Differential Expression of Cytokines in UV-B–Exposed Skin of Patients With Polymorphous Light Eruption

Correlation With Langerhans Cell Migration and Immunosuppression

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Background: Disturbances in UV-induced Langerhans cell migration and T helper (Th) 2 cell responses could be early steps in the pathogenesis of PLE.

Objective: To establish whether UV-B exposure induces aberrant cytokine expression in the uninvolved skin of patients with polymorphous light eruption (PLE).

Design: Immunohistochemical staining and comparison of microscopic sections of skin irradiated with 6 times the minimal dose of UV-B causing erythema and the unirradiated skin of patients with PLE and of healthy individuals.

Setting: University Medical Center (Dutch National Center for Photodermatoses).

Patients: Patients with PLE (n=6) with clinically proven pathological responses to UV-B exposure and normal erythematous sensitivity. Healthy volunteers (n=5) were recruited among students and hospital staff.

Main Outcome Measures: Expression of cytokines related to Langerhans cell migration (interleukin [IL] 1, IL-18, and tumor necrosis factor [TNF]-α; Th2 responses (IL-4 and IL-10); and Th1 responses (IL-6, IL-12, and interferon γ). Double staining was performed for elastase (neutrophils), tryptase (mast cells), and CD36 (macrophages).

Results: The number of cells expressing IL-1β and TNF-α was reduced in the UV-B–exposed skin of patients with PLE compared with the skin of healthy individuals (P<.05 for TNF-α). No differences were observed in the expression of Th1-related cytokines but fewer cells expressing IL-4 infiltrated the epidermis of patients with PLE 24 hours after irradiation (P = .03). After UV exposure TNF-α, IL-4, and, to a lesser extent, IL-10 were predominantly expressed by neutrophils.

Conclusions: The reduced expression of TNF-α, IL-4, and IL-10 in the UV-B–irradiated skin of patients with PLE appears largely attributable to a lack of neutrophils, and is indicative of reduced Langerhans cell migration and reduced Th1 skewing. An impairment of these mechanisms underlying UV-B–induced immunosuppression may be important in the pathogenesis of PLE.

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Ultraviolet Radiation (UV), especially UV-B radiation (280-315 nm), can modify proteins and other organic molecules that may act as neoantigens to provoke an autoimmune reaction.1,2 This adverse immune reaction can be suppressed by another effect of UV radiation, ie, the induction of cellular immunosuppression.3,4 A defect in this immunosuppression might therefore allow an adverse immune reaction to UV radiation, and polymorphous light eruption (PLE) could thus arise.

Key players in the UV-induced modulation of the skin immune system are cytokines. UV-B radiation can, directly or indirectly, induce the release of cytokines in the skin, resulting in the efflux or influx of several cell types. Interleukin (IL) 1β, tumor necrosis factor (TNF)-α, and IL-18 release, for instance by keratinocytes, can induce Langerhans cell migration out of the skin5,7; and IL-10 is also involved in Langerhans cell migration, although it is still not clear whether it enhances8 or impairs the migration.9

Concurrently with the depletion of Langerhans cells after UV exposure, macrophages expand in the dermis and infiltrate the epidermis.10 Kang et al11,12 showed that these CD11b+ cells produce the immunosuppressive cytokine IL-10. Not only IL-10 from macrophages but also IL-10 from keratinocytes13 and TNF-α from dermal mast cells14 contribute to an immunosuppressive environment in the UV-exposed skin.15 Additionally, IL-4 was shown to be involved in the UV-induced suppression of delayed-type hypersensitivity16 and contact hypersensitivity.17
The development of a T<sub>H</sub>2 milieu in the UV-B–exposed skin is supported by the influx of IL-4–expressing neutrophils and by the production of IL-10, which counteracts IL-12 activity and inhibits the activation of T<sub>H</sub>1 cells but allows activation of T<sub>H</sub>2 cells. The production of IL-12 by monocytes/macrophages is decreased after UV-B irradiation, as is the production of IL-10, the natural antagonist of IL-12, by keratinocytes and Langerhans cells is increased after UV exposure. Most importantly, a decrease in IL-12 activity and an increase in IL-4 and IL-10 activity favors the differentiation of T<sub>H</sub>0 cells into T<sub>H</sub>2 cells.

