheless, the link between the immediate biological effects of UVB via its cellular receptors (DNA, reactive oxygen species, membrane changes, aryl-hydrocarbon receptor, and inflammasome) and its antipsoriatic/anti-inflammatory effects has yet to be identified.

Type I IFNs are mainly produced by plasmacytoid DCs in autoimmune diseases such as systemic lupus erythematoses (Banchereau and Pascual, 2006), and during the innate immune response to viral infections. Type I IFNs are critical in the pathogenesis of psoriasis (Funk et al., 1991; Pauluzzi et al., 1993; Downs and Dunnill, 2000; van der Fits et al., 2004; Ketikoglou et al., 2005). Treatment of patients with pre-existing psoriasis or with a familial predisposition for psoriasis with IFN-α can induce or exacerbate the disease (Funk et al., 1991; Pauluzzi et al., 1993; Downs and Dunnill, 2000; Ketikoglou et al., 2005). We have previously shown that the type I IFN pathway is activated in lesional psoriatic skin (van der Fits et al., 2004). The pathophysiological relevance of type I IFN was demonstrated in a xenograft murine model of psoriasis, where blocking of IFN-α prevented the development of psoriatic lesions in transplanted nonlesional skin (Nestle et al., 2005). IFN-γ, a type II IFN, is mainly produced by activated Th1 cells present in psoriatic lesions and it has been shown to induce the regenerative psoriatic phenotype in healthy skin (Wei et al., 1999). NB-UVB is known to deplete hematopoietic cells such as T lymphocytes and Langerhans cells from the skin (Ozawa et al., 1999; DeSilva et al., 2008). It is conceivable that the observed inhibition of both type I and type II IFN signaling pathways are because of depletion or inhibition of lymphocytes and plasmacytoid DCs from psoriatic lesions by NB-UVB.

Psoriatic lesions show several signs of altered epidermal differentiation clinically and histologically (Tscharcler, 2007). The importance of keratinocyte differentiation in the pathogenesis of psoriasis is highlighted by the fact that genetic alterations in the epidermal differentiation complex on chromosome 1q21 (de Cid et al., 2009; Zhang et al., 2009) and around the corneodesmosin gene on chromosome 6p21 (Orru et al., 2005) are closely associated with psoriasis. NB-UVB phototherapy drives the expression of epidermal differentiation-associated genes in the psoriatic lesions toward the expression observed in normal, healthy skin.

A surprising finding in our study was that at 6 hours after the first UV irradiation, many more genes were differentially
Th1 and Th17 Pathways Are Suppressed during UVB Therapy

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PATIENTS AND METHODS

Patients and treatments

A total of 11 patients with psoriasis were recruited. All participants gave written informed consent. The Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam, The Netherlands, approved this study under the registration number 234.237/2003/210. Patients (10 men and 1 woman, aged 20–73 years) were recruited. All of them gave written informed consent. The Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam, The Netherlands, approved this study under the registration number 104.050/SPO/1990/30. Patients were treated with ustekinumab (Stelara, Janssen-Cilag BV, Tilburg, The Netherlands), a monoclonal antibody, and at weeks 4 and 16.

Biopsy samples, RNA extraction, and microarray analysis

Biopsies (3 mm) were taken from psoriasis lesional and nonlesional skin before the start of NB-UVB therapy and after the last treatment session. To investigate the short-term effects of UVB, biopsies were taken 6 hours after the first irradiation. When PASI scores were reduced to 50% of the baseline score, additional biopsies from lesional and nonlesional skin were taken before and 6 hours after UVB irradiation. For CPD immunohistochemistry, lesional and nonlesional skin biopsies were taken from three patients before and 15 minutes after the first treatment session of NB-UVB phototherapy. From patients treated with ustekinumab, 3-mm biopsy samples were taken from nonlesional skin before the start of the treatment and at 50% PASI score reduction. For details on RNA extraction, see Supplementary Material online, as well as for details on array hybridization and analysis.

Pathway analysis

The lists of genes differentially expressed in psoriasis lesions before and after NB-UVB therapy were subjected to Ingenuity Pathway Analysis (Ingenuity Systems) to identify signaling pathways represented by these genes. In addition, functional annotation of these genes was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID; Dennis et al., 2003).

