Molecular Targets of Narrow-Band UVB Phototherapy in Psoriasis

Moleculaire doelwitten van smalband UVB fototherapie in psoriasis

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General Introduction

Including: Molecular pathophysiology of psoriasis and molecular targets of anti-psoriatic therapy.

Emőke Rácz and Errol P. Prens

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GENERAL INTRODUCTION

Psoriasis is a chronic inflammatory skin disease affecting about 2-3% of the Western population. It is characterized clinically by sharply demarcated, elevated red scaly plaques (Figure 1) preferentially occuring at specific body sites, such as the elbows, knees and scalp. In severe cases the whole body may be involved, this serious condition is called erythroderma. Symptoms usually occur first at 20-30 years of age. Twenty to 30% of patients with psoriasis suffer from psoriatic arthritis. Psoriasis is a multifactorial disease resulting from polygenic predisposition and environmental triggering factors, such as medications, infections or trauma. During the last decades there is quickly growing understanding of the cellular processes driving inflammation in psoriasis. Importantly, this knowledge might apply for other organ-specific autoimmune diseases as well, such as for Crohn's disease, rheumatoid arthritis and multiple sclerosis, where the affected organs are less accessible than the skin.

Several local and systemic therapies are available for psoriasis. Still, treatment of patients with psoriasis is often challenging as the long-term use of currently available treatments is limited by side-effects. About 40% of patients with psoriasis are not satisfied with the available care (1).

Narrow-band UVB (NB-UVB) therapy is one of the standard systemic treatments, and it is highly effective. During NB-UVB therapy patients receive increasing doses of UVB three times weekly for about 12 weeks, starting with a low UVB dose to avoid sunburn. For this, patients stand or lie for a few minutes in cabins equipped with TL-01 lamps that emit UVB radiation with a peak emission at 311 nm.

The mechanism of action of NB-UVB therapy in psoriasis is poorly understood. Much of the current knowledge is based on *in vitro* and mouse experiments. In this thesis molecular

Figure 1. Clinical appearance of psoriatic plaques.
See page 149 for a full-color representation of this figure.

effects of NB-UVB therapy *in vivo* in patients with psoriasis are analyzed. The aim was to identify molecular pathways that are responsible for the beneficial effects of UVB in psoriasis. This can help to better understand the pathophysiology of the disease, and to detect potential novel therapeutic targets.

The general introduction will discuss the current understanding of the molecular pathophysiology of psoriasis and the molecular targets of available treatments. Subsequently the mode of action of UVB phototherapy is summarized as we now understand it. Since a major part of the thesis is based on global gene expression profiling, a chapter is included in the introduction showing what such microarray studies tought us about psoriasis and about the effects of UVB in human cells prior to our own investigations. The general introduction ends with a summary of the objectives of our research.

MOLECULAR PATHOPHYSIOLOGY OF PSORIASIS

Psoriatic plaques are defined histologically by epidermal hyperplasia, epidermal and dermal infiltration by leukocytes and changes in dermal microvasculature (2). In lesional psoriatic keratinocytes the differentiation and activation program is disturbed. The pool of proliferating keratinocytes is increased, which is accompanied by enhanced production of proinflammatory cytokines, adhesion molecules and antimicrobial peptides. In psoriasis lesions the number of T cells, myeloid and IFN-α-producing plasmacytoid dendritic cells (DC) is markedly increased. Myeloid DC produce IL-23, tumor necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS), which are critical cytokines in the pathogenesis of psoriasis. IL-23 stimulates the secretion of IL-22 by T helper (Th)17 cells, and IL-22 induces epidermal hyperplasia. The cross-talk between keratinocytes and leukocytes via their pro-inflammatory cytokines create the vicious circle of chronic skin inflammation in psoriasis.

Genomwide linkage analysis identified at least nine chromosomal loci with statistically significant linkage to psoriasis, called PSORS1 through PSORS9 (3). These are probably responsible for the increased incidence of psoriasis among first- and second-degree relatives of patients compared to the general population (4). PSORS1 on chromosome 6p might account for 35 to 50% of the hereditability of the disease (2). Within this region *HLA-C* (HLA-Cw6) encoding an MHC class I protein, and corneodesmosin, an epidermal protein have been extensively studied as possible causative genes, but disease-specific mutations have not been identified (5-7). Genetic variations within loci containing epidermal defense genes *DEFB4* (encoding human β-defensin 2) and *LCE3C/3D* (encoding late cornified envelope proteins 3B and 3C) have been found to be associated with psoriasis (8, 9). Recently, novel genetic regions were identified that confirm the involvement of the immune system in the pathogenesis of psoriasis: both IL-23 subunits and the IL-23 receptor, *TNFAIP3* and *TNIP1*, regulators of TNF-α signaling, and *IL4/IL13*, genes whose products support development of Th2 cells (10-12).

Below, contribution of epidermal keratinocytes and leukocytes to the pathogenesis and pathophysiology of psoriasis is discussed separately.

The epidermal compartment in psoriasis: the regenerative or wound healing phenotype

The epidermis is a stratified squamous epithelium consisting of keratinocytes. Keratinocytes undergo a tightly regulated differentiation program to form a mechanical, permeability, as well as antimicrobial barrier to the external environment. Basal epidermal cells contain stem cells that retain their proliferative potential but can also differentiate to generate multiple suprabasal layers. Cells of the spinous layer synthesize keratin filaments interconnected to desmosomes to generate an integrated mechanical structure, while granular cells produce lipid-rich lamellar granules. Granular cells also make proteins that become irreversibly cross-linked to form the cornified envelope (13). The stratum corneum of normal human epidermis is a cross-linked structure of insoluble proteins and lipids and as such represents a barrier against invasion by microbial agents. The skin barrier function is enhanced by incorporation of proteins with bactericidal or bacteriostatic properties that are constitutively or inducibly expressed (14).