As a whole, the cytokine balance in the UV-exposed skin is shifted toward an immunosuppressive reaction in healthy individuals.

One of the cellular mechanisms related to this immunosuppression is the disappearance of Langerhans cells. In previous experiments, we showed that Langerhans cells persisted in the skin of patients with PLE after UV-B overexposure, in contrast to what was observed in healthy individuals. The main mechanism responsible for Langerhans cell depletion in healthy individuals is migration, found to be impaired in patients with PLE. Only when patients with PLE were exposed to a larger UV dose did Langerhans cells disappear from the epidermis, thus revealing a disturbance in the balance between Langerhans cell migration and the erythemal response.

In the context of a potential defect in UV-induced immunosuppression in patients with PLE, these observations evolve into 2 hypotheses. First, the expression of the cytokines involved in Langerhans cell migration (eg, IL-1 and TNF-α) is reduced in the skin of patients with PLE. Second, we speculate that patients with PLE show an imbalance between proinflammatory and T<sub>H</sub>1-skewing cytokines (eg, IL-12 and IFN-γ), and the cytokines involved in T<sub>H</sub>2-skewing and T<sub>H</sub>1 suppression (eg, IL-4 and IL-10), ultimately shifting the balance toward proinflammatory and T<sub>H</sub>1-skewed immune responses in the skin.

To test these hypotheses, unaffacted buttock skin of 6 patients with PLE and 5 healthy volunteers was overexposed to UV-B. Skin biopsy specimens were obtained from the UV-B–exposed skin 24 hours and 48 hours after irradiation, and from the unexposed skin as well as control specimens. Immunohistochemical staining was performed on frozen skin sections for a series of cytokines to locate and identify cytokine-expressing cells.

**METHODS**

**SUBJECTS**

Six patients with PLE and normal sunburn sensitivity (1 man and 5 women aged between 32 and 64 years) and 3 healthy volunteers (2 men and 3 women aged between 19 and 21 years) participated in the study. Skin types ranged from type I to type III in both groups. In previous studies we found fewer Langerhans cells in the skin of individuals older than 60 years, with a somewhat lessened depletion of Langerhans cells after UV irradiation. We did not find any age dependence with respect to epidermal Langerhans cell density in healthy volunteers aged between 19 and 55 years, nor did we find a diminished Langerhans cell migration in this age range. In this study the diagnosis of PLE was based on patients' history and clinical photoprovocation with daily exposure of UV-A 1, UV-B, and visible light on restricted areas of the upper arms. All recruited patients developed papules or vesicles in the UV-B-irradiated skin area, with or without a similar reaction in the UV-A 1-irradiated skin. Patients who were taking medication (eg, corticosteroids) or who had received phototherapy were excluded from the study. Patients and healthy volunteers whose buttock skin was exposed to sunlight less than 2 months previously were also excluded. The medical ethics committee of the University Medical Center Utrecht approved the study.

**PHOTOTESTING PROCEDURES**

The UV-B dose giving a minimal perceptible redness of the skin (minimum erythema dose [MED]) was determined on the buttock skin of each participant using a Philips TL-12 lamp (Philips, Eindhoven, the Netherlands). The TL-12 lamp emitted 58% of its UV output in UV-B (280–315 nm) and 9% in UV rays less than 290 nm. Erythemally weighted, 98% of the lamp's effectiveness stemmed from the UV-B band (18% of rays below 290 nm). For MED determination a test device with 9 windows (3 × 10 mm) was used. These windows opened and closed sequentially, exposing the skin to UV-B for different lengths of time (1.2–200 seconds). The erythema was assessed 24 hours after irradiation. In patients with PLE, MEDs ranged from 483 to 945 J/m<sup>2</sup> (median, 654 J/m<sup>2</sup>; 95% confidence interval [CI], 396–1080 J/m<sup>2</sup>) and in healthy controls, they ranged from 490 to 980 J/m<sup>2</sup> (median, 734 J/m<sup>2</sup>; 95% CI, 421–1279 J/m<sup>2</sup>). Subsequently, the unaffected buttock skin of each subject was exposed to 6 MEDs of UV-B with the Philips TL-12 lamp, resulting in a sunburn reaction but not in a pathologic PLE-related reaction (ie, there were no papular or vesicular responses in patients with PLE). Three-millimeter punch biopsy specimens were obtained from the UV-exposed skin 24 hours and 48 hours after irradiation, together with 1 control biopsy specimen from the normally unexposed buttock skin. The specimens were snap-frozen in liquid nitrogen, embedded in ornithine carbamyl transferase compound (Tissue-Tek OCT compound; Sakura, Zoeterwoude, the Netherlands), and stored at −80°C until further processing.