Immunohistochemistry

To validate the microarray results at the protein level, immunostainings were performed on biopsies from psoriasis lesions as described previously (van der Fits et al., 2004). For immunofluorescent staining, cryosections were fixed for 10 minutes in 4% paraformaldehyde in phosphate-buffered saline. Before staining with anti-CPD antibody, DNA was denatured using 0.07 M NaOH in 70% ethanol, and slides were preincubated with 5% normal rabbit serum in phosphate-buffered saline with 0.1% BSA. Primary antibodies and the applied dilutions are shown in Supplementary Table S7 online. Relevant FITC-, Cy5-, or TxR-conjugated antibodies (1:100; Abcam, Cambridge, UK) were used to detect primary antibodies. All fluorescent images were made with an Axio Imager (Carl Zeiss BV, Sliedrecht, The Netherlands) fluorescence microscope.

Skin organ culture

Normal skin biopsies were obtained from healthy volunteers undergoing breast surgery in the Department of Plastic Surgery of the Sint Franciscus Gasthuis, Rotterdam, The Netherlands, after shaving back skin for 5 days. Every other day, starting on the first day of the experiment, mice were irradiated with a Waldmann irradiation device equipped with TL-01 UV 236-01 lamps (Waldmann Medizintechnik, Villingen-Schwenningen, Germany), or were sham irradiated. The applied UV dose was 70% of the minimal erythema dose on the first day and it was increased each time by 10%. Scoring of the severity of skin inflammation was performed as described previously (van der Fits et al., 2009). On the sixth day, mice were killed and 3-mm biopsy samples were taken from the back skin. Details on the determination of the minimal erythema dose in mice are provided in the Supplementary Material online. All animal work was approved by the animal ethical committee of the Erasmus University Medical Center Rotterdam, The Netherlands, under approval number DEC EUR 851 (OZP 128-06-07).

Mice and treatments

Induction of skin inflammation by daily imiquimod application in BALB/c mice was performed as described previously (van der Fits et al., 2009). Briefly, mice were treated daily with imiquimod on the shaved back skin for 5 days. Every other day, starting on the first day of the experiment, mice were irradiated with a Waldmann irradiation device equipped with TL-01 UV 236-01 lamps (Waldmann Medizintechnik, Villingen-Schwenningen, Germany), or were sham irradiated. The applied UV dose was 70% of the minimal erythema dose on the first day and it was increased each time by 10%. Scoring of the severity of skin inflammation was performed as described previously (van der Fits et al., 2009). On the sixth day, mice were killed and 3-mm biopsy samples were taken from the back skin. Details on the determination of the minimal erythema dose in mice are provided in the Supplementary Material online. All animal work was approved by the animal ethical committee of the Erasmus University Medical Center Rotterdam, The Netherlands, under approval number DEC EUR 851 (OZP 128-06-07).

In conclusion, clinically effective NB-UVB therapy is associated with downregulation of the critical Th17, type I, and type II IFN signaling pathways and genes involved in keratinocyte differentiation in lesional psoriatic skin. In addition, several anti-inflammatory pathways, such as glucocorticoid, vitamin D, peroxisome proliferator-activated receptor, and IL-4 signaling, are modulated by NB-UVB therapy. Our data underscore the importance of these pathways in the pathogenesis of psoriasis and identify them as targets of NB-UVB in the resolution of psoriatic inflammation.

PATIENTS AND METHODS

Patients and treatments

A total of 11 patients with psoriasis were recruited. All participants gave written informed consent. The Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam, The Netherlands, approved this study under the registration number 234.237/2003/210. Patients (10 men and 1 woman, aged 20–73 years) had PASI scores of at least 10 (Supplementary Table S4 online) and did not receive systemic therapy for at least 1 month or topical therapy for at least 2 weeks before the start of the study. For treatment specifications, see Supplementary Material online.

In a parallel unrelated study, 8 patients with psoriasis (6 women, 5 men, mean age 49 years, range 29–71 years) were recruited. All of them gave written informed consent. The Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam, The Netherlands, approved this study under the registration number 104.050/SPO/1990/30. Patients were treated with ustekinumab (Stelara, Janssen-Cilag BV, Tilburg, The Netherlands), a monoclonal antibody, and at weeks 4 and 16.

The study was conducted according to the Declaration of Helsinki Principles.
Informed consent. Biopsies were cultured as described previously (Companjen et al., 2001). Recombinant human IL-22 (50 ng ml⁻¹; R&D Systems, Abingdon, UK) was added to the culture medium, and 16 hours later biopsies were irradiated with NB-UVB using a small Waldmann irradiation device equipped with TL-01 UV 236-01 lamps (Waldmann Medizintechnik). In all experiments, a single NB-UVB dose of 600 mJ cm⁻² was used, representing a dose that is usually reached within 3–9 clinical UVB therapy sessions (1–3 weeks of clinical treatment). Biopsies were collected 6 hours after UV irradiation. One of the four biopsies was snap-frozen, whereas the epidermis of the three other biopsies was separated from the dermis and total messenger RNA was isolated as described above.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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