In psoriasis the epidermal balance between proliferation and differentiation is disturbed (15) (Figure 2). The large, silvery scales are a consequence of altered differentiation (hyper- and parakeratosis: thickening of the stratum corneum with retention of cell nuclei), whereas thickening of the epidermis is due to a strongly increased pool of proliferating keratinocytes. Altered differentiation of psoriatic keratinocytes is characterized by focal absence of the granular layer and hereby a downregulation of late keratinocyte differentiation markers (filaggrin, loricrin, caspase 14) and upregulation of early differentiation markers (involucrin, small proline-rich proteins) (15). These alterations in keratinocyte differentiation lead to impairment of the skin barrier function, which was shown to correlate with the severity of the disease (16).

Differentiation of epidermal cells is controlled by several signalling pathways, e.g. by Notch and Wnt signalling and the transcription factors *peroxisome proliferator-activated receptor (*PPAR)-α, transcription factor AP2, and CCAAT-enhancer-binding protein (C/EBP)-α/β (Figure 2). Proliferation of epidermal stem cells is maintained by p63, c-Myc, β1-integrin and transforming growth factor (TGF)-α signalling pathways, and negatively regulated by TGF-β signalling (13). Signalling through insulin-like growth factor receptor (IGFR) and epidermal growth factor receptor (EGFR) also regulate proliferative behaviour in the epidermis (13). Notably, all of these pathways are differentially expressed in lesional psoriatic skin (17-23), indicating a complex network of regulatory pathways in which a primary pathogenic switch is difficult to pinpoint.

Epidermal keratinocytes release several factors upon microbial invasion or physical wounding and also express receptors that alert the immune system to danger signals, as part of an innate immune response (24). Psoriasis is often considered a genetically determined dysregulation of innate and adaptive immunity (25-27). A dysregulation of the innate detection and response system can result in the epidermal phenotype and the abnormal T cell function that characterize the disease (27). Examples for abnormalities of the innate response system in psoriasis are the increased expression of antimicrobial peptides (8), activation of type I interferon (IFN) system (28, 29), and dysregulation of the expression of the IL-1 family of cytokines (30). In addition, psoriasis is often compared to the normal skin wounding response, or the regenerative epidermal phenotype (31). As part of this regenerative or "stress" response,

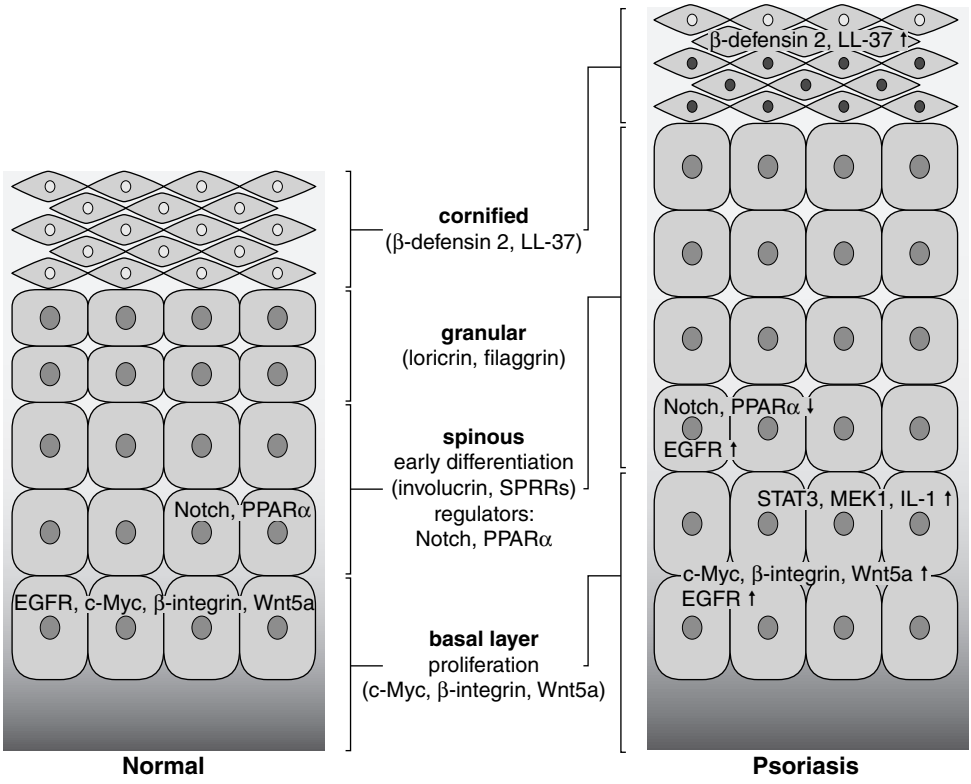

Figure 2. Epidermal alterations in psoriasis.
In psoriasis (right panel) signaling pathways that are associated with keratinocyte differentiation, such as Notch and PPAR-α signaling, are suppressed, whereas proliferation-associated pathways (e.g. c-Myc, β-integrin EGFR and Wnt5a), are induced. STAT3, MEK1 and IL-1 signalling pathways are responsible for the regenerative epidermal phenotype, involving an increased expression of antimicrobial peptides, such as β-defensin and LL-37.

some of the antimicrobial peptide molecules (elafin, psoriasin, LL-37 and β-defensin-2) are highly induced. In addition, neutrophils, macrophages, dendritic cells and natural killer cells initiate an innate immune response to defend the organism from invading pathogens.

Several molecular processes have been described driving this epidermal regenerative or stress response both during wound healing and in psoriasis, signal transducer and activator of transcription (STAT)3 and the mitogen-activated protein kinase (MAPK) cascade being the most important. In healing wounds, STAT3 was found to be activated, while epidermis-specific disruption of STAT3 in mice led to delayed wound healing (32, 33). In psoriatic patients, STAT3 is activated in lesional keratinocytes (34). Transgenic mice with constitutively active STAT3 in keratinocytes spontaneously develop skin lesions resembling human psoriasis (34). These data indicate that activation of STAT3 plays a role in the development of the regenerative epidermal phenotype observed in psoriasis. MAPK-activated protein kinase 2 and mitogen- and stress-activated protein kinase (MSK)1 regulate the expression of tumor necrosis factor

(TNF)-α and other pro-inflammatory mediators in psoriatic lesions (35, 36). Integrin-induced activation of MAPK was considered responsible for epidermal hyperproliferation in psoriasis and wound healing, either directly or through increased IL-1α production (37). More recently expression of IL-20, a cytokine with high expression in psoriasis, was shown to be induced by p38 MAPK in keratinocytes (38, 39). IL-20 induces the regenerative epidermal phenotype characteristic of psoriais (40).