**ANTIBODIES**

We used as primary antibodies monoclonal antibodies against IL-1α and IL-1β conjugated with biotin (Immunological Research Institute of Siena, Siena, Italy): IL-4 and IL-6 (Genzyme, Cambridge, Mass; dilution, 1:100 and 1:50, respectively); IL-10 (Intruchemie, Delfzijl, the Netherlands; dilution, 1:400); IL-12p40/p70 (BD Biosciences Pharmingen, Erembodegem, Belgium; dilution, 1:150); IL-18 (MBL, Nagoya, Japan; dilution, 1:200); IFN-γ (U-Cytech, Utrecht, the Netherlands; dilution, 1:100); TNF-α (Biosource, Nivelles, Belgium; dilution, 1:50); horseradish peroxidase (HRP)–conjugated elastase as a neutrophil marker (Dako, Glostrup, Denmark; dilution, 1:10); alkaline phosphatase–conjugated AA1 against tryptase (Chemicon, Temecula, Calif; dilution, 1:50); and fluorescein isothiocyanate–labeled CD36 (Immunotech, Marseille, France; dilution, 1:800). Biotinylated rabbit antimouse immunoglobulin (Ig) (Dako; dilution, 1:400) or biotinylated horse antimouse Ig (Vector, Burlingame, Calif; dilution, 1:800) were used as secondary antibodies. Alkaline phosphate– or HRP-conjugated avidine-biotine complex (Dako; dilution, 1:50), HRP-conjugated rabbit antimouse Ig (IL-10; Dako; dilution, 1:100), or AP-conjugated F(ab) fragments of sheep anti-FITC (Boehringer Mannheim GmbH, Mannheim, Germany) were used as detecting reagents.

**IMMUNOHISTOCHEMISTRY**

**Single Staining of Cytokines**

The technique to detect cytokines by immunohistochemistry in the cytoplasm of cells in microscopic sections has been de-
scribed in detail by Hoefakker et al.20 Frozen skin sections (thickness, 6 µm) on 3-aminopropyltriethoxysilane–coated glass slides were fixed for 10 minutes in 65 mL of acetone containing 100 µL of 30% hydrogen peroxide. Endogenous peroxidase activity was additionally blocked by incubating the slides for 15 minutes in 65 mL of 0.05M Tris hydrochloride (pH 7.6) containing 26 mg of 4-Cl-1-naphthol dissolved in absolute ethanol.

Primary antibodies for cytokine detection were all diluted in phosphate-buffered saline solution with 0.1% bovine serum albumin (PBS/0.1% BSA) and incubated overnight. Slides were then incubated for 30 minutes with biotinylated rabbit antitryptase Ig diluted in PBS/0.1% BSA normal human serum, for 60 minutes with HRP-conjugated rabbit antitryptase Ig diluted in PBS/0.1% BSA for IL-10 staining. After incubation with biotinylated rabbit antitryptase Ig, slides were incubated for 60 minutes with an HRP-conjugated avidin-biotin complex diluted in PBS/0.1% BSA. Antibody binding was visualized by incubating all skin sections simultaneously in 65 mL of 0.05M acetate buffer (pH 5.0) containing 26 mg of 3-amino-9-ethyl-carbazole (AEC; Sigma-Aldrich Corp, St Louis, Mo) and 32.5 µL of 30% hydrogen peroxide, resulting in red staining. The sections were counterstained with Mayer hematoxylin.