In summary, psoriasis is characterized by hyperproliferation and disturbed differentiation of epidermal keratinocytes. Keratinocytes have an activated phenotype in psoriasis, which is similar to their condition during wound healing (Figure 2). Keratinocytes produce mediators that activate cells of the immune system and endothelial cells in the dermis. On the other hand, leukocyte-derived cytokines may activate keratinocytes, as discussed below.

The contribution of leukocytes in the pathogenesis of psoriasis

The immune system is strongly implicated in the pathogenesis of psoriasis, including both acquired immunity (T cells) and innate immunity (antigen-presenting cells (APC), neutrophils, mast cells, and keratinocytes). In psoriasis lesions the number of T cells and $CD11c^+$ dendritic cells (DC) is markedly increased (41). T cells, especially CD8+ T cells migrate into all layers of the epidermis. CD11c+ DC also infiltrate the epidermis, but remain confined to the lower cell layers (42). The number of immature Langerhans cells are only slightly reduced, however, mobilization of these cells in response to stimuli that normally induce migration (chemical allergen, TNF-α, and IL-1β) is largely absent (43). Neutrophils accumulate in small aggregates called Munro abscesses beneath the corneal layers (44, 45) (Figure 3). As a consequence of the activation of leukocytes, levels of proinflammatory cytokines (IFN-α, IFN-γ, TNF-α, IL-1, IL-6, IL-23, IL-22, IL-17) are high in psoriasis lesions (29, 46-49). These cytokines drive the activation of leukocytes, as well as contribute to the epidermal alterations described above. Chemokines, which are responsible for the chemoattraction of leukocytes from the circulation into the skin, form a bridge between the epidermal compartment and the immunological components of the psoriatic pathology (Figure 3).

Dendritic cells are a heterogeneous group of antigen-presenting leukocytes that are important in activation of both the innate and adaptive arms of the immune system. DC are defined by their capacity for antigen uptake and presentation and activation of naive T-cells. In non-inflamed human skin there are three main DC populations: epidermal Langerhans cells, (mature) dermal myeloid (CD11c+) DC, and sporadically dermal plasmacytoid DC (pDC) (50).

Although pDC have the capacity to present antigen, they are characterized by their ability to produce high amounts of type I IFN during viral infection, and in autoimmune diseases. One of the earliest events driving the inflammatory eruption in psoriasis is the secretion of IFN-α, a type I IFN from pDC (29). pDC are only sporadically seen in normal skin, but are clearly increased in psoriasis lesions (51). We have previously shown that the type I IFN pathway is activated in psoriatic lesions (28). Treatment of non- or pre-psoriatic patients with IFN-α can induce or exacerbate psoriasis (52-55). A functional role of IFN-α/β in the initiation of psoriasis has been demonstrated using a xenograft murine model of psoriasis. Blocking of IFN-α signalling prevented the T-cell dependent development of psoriatic lesions in non-