The skin sections were evaluated using 30% hydrogen peroxide (original magnification × 250). The numbers of cells expressing cytokines (TNF-α and IL-4) were assessed per square millimeter of epidermis or dermis. Double Staining of TNF-α, IL-4, or IL-10 With Elastase

After a 10-minute fixation in 65 mL of acetone containing 100 µL of 30% hydrogen peroxide and a 20-minute preincubation in 10% normal human serum and 10% normal horse serum, the skin sections were incubated overnight with antibodies against TNF-α, IL-4, or IL-10 diluted in PBS/0.1% BSA. Subsequently, the slides were incubated for 60 minutes with biotinylated horse antitryptase Ig diluted in PBS with 1% normal human serum and 1% normal horse serum, followed by a 30-minute incubation with an AP-conjugated avidin-biotin complex. After incubation with a 10% normal mouse serum, the slides were incubated for 60 minutes with HRP-conjugated antitryptase. Alkaline phosphatase activity was visualized by incubating the skin sections in a Tris hydrochloride buffer (pH 8.5) containing 23 mg of Fast Blue BB salt (Sigma-Aldrich), 12.5 mg of naphthol AS-MX phosphate, and 35 mg of levamisole (Sigma-Aldrich), resulting in a blue staining. For visualization of the peroxidase activity the slides were then incubated with a 3-amino-9-ethyl-carbazole solution, as described above.

Double Staining of TNF-α With Tryptase and of IL-10 With CD36

Immunohistochemical staining for TNF-α and IL-10 was performed with the double staining technique just described, except that a HRP–rather than an AP–conjugated avidin-biotin complex was used as a detecting reagent. The slides were then incubated for 20 minutes with 10% normal mouse serum. Subsequently, the skin sections were incubated for 60 minutes with AP-labeled anti-AA1 (against tryptase) for TNF-α double staining or with FITC-labeled CD36 for IL-10 double staining. Incubation of the slides with CD36-FITC was followed by a 60-minute incubation with AP-conjugated F(ab) fragments of sheep anti-FITC. Alkaline phosphatase and HRP activity was visualized as described above.

The antibodies used in this study have been extensively tested for their suitability to stain microscopic skin sections. The primary antibody was omitted or replaced by an irrelevant antibody of the same isotype for negative controls. Sections of an inflamed human tonsil were used as positive staining controls. All antibody incubations were performed in a humidified chamber at room temperature except for incubations with the anticytokine antibodies, which were carried out at 4°C. After each antibody incubation, slides were rinsed in PBS containing 0.05% polysorbate (Tween) 20.

**STATISTICAL ANALYSIS**

A t test was performed to ascertain the observed differences (significance level, P < .05). The numbers of cells expressing TNF-α and IL-4 in the UV-exposed skin and in the unexposed control skin were compared. Furthermore, the numbers of cells expressing cytokines (TNF-α and IL-4) in the exposed and unexposed skin of patients with PLE were compared with the number of the corresponding cells in the skin of healthy controls. A Spearman correlation test was carried out to reveal a possible correlation between the number of cells expressing TNF-α or IL-4 and an individual’s absolute UV dose received (MED in joules per square meter).

**RESULTS**

**REDUCED EXPRESSIONS OF CYTOKINES INVOLVED IN LANGERHANS CELL MIGRATION IN PATIENTS WITH PLE**

Previous experiments showed that, in contrast to what happens in healthy individuals, Langerhans cells persist in the epidermis of patients with PLE after overexposure of the skin to UV-B, and only a small number migrates out of the skin.23 Therefore, we studied the expression of the proinflammatory cytokines IL-1α, IL-1β, TNF-α, and IL-18, which are involved in the migration of Langerhans cells out of the skin.

No appreciable change in the number of cells expressing IL-1β was detected in the epidermis after UV exposure, but an increase was observed in the dermis of all participants. This increase, however, was much less in patients with PLE than in healthy controls (data not shown). Unfortunately, the variable and, at times, strong background staining hampered any good overall quantification of IL-1β expression. A semiquantitative scoring from very positive (++) to negative (−) was too crude to objectify this difference in any measure of statistical significance.

Constitutive expression of IL-1α and IL-18 was observed in the epidermis as IL-1α appeared to be diffusely spread whereas IL-18 was restricted to basal cells. The specimens from patients with PLE tended to show less IL-18 staining. A considerable number of cells in the dermis also stained positive for IL-1α. No change in the number of IL-1α–positive cells was apparent after UV exposure, as only a slightly weaker staining was observed. The expression of IL-18 became more diffuse and spread to suprabasal cells in the UV-exposed skin (data not shown). The present technique is, however, not suitable to quantify these observations on IL-1α and IL-18.