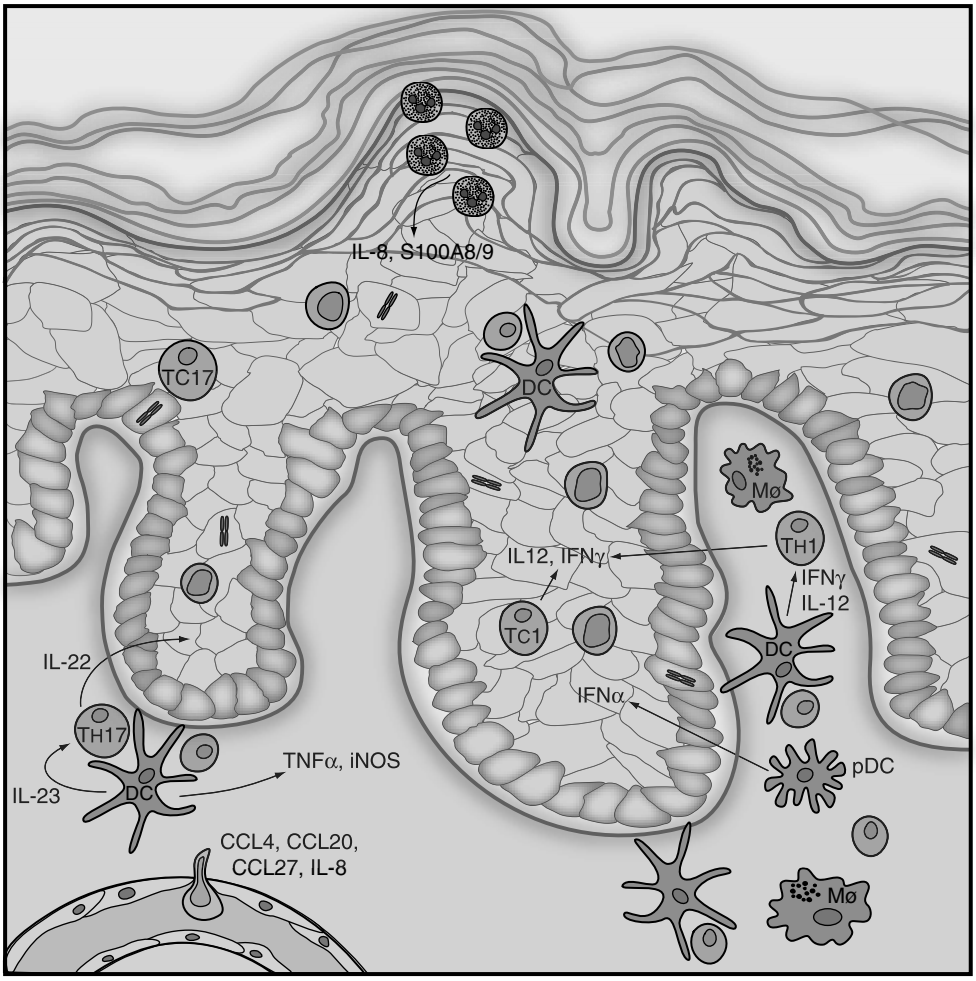

Figure 3. The role of leukocytes in the pathogenesis of psoriasis.
A chemotactic gradient is formed by chemokines, such as CXCL8/IL-8, CCL20 and CCL27, which aids the extravasation of leukocytes into the skin. Myeloid dendritic cells (DC) produce IL-12, IL-23, TNF-α and iNOS, activating hereby T helper (Th) cells to produce IL-21, IL-22, IL-12 and IFN-γ. Plasmacytoid DC (pDC) produce large amounts of IFN-α, which plays a role in the initiation of psoriatic plaque formation. Neutrophils in the stratum corneum are a source of chemokines. Cytotoxic T cells (Tc1, Tc17) infiltrate the epidermis, where they produce cytotoxic molecules, as well as proinflammatory cytokines.

lesional skin transplanted onto immunocompromised mice (29). Recently, LL-37 complexed to self DNA, was shown to trigger pDC to produce large amounts of IFN-α (56), providing the first clear illustration of the autoimmune character of psoriasis.

$CD11c^+$ myeloid DC are dramatically increased in the psoriatic dermis (approximately equal to T-cell numbers) (57). This change is not due to an increase in DC that are also present

in steady state, but to the occurrence of a novel, immature population of DC (42). Thus, psoriatic lesional skin has two populations of dermal DC: (1) $CD11c^+BDCA\text{-}1^+$ cells, which are phenotypically similar to those contained in normal skin and (2) $CD11c^+BDCA\text{-}1^-$ cells, which are phenotypically immature and produce inflammatory cytokines. This second group may be derived from circulating DC precursors (e.g. monocytes or pre-DC) migrating into the skin due to inflammatory and chemotactic signals (50). Importantly, in perilesional, normal-appearing skin of psoriatic patients an increase in the number of activated DC was observed, suggesting a role of these cells in the provocation/initiation of epidermal inflammation (19).

$CD11c^+BDCA1^-$ myeloid dermal DC in psoriasis are called "inflammatory" DC, or TNF- and inducible nitric oxide synthase (iNOS)-producing DC (alias TIP-DC) (58). In addition, these inflammatory DC produce IL-20, a cytokine inducing the regenerative epidermal phenotype as observed in psoriasis (57, 59), and IL-23 which is important for induction of IL-17 and IL-22 in T lymphocytes. Myeloid dermal DC populations induce T-cell proliferation, and polarize T cells to become T helper 1 (Th1) and T helper 17 (Th17) cells (42).

T lymphocytes are the major effector cells of the adaptive immune system. CD8+ cytotoxic T cells kill virus-infected cells in a MHC class I restricted manner, whereas MHC class II restricted CD4+ T helper (Th) cells activate other cells such as B cells and macrophages. T cells are activated by APC via interaction between co-stimulatory molecules on APC and their receptors on the surface of T cells (25). Activated T cells secrete proinflammatory cytokines, such as IFN-γ, IL-17, IL-22, TNF-α, IL-4 or IL-13, depending on their phenotype. In normal skin, a considerable number of T cells are found in the dermis ($\sim 2x10^6$ cells/cm^2; which means that there are about twice as many T cells in the skin than in the circulation), but there are almost no T cells located in the epidermis (60).

In psoriatic skin lesions, both CD4+ and CD8+ T cell subsets are present with CD8+ cells predominant in the epidermis (61). Cytotoxic CD8+ T cells in psoriasis produce cytotoxic granule components Tia-1, granzyme B and perforin, although their pathogenic action is mostly related to cytokine secretion (25). The psoriasis susceptibility gene *HLA-C* product HLA-Cw6 may be involved in the activation of CD8+ T cells by dendritic cells. Activated CD8+ T cells may recognize keratinocyte antigens presented in the context of HLA-Cw6 (62). The antigen specificity of these cytotoxic T lymphocytes in psoriasis has not been identified (25), although they were shown to be stimulated by lesional psoriatic autologous epidermal cells and epidermal cell extracts (63). CD4+ Th cells with Th1 and Th17 phenotype accumulate in psoriasis lesions (64). When activated, Th1 cells produce IFN-γ, TNF-α, while Th17 cell secrete IL-17 or IL-22 (and also some IFN-γ).

T cells are thought to be critical pathogenic triggers of psoriasis. Therapies that inhibit T cell activation and trafficking are effective in treating psoriasis. Immunosuppressive drugs with preferential activity towards T cells, such as cyclosporine, have been shown to be highly efficacious and are widely used for systemic therapy of psoriasis. In addition, psoriasis can be induced in xenograft murine models by the injection of T cells from human skin lesions. The genetic association of psoriasis with certain MHC alleles including HLA-Cw6 also suggest a pathogenic role of T cells (65). Furthermore, psoriasis may develop for the first time after bone marrow transplantation from a donor with psoriasis, or may not recur in patients with psoriasis after bone marrow transplantation from healthy donors (66, 67).

Infiltrating macrophages in psoriatic plaques are accumulated along the basal membrane of the epidermitis ("lining cells") (68). The importance of these cells in the maintenance of psoriatic inflammation has been demonstated in different psoriasis-like dermatitis mouse models, where depletion of macrophages led to significant attenuation of psoriasis-like skin changes (69, 70).

Leukocytes are recruited to the skin by transmigration through activated endothelial cells. In addition, resident skin leukocytes might also expand to create the dense infiltrates seen in psoriatic lesions (71). For transmigration of cells, a chemotactic gradient towards the target tissue is needed, as well as homing receptors and ligands on the surface of the migrating cells and endothelium. Under the influence of T-cell derived proinflammatory cytokines, keratinocytes express a broad array of mediators, thereby amplifying the inflammatory response. Keratinocyte-derived chemokines play a key role in the recruitment of the inflammatory infiltrate. Several chemokines and their corresponding receptors have been shown to be associated with the psoriatic phenotype and may support leukocyte recruitment (72). CCL27 is exclusively produced by epidermal keratinocytes and binds CCR10. More than 90% of skin infiltrating lymphocytes in psoriasis and in atopic and contact dermatitis express CCR10, suggesting a pivotal role of CCL27-CCR10 interactions during the pathogenesis of T cell mediated skin inflammation (73). Chemokines that are highly expressed in psoriasis (and not elevated in atopic dermatitis) are CCL4, CCL20, CXCL2, CXCL8/IL-8 (74). Neutrophils, a defining feature of psoriasis, are an important source of IL-8, CXCL10 and the S100 protein S100A8/9.

Leukocyte-derived cytokines that can induce the epidermal changes seen in psoriatic plaques are IL-21, IL-22 and IFN-γ (46, 75–79). The prototypic Th17 cytokine IL-22 stimulates keratinocyte proliferation and of the production of antimicrobial peptides; therefore it is a key effector cytokine in the pathogenesis of psoriasis (76-78). Elevated levels of IL-22 are found in the blood of patients with psoriasis (80). IL-22 inhibits keratinocyte terminal differentiation, induces STAT3 upregulation, and causes psoriasis-like morphological changes in human skin equivalents (40). IL-22 secretion is induced by IL-23. IL-23 emerged recently as key cytokine in the pathogenesis of psoriasis (81). IL-23 is produced in great quantities in psoriasis lesions by dendritic cells and keratinocytes, and it stimulates Th17 cells to produce Th17 cytokines (48, 82, 83). The importance of this immunological pathway is underlined by the facts that polymorphisms in genes encoding IL23R and IL12/IL23p40 are associated with susceptibility to psoriasis (10, 84). In addition, it is noteworthy that monoclonal antibodies targeting IL12/IL23p40 are highly effective in treating psoriasis (85). We have shown that a psoriasis-like dermatitis in mice induced by the TLR7/TLR8 agonist imiquimod is critically dependent on the IL-23/IL-17 axis (86). Cyclosporine A, a standard therapy for psoriasis, was shown to downregulate the Th17 pathway in psoriatic skin (87). This modulation was observed within 2 weeks after the start of treatment and correlated well with the clinical improvement of the disease (87). Also etanercept (TNF receptor - immunoglobulin fusion protein), another effective treatment modality in psoriasis, was shown to inhibit the Th17 pathway (57).

IFN-γ, a type II IFN, is mainly produced by activated Th1/Tc1 cells and is mainly involved in adaptive immune responses. IFN-γ can induce the regenerative psoriatic phenotype in epidermal reconstructs (79). Interestingly, the TNF-α-inhibitor infliximab inhibits the synthesis of IFN-γ by Th1 cells (88). Thus, IFN-γ secretion might be downstream of TNF-α. TNF-α has

been called a sentinel cytokine or "the body's fire alarm", as it initiates the defense response to local injury (89). Many different immune and nonimmune cell types can produce TNF, including macrophages, T cells, mast cells, granulocytes, natural killer (NK) cells, fibroblasts, neurons, keratinocytes and smooth muscle cells. At low concentrations in tissues, TNF is thought to have beneficial effects, such as the augmentation of host defense mechanisms against infections. In disease states, TNF mediates a variety of direct pathogenic effects and induces the production of other mediators of inflammation and tissue destruction, placing it in a key position in the inflammatory cascade within an inflammatory network (89).

In conclusion, components of innate and adaptive immunity are activated in psoriasis (Figure 3). Plasmacytoid dendritic cells produce IFN-α, hereby activating other cells of the immune system. Dermal dendritic cells acquire an inflammatory phenotype and produce IL-23, TNF-α and iNOS, resulting in the activation of Th1 and Th17 cells. Active Th1 and Th17 cells synthesize IFN-γ and IL-22, respectively, and these cytokines stimulate the activation and proliferation of epidermal keratinocytes, hereby contributing to the vicious circle of psoriatic skin inflammation.

Endothelial cell activation in psoriasis

A hallmark of psoriatic skin is the transformation of the local microvascular system, characterized by dilation and tortuosity of capillaries, increased permeability, and high endothelial venule formation which is usually seen in lymph nodes. Extravasation of leukocytes into the dermis is aided by this transformation of the capillaries, as well as by the increased expression of adhesion molecules (e.g. intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-Selectin) by endothelial cells.

Accumulating evidence indicates that many of the cells involved in inflammatory processes release factors that act directly or indirectly on vascular endothelial cells. The interplay between chronic inflammation and angiogenesis is accomplished in the first place by the production of nitric oxide (NO), a well-established inflammatory agent produced by the activation of iNOS in inflammatory DC. NO stimulates vessel dilation and permeability, a feature required for leukocyte extravasation (90). In addition, cyclo-oxygenase-2 (COX-2), an inducible enzyme responsible for the increase in prostaglandin biosynthesis during inflammation, also contributes to increased angiogenesis by up-regulating the production of vascular endothelial growth factor (VEGF) (90).

VEGF plays a crucial role in neo-angiogenesis as occurs in psoriasis (90), inducing migration, survival, and proliferation of endothelial cells. VEGF acts by engaging with its tyrosin kinase receptors VEGFR1 and VEGFR2 on endothelial cells. Although VEGF binds to both receptors, it appears that most of its biological functions are mediated via VEGFR2. VEGF is secreted by keratinocytes, and VEGF serum levels are increased in patients with psoriasis (90). Distinct single nucleotide polymorphisms of the *VEGF* gene occur more frequently in subsets of psoriasis patients, and these haplotypes may contribute to the elevated VEGF levels observed in these patients (91). Furthermore, overexpression of VEGF in mouse skin results in a psoriasis skin phenotype, which implies a complex interplay between blood vessels, keratinocytes and leukocytes (92).

Although vascular remodelling is a hallmark of many chronic inflammatory disorders, anti-vascular strategies for treating these conditions have received little attention to date. In psoriasis, selective photothermolysis of the dermal microvasculature by use of the 585 nm pulsed-dye laser resulted in partial or complete disappearance of psoriasis plaques (93-96). The anti-angiogenic agents paclitaxel and AE-941 (Neovastat) demonstrated therapeutic activity in patients with severe psoriais (97, 98). Recently, a VEGFR antagonist - already in clinical use for the treatment of malignancies and for the inhibition of retinal neovascularisation - was shown to inhibit psoriasis-like skin inflammation in mice (99).