In the unexposed skin of patients with PLE and healthy controls, a limited number of cells expressing TNF-α (weak positive staining) were observed scattered throughout the dermis (mean ± SEM, 29 ± 9 cells/mm² and 42 ± 19 cells/mm², respectively) (Figure 1), but not the epidermis. However, 24 hours after UV-B exposure we observed a significant increase in the number of these cells in the dermis of healthy controls (mean ± SEM, 157 ± 17 cells/mm² [P = .02]) as well as an influx into the epidermis.
mis (mean±SEM, 34±6 cells/mm² \( P = .004 \)). In patients with PLE we observed a small but significant increase in the number of dermal cells expressing TNF-α 24 hours and 48 hours after irradiation (mean±SEM, 64±13 cells/mm² \( P = .02 \) and 68±16 cells/mm² \( P = .04 \), respectively). However, no change could be observed in the number of cells expressing TNF-α in the epidermis 24 hours and 48 hours after UV irradiation (mean±SEM, 12±5 cells/mm² and 7±3 cells/mm², respectively). Twenty-four hours after UV exposure the number of dermal and epidermal cells expressing TNF-α was significantly smaller (\( P = .001 \) and \( P = .01 \), respectively) in patients with PLE than in healthy controls. No correlation was observed between the number cells expressing TNF-α and the absolute UV dose to which the individual’s skin was exposed.

SIMILAR EXPRESSION OF TH1-SKEWING CYTOKINES IN PATIENTS WITH PLE AND HEALTHY CONTROLS

Only very few epidermal and dermal cells expressing IL-6 were detected in the unexposed skin of patients with PLE and healthy controls, and we did not observe any difference in the number of cells expressing IL-6 between the unexposed and UV-B–exposed skin of all participants (data not shown).

All epidermal cells constitutively expressed IL-12, although some cells showed a more pronounced IL-12 expression (data not shown). After UV exposure the epidermal IL-12 expression became more focal. A few cells with a strong expression of IL-12 could be observed in the upper epidermis. The number of dermal cells expressing IL-12 did not differ between unexposed and UV-B–exposed skin. The above observations were made in patients with PLE as well as in healthy controls.

The number of epidermal and dermal cells expressing IFN-γ was very low in the unexposed and UV-B–exposed skin of patients with PLE and of healthy controls, and we did not detect any difference in IFN-γ expression between unexposed and UV-B–exposed skin or between patients with PLE and healthy controls (data not shown).

REDUCED EXPRESSION OF TH2-SKEWING AND IMMUNOSUPPRESSIVE CYTOKINES IN PATIENTS WITH PLE

The number of dermal cells expressing IL-4 in the unexposed skin of patients with PLE and healthy controls was very low (mean±SEM, 4±2 cells/mm² and 1±0.5 cells/mm²) (Figure 2) and no IL-4–expressing cells could be detected in the epidermis. The number of dermal cells expressing IL-4 increased significantly 24 hours after irradiation in the skin of patients with PLE and healthy controls (mean±SE, 208±49 cells/mm² \( P = .008 \) and 246±20 cells/mm² \( P < .001 \)). At 48 hours after UV exposure the number of dermal cells expressing IL-4 decreased, but not significantly, in the skin of patients with PLE and healthy controls (mean±SEM, 103±28 cells/mm² and 196±46 cells/mm²). At 24 hours and 48 hours after UV exposure cells expressing IL-4 infiltrated the epidermis of healthy controls (mean±SEM, 47±13 cells/mm² and 55±26 cells/mm²) and, to a lesser extent, of patients with PLE (mean±SEM, 11±3 cells/mm² and 9±4 cells/mm² \( P = .03 \) and \( P = .05 \)). Twenty-four hours after irradiation, the num-

![Figure 1: Tumor necrosis factor α expression in a healthy control (A [unexposed skin] and B [24 hours after UV exposure]) and in a patient with polymorphous light eruption (C [unexposed skin] and D [24 hours after UV exposure]) (hematoxylin-3-amino-ethylcarbazole; scale bar, 50 µm).](http://archderm.jamanetwork.com/pdfaccess.ashx?url=/data/journals/derm/11761/)
Number of epidermal cells expressing IL-4 differed significantly from the number found in the unexposed skin of patients with PLE (P = .02) and of healthy controls (P = .02). To summarize, the number of cells expressing IL-4 increased after UV exposure and was significantly less in the epidermis of patients with PLE 24 hours after irradiation than in the epidermis of healthy controls. No correlation was observed between the number of cells expressing IL-4 and the absolute UV dose.

Cells expressing IL-10 were present in the basal layer of the epidermis and in the dermis of the unexposed skin (Figure 3) of patients with PLE and healthy controls. At 24 hours postirradiation the expression of IL-10 in the basal layer had increased, and decreased again at 48 hours in most of the participants. A few cells that strongly expressed IL-10 had appeared in the epidermis at 24 hours in most of the healthy controls or at 48 hours in most of the patients with PLE. There was no difference in the number of dermal cells expressing IL-10 in the unexposed and UV-exposed skin of all participants. The diffuse staining of IL-10 in the epidermis prevented a good quantification of this expression; moreover, the difference seemed to be more in a delayed occurrence of densely stained cells in the skin of patients with PLE than in the absolute number of these cells, as is shown in Figure 3.

**EXPRESSION OF IL-10 BY KERATINOCYTES AND TNF-α AND IL-4 BY NEUTROPHILS**

Because Meunier et al reported that CD36⁺, CD11b⁺, and CD1a⁻ cells infiltrate the skin after UV exposure and that the CD11b⁺ CD1a⁻ cells produce IL-10 and are involved in UV-induced immunosuppression, we determined whether the cells strongly expressing IL-10 in the epidermis of the UV-exposed skin were CD36⁺ macrophages. Double staining was performed for IL-10, CD36, and elastase (a neutrophil marker). At 24 hours after irradiation almost all epidermal cells expressing IL-10 in healthy controls were neutrophils. However, the epidermal cells with a strong IL-10 expression in the UV-exposed skin of patients with PLE and healthy individuals did not coexpress elastase 48 hours after irradiation (Figure 4). Only very few cells expressing IL-10 were CD36⁺ macrophages. Therefore, the cells expressing IL-10 in the basal layer of the epidermis, and probably also a fraction of the cells that expressed IL-10 very strongly, were most likely keratinocytes.

Mast cells were shown to release large amounts of TNF-α upon activation by UV-B radiation. However, the pattern of TNF-α expression that we observed was not indicative of mast cells, as it resembled the pattern of the neutrophil influx. Therefore, double staining was performed for TNF-α and tryptase (a mast cell marker) or elastase (a neutrophil marker). All dermal and epidermal cells expressing TNF-α in the skin of healthy controls and patients with PLE proved to be neutrophils (Figure 4). Only a small minority of the neutrophils did not express TNF-α.

Teunissen et al showed that neutrophils are the main source of IL-4 in the UV-B–exposed skin. Hence, we performed a double staining for IL-4 and elastase to characterize the IL-4-expressing cells. All cells expressing IL-4 in the skin of healthy controls and patients with PLE were neutrophils (Figure 4), and a minority of the neutrophils did not express IL-4.

**Figure 2.** Interleukin 4 expression in a healthy control (A [unexposed skin] and B [24 hours after UV exposure]) and in a patient with polymorphous light eruption (C [unexposed skin] and D [24 hours after UV exposure]) (hematoxylin–3-aminoethylcarbazole; scale bar, 50 µm).
We investigated whether the diminished migration of Langerhans cells from the UV-exposed epidermis of patients with PLE could be related to a lack of essential cytokines such as IL-1, TNF-α, and IL-18. The number of cells expressing IL-1β in the UV-exposed skin appeared to be lower in patients with PLE than in healthy controls. Some investigators reported that the UV-induced increase in IL-1β was mainly seen in keratinocytes and Langerhans cells, but we observed an increase in cells expressing IL-1β scattered throughout the epidermis.
dermis. The increase in cells expressing TNF-α after UV exposure was significantly higher in healthy controls than in patients with PLE. In contrast to what happened in healthy controls, hardly any cells expressing TNF-α infiltrated the epidermis of patients with PLE. It has been reported that TNF-α is produced by keratinocytes and mast cells upon UV exposure. However, the TNF-α–expressing cells that we observed were neutrophils. Overall, the reduced expression of IL-1β and TNF-α derived from neutrophils supports our hypothesis that the cytokines inducing Langerhans cell migration are lacking in the UV-exposed skin of patients with PLE. This could explain the impaired Langerhans cell migration that we observed in patients with PLE using the same irradiation protocol as in the present study.