MOLECULAR TARGETS OF CURRENT TREATMENTS FOR PSORIASIS

Most established treatments for psoriasis have been developed empirically. Biologics, on the other hand, specifically target key mechanisms of psoriasis pathogenesis. Most of these compounds are monoclonal antibodies, anti-cytokines or fusion proteins that block T cell migration and adhesion, or antagonize/neutralize the effects of cytokines.

Established empirical treatments for psoriasis include topical and systemic therapies. In the majority of patients with psoriasis (approximately 60%) the disease is managed by topical therapy alone, although many patients are dissatisfied with this type of treatment. Corticosteroids and Vitamin D_3 are the most frequently used topical treatments for psoriasis. Topical corticosteroids can be very effective in controlling mild to moderate psoriasis lesions. A recent study on the effects of corticosteroids on keratinocytes showed that in addition to downregulation of pro-inflammatory genes, corticosteroids regulate keratinocyte motility and metabolism, suppress the expression of essentially all IFN-γ-regulated genes, including IFN-γ receptor and STAT1, promote terminal epidermal differentiation while simultaneously inhibiting early stage differentiation (100). Because of their side effects upon prolonged use, corticosteroids are combined with other topical treatments in psoriasis. Vitamin D_3 analogues (calcipotriol and tacalcitol) and the topical retinoid tazarotene, all of which affect keratinocyte functions and the immune response, are nowadays more widely used than either anthralin or coal tar (101). Moderate-to-severe psoriasis may be treated with UV phototherapy or systemic drugs. The mode of action of traditional systemic therapies is incompletely understood. Some of these, such as cyclosporine A and fumarates inhibit immune functions, whereas others (retinoids and methotrexate) also target keratinocyte functions (101).

Novel therapeutic agents are characterized by more specific targeting of defined molecules in the pathological pathways. The T-cell targeted alefacept, a lymphocyte function-associated antigen (LFA)-3-immunoglobulin fusion protein interferes with LFA-3:CD2 interactions on T cells and antigen presenting cells. Alefacept is an effective therapy in selected patients and may induce relatively long remissions. In responding patients, alefacept induces a reduction in $CD11c^+$ DC as well as T cells (102). A range of inflammatory genes are reduced. T cells are the primary target for therapy, but DC and a spectrum of Th1 inflammatory genes are coordinately suppressed (103).

Cytokine-targeted interventions include anti-TNF-α and anti-IL-12/IL-23p40 approaches. Infliximab, adalimumab and etanercept target TNF-α; all are indicated for several immune-

mediated inflammatory diseases. All three TNF antagonists are parenterally administered protein therapeutics (biologics); infliximab and adalimumab are monoclonal antibodies (mAbs) that specifically bind TNF; and etanercept is a TNF-receptor-fusion protein that binds TNF and lymphotoxin (LT) family members. Infliximab not only blocks TNF-α but also (indirectly) inhibits IFN-γ synthesis by T cells, blocking hereby Th1 pathway (88), in which aspect it differs from etanercept (104). Adalimumab is a fully human recombinant anti-TNF antibody, and, theoretically, has similar actions and effects as infliximab. Etanercept downregulates Th17 responses and induces apoptosis of dermal dendritic cells in patients with psoriasis (57, 105). The response of psoriasis to three distinct TNF inhibitors certainly suggests that this cytokine has a key role in disease pathogenesis. New TNF-α blockers are golimumab, a fully human anti-TNF-α antibody, and certolizumab pegol. As certolizumab does not have an Fc region, unlike infliximab and adalimumab, it should not fix complement or cause antibody-dependent cell-mediated cytotoxicity *in vitro* (106).

Ustekinumab is a fully human monoclonal antibody against the p40 subunit of IL-12/IL-23 (107, 108). It binds with high affinity and specificity to the p40 subunit of IL-12 and IL-23, preventing these cytokines from binding to IL-12 and IL-23 receptor 1 (IL-12R1) on the surface of leukocytes. Ustekinumab appears to be as effective as the anti-TNF antibodies during induction therapy and the available long-term data indicate a stable clinical response over time, with no rebound after withdrawal of the drug (109). Another monoclonal antibody against IL-12/IL-23 p40 is ABT-874 has also been tested in phase II clinical trials and was found to be effective and comparable to ustekinumab (110). The clinical efficacy of anti-p40 antibodies is much longer than its serum half-life, which suggests that this intervention collapses a key population of cells. This long-lasting effect makes these drugs highly practical, as they require only 4 to 5 injections per year. At present, information on the safety of ustekinumab is based on smaller patient numbers and shorter duration of exposure compared with the TNF antagonists.

Meta-analysis of trials with new and established therapies showed that these novel, specific treatments are more effective than traditional systemic treatments (111). Infliximab appears to be the most effective treatment for moderate-to-severe psoriasis, followed by adalimumab. Etanercept induces stable long-term response rates (111).

Several other approaches for the treatment of psoriasis are currently under clinical investigation. The PPARγ antagonist pioglitazone is effective in clearing psoriasis (112), and in combination therapies it enhanced the efficacy of acitretin (113). Janus kinase (JAK) inhibitors lestaurtinib (CEP-701) and INVB18424 aim to block proinflammatory JAK/STAT signalling pathways. The pan-selectin antagonist Bimosiamose prevents leukocytes to enter the tissues from the circulation. Use of recombinant alfa-fetoprotein (MM-093) is based on the observation that psoriasis, as most autoimmune diseases tends to improve during pregnancy (114).

UVB PHOTOTHERAPY FOR THE TREATMENT OF PSORIASIS – MECHANISM OF ACTION

The development of UVB phototherapy for psoriasis was based on the observation that exposure to sunlight improves the symptoms of psoriasis. Artificial light sources have been

used for the treatment of psoriasis since the 1920s. The most frequently applied regimen was the combination of topical coal tar and subsequent UVB radiation, introduced by Goeckerman in 1925. Broad-band UVB alone is used since the 1970s (115). Also in the 1970s psoralen plus UVA therapy (PUVA) was introduced. Psoralens, plant-derived photosensitizers, can be applied topically or orally. Subsequent UVA irradiation causes a therapeutically beneficial phototoxic reaction in the skin. PUVA therapy has anti-inflammatory and antiproliferative effects, and is highly efficacious for the treatment of psoriasis, inducing response rates from 74 to 100% (116). UVA effects are different from UVB effects (117). Effects of UVA radiation and PUVA therapy are not the subject of this thesis. Further forms of UV phototherapy for psoriasis are climatotherapy (also called heliotherapy), and the 308 nm excimer laser. Climatotherapy involves intentional gradual daily exposure to natural sunlight to get the therapeutic benefits of the included UVB radiation. Climatotherapy at the Dead Sea is combined with daily bathing in Dead Sea water. Treatment is usually for 4 weeks and results in decreases in PASI scores by 75% or more. Most of the benefit of climatotherapy at the Dead Sea has been attributed to the sunlight at the Dead Sea (118). Balneophototherapy, which involves salt water baths and artificial ultraviolet radiation, can be used as an alternative to climatotherapy at the Dead Sea (118). The 308 nm excimer laser is used for the treatment of psoriasis since the late 1990s (119). This laser emits monochromatic light adjacent to those of NB-UVB, and probably has similar biological and clinical effects (120).

Narrow-band UVB phototherapy using Philips TL-01 fluorescent lamps was introduced in 1988 for the treatment of psoriasis, following examinations of efficacy of discrete wavelengths in clearing psoriasis by Fisher and Parrish (121, 122). Several studies reported a superior clinical efficacy of NB-UVB to BB-UVB (123-126). Some other studies found that BB-UVB and NB-UVB are equally effective (127, 128).

Minimal erythema dose (MED) is the lowest radiation exposure that is sufficient to produce just perceptible erythema on exposed skin after 24 h. MEDs reported for NB-UVB and BB-UVB are shown in Table 1.

Table 1. Minimal erythema dose with narrow-band and broad-band UVB.

Study	Skin phototype[1]	MED BB-UVB (mJ/cm^2)	MED NB-UVB (mJ/cm^2)
Van Weelden (114)	II	76	410
Johnson (115)	II	100	500
Karvonen (116)	II	230	970
Storbeck (111)	II	114	1034
Srinivas (117)	IV	21	300
Tejasvi (118)	III-V	-	1000
Yuon (119)	III-V	-	750-1075
Morita (120)	IV	-	700

[1] Types I-IV are determined by sunburn history (I: always burn, never tan; II: always burn, but sometimes tan; III: sometimes burn, but always tan; IV: never burn, always tan). Types V and VI by physical examination (V: moderately pigmented skin, VI: darkly pigmented skin) (100).

Thus, at least 5-times higher doses of NB-UVB are needed for the induction of erythema than of BB-UVB. NB-UVB doses required for the induction of hyperplasia, edema, sunburn cell formation and Langerhans cell depletion are 5-7 times higher than equally effective BB-UVB doses (136). Since in the clinical setting the applied doses are calculated based on the induction of erythema, much higher UV doses are given when NB-UVB is used than in the case of BB-UVB. Although in mice NB-UVB was more carcinogenic than BB-UVB at equally erythemogen doses (137), follow-up of patients receiving NB-UVB for psoriasis did not show an increase in skin cancer incidence as compared to controls (138-141). In addition, although NB-UVB induced the expression of matrix metalloproteinases and reduced the synthesis of collagens by dermal fibroblasts, factors that add to enhanced aging of the skin, these effects were weaker than induced by BB-UVB (142).

A further difference between NB-UVB and BB-UVB was that NB-UVB radiation did not suppress contact hypersensitivity response in mice, even at 7 times higher doses than the effective BB-UVB dose (136), demonstrating that BB-UVB and NB-UVB have different biological effects.

In summary, several forms of phototherapy are available for the treatment of psoriasis. Wavelengths between 311-313 nm are probably the most effective in clearing psoriasis. Studies in mice indicated that BB-UVB and NB-UVB have different biological effects. The mode of action of NB-UVB clearing psoriasis is incompletely understood.

UVB effects in healthy human skin

Primary molecular targets of UVB in the skin

The epidermis is the primary target of UVB radiation. UVB radiation is absorbed to the greatest extent by chromophores (light absorbing molecules) in the upper layers of the skin, mostly in the epidermis (143) (Figure 4). Light absorption in chromophores induces structural changes, thereby changing their functionality. Molecules which underwent light-induced structural changes are called photoproducts.

DNA damage

DNA absorbs light with wavelengths between 230 and 340 nm, and thereby it is a chromophore for UVB radiation (144). When DNA absorbs UVB radiation, different types of photoproducts are formed, the most frequent being cyclobutyl pyrimidine dimers (CPD) and (6-4)-photoproducts (145). These UVB signature molecules can be observed in both keratinocytes and Langerhans cells upon UVB radiation (145) (Figure 4). Involvement of DNA photoproduct formation in UV-induced processes can be studied by external or transgenic application of DNA repair photolyase enzymes. DNA photoproduct formation is involved in a specific biological effect when general or cell-specific repair of the UV signature mutations abolishes that biological effect. With this approach CPD formation was shown to play a role in UVB-induced apoptosis, inflammation, immunosuppression and photocarcinogenesis (146). Interestingly, UV-induced tumor formation, but not UV-induced immunosuppression could be prevented by repair of CPD in the basal keratinocytes only, demonstrating that different cell types mediate different biological effects of UV radiation (146).

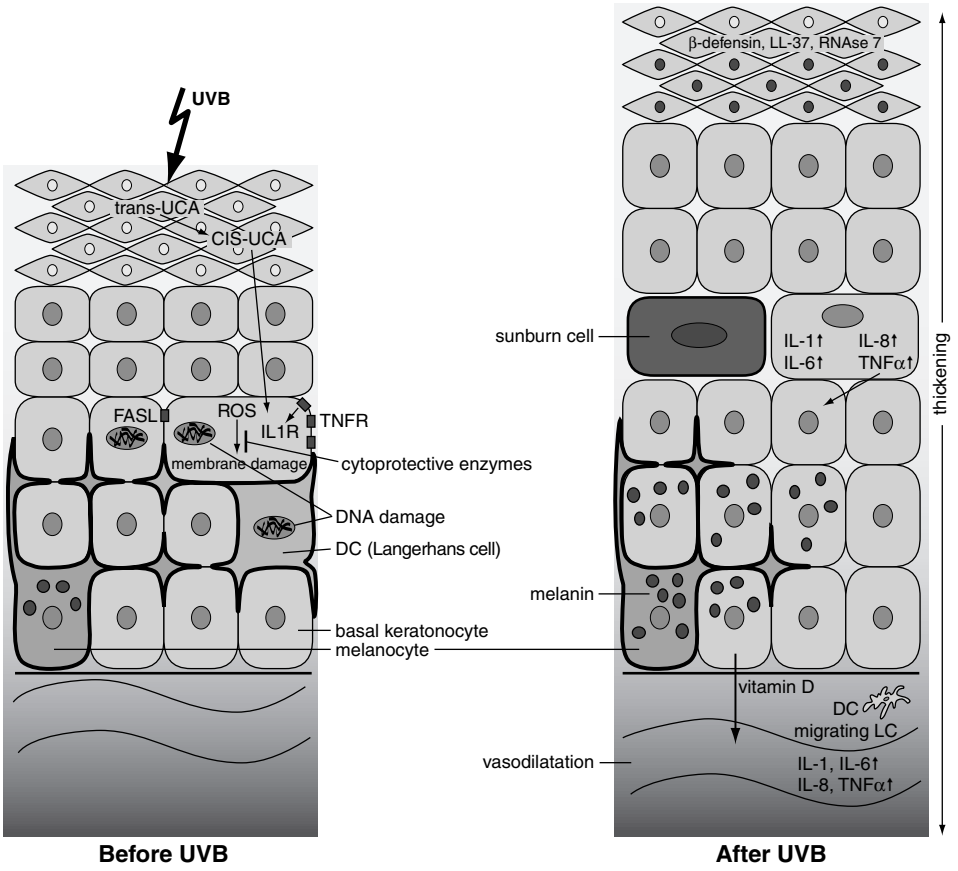

Figure 4. Acute effects of UVB in normal human skin.
The primary molecular effects of UVB are the isomerisation of urocanic acid, the formation of DNA photoproducts and reactive oxygen species (ROS), and ligand-independent activation of certain transmembrane receptors. These primary changes lead to the secretion of proinflammatory cytokines, to apoptosis of keratinocytes with accumulated DNA damage, to the synthesis of vitamin D, enhanced pigmentation, epidermal hyperplasia, and the migration of Langerhans cells to the lymph nodes.

Reactive oxygen species (ROS)

Formation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and singlet oxygen is the result of active cellular metabolism in the presence of oxygen. Reactive oxygen species are extremely instable, react with other molecules and hereby cause a variety of damages in the cell such as lipid peroxidation, DNA breaks and DNA-protein cross-links (147). To counteract oxidative damage, cells have an antioxidative defence system, comprising of radical scavengers (e.g. tocopherol, vitamin A) and different enzymes (e.g. superoxide dismutase, glutathione peroxidase, and catalase). UVB radiation induces enhanced ROS production (Figure 4). Cells respond with upregulation of scavenger enzyme activity and synthesis. Hereby UVB radiation drives the cells into a complex stress response.

Membrane changes

UVB radiation leads to clustering and internalization of cell membrane receptors for epidermal growth factor (EGF), TNF and IL-1, resulting in ligand-independent activation of members of the MAPK family (148). Furthermore, CD95 or FAS, another cell surface receptor is also activated by UVB radiation on a ligand-independent manner, contributing to UVB-induced apoptosis (149).

Urocanic acid

Urocanic acid (UCA) is generated in the skin from histidin, and accumulates in the stratum corneum of the epidermis. The major source of UCA in the epidermis is filaggrin, a histidin-rich basic protein. Cleavage of filaggrin by caspase 14 produces UCA that absorbs UV radiation, and carboxylic-pyrrolidone acid that support cutaneous hydratation. Upon UV radiation the naturally occurring trans-UCA isoform converts to cis-UCA. The action spectrum for the production of cis-UCA in human skin is between 280 and 310 nm (150). UCA was first identified as a chromophore responsible for UVB-induced suppression of contact hypersensitivity (151). Upon UVB radiation in humans, cis-UCA is detectable in the skin and in the urine (152). Recently, the 5-HT_{2A} serotonin receptor was identified as receptor for cis-UCA (153). In addition, a 5-HT_{2A} receptor antagonist inhibited cis-UCA-induced suppression of delayed-type hypersensitivity response in mice (153). Interestingly, although cis-UCA stimulates the production of IL-6, TNF-α and PGE2 by keratinocytes, these effects were independent of the 5-HT_{2A} receptor, indicating that there has to be another receptor for cis-UCA in these cells, and that keratinocytes might not be the effector cells of cis-UCA-induced immune suppression (154).

Secondary events following UVB irradiation of the skin

Inflammation

Sunburn inflammation (erythema), a well-known acute effect of UV exposure, reaches its maximum 6 to 24 h after UV exposure. Wavelengths shorter than 320 nm are the most erythemogenic. During the development of sunburn erythema DNA seems to be the most important chromophore, based on the similarity of action spectra (144). UVB-exposed keratinocytes synthesize a set of cytokines, such as IL-1, IL-6, IL-8, IL-18, TNF-α and PGE2. These cytokines are responsible for the onset of inflammation (155). Secretion of IL-1β and IL-18 upon UVB exposure is dependent on components of the inflammasome (NALP3, NALP1, pro-caspase 1), which become activated upon UV-induced elevation of the cytoplasmic Ca^{2+} concentration (156).

Epidermal thickening

UV-induced thickening of