Interestingly, exposure of the skin of patients with PLE to 8.4 MEDs resulted in a near complete depletion of Langerhans cells 48 hours after irradiation. In addition, the expression of IL-1β, TNF-α, and IL-4 was considerably higher than with 6 MEDs and more cells expressing TNF-α and IL-4 infiltrated the epidermis (data not shown). These observations indicate that Langerhans cells are not defective in their ability to migrate in patients with PLE but that the balance between erythemal and immunosuppressive responses is disturbed.

Another hypothesis that we wanted to investigate was whether the TH1-skewing cytokines were expressed in larger amounts in the UV-exposed skin of patients with PLE in comparison with healthy controls, thereby contributing to the pathogenesis of PLE. There are limited data on the cytokine profile of patients with PLE, and only on lesional skin. Norris et al showed an increased activity of IL-6, IL-8, and, possibly, IL-1 in experimentally provoked PLE lesions. However, these changes need not be identical to those preceding the development of a lesion. We did not observe any difference in the number of cells expressing IL-6 between the unexposed and the UV-exposed skin, nor between patients with PLE and healthy controls. Interleukin 12 was constitutively expressed by keratinocytes, which has been described by Yawalkar et al. The dose of UV radiation did not appear to change the level of IL-12 expression; it changed the expression pattern, however, as expression became more focal and stronger in some epidermis cells. Only very few IFN-γ–expressing cells were observed in the UV-exposed or unexposed skin of all participants. Collectively, these findings show that the expression of TH1-skewing cytokines is similar in the UV-B–exposed skin of patients with PLE and of healthy individuals.

To further investigate the balance between TH1-skewing and TH2-skewing cytokines we performed immunohistochemical stainings for IL-4 and IL-10. In line with the results from Teunissen et al we found a UV-induced increase in dermal IL-4–expressing neutrophils and an influx of these cells into the epidermis of healthy controls. The number of IL-4–expressing neutrophils in the UV-exposed epidermis of patients with PLE was significantly lower than in healthy controls, indicating that the balance is somewhat shifted toward a TH1 response in patients with PLE. UV radiation can induce the production of IL-10 by keratinocytes and macrophages. Treatment with IL-12 to inhibit IL-10 release or treatment with anti-CD11b to deplete macrophages reversed the UV-mediated immunosuppression. Our results showed that IL-10 is predominantly expressed by cells in the basal layer of the epidermis. This expression is increased after UV exposure and cells that strongly express IL-10 can be observed in the epidermis of patients with PLE (mostly at 48 hours) and healthy controls (mostly at 24 hours). It was less the number of cells expressing IL-10 that differed between patients with PLE and healthy controls than the timing of their appearance in the epidermis. Only very few IL-10–expressing cells were CD36+ macrophages. The epidermal cells that strongly expressed IL-10 in healthy controls 24 hours after UV exposure were neutrophils. However, 48 hours after irradiation and in the UV-exposed skin of patients with PLE, cells expressing IL-10 coexpressed neither CD36 nor elastase and hence were most probably keratinocytes. CD11b is also expressed by neutrophils, and anti-CD11b treatment will therefore also deplete neutrophils expressing IL-10, resulting in rever-
one could determine cytokine mRNA levels by quantitative polymerase chain reaction. A disadvantage of this technique is that gene expression of cytokines does not necessarily lead to protein synthesis.14

Based on the present cytokine data and on previous experiments we speculate that the following events occur in the skin of patients with PLE upon UV exposure: Langerhans cells residing in the epidermis capture UV-induced neo-antigens. Subsequently, these cells migrate out of the skin but their migration is impaired by low levels of IL-1β and TNF-α. The Langerhans cells that migrate increase their expression of costimulatory molecules and HLA-DR. Because of the impaired migration, activated Langerhans cells move slowly and accumulate in the dermis where they can stimulate memory T cells. A reduced expression of IL-4, due to a failure of neutrophils to infiltrate in adequate numbers the skin of patients with PLE, favors the development of a Th1 response, in contrast to a pronounced Th2 response in healthy controls. Together, these events may contribute to the pathogenesis of PLE. Clearly, this model of PLE pathogenesis needs further substantiation but provides a framework for well-targeted experiments.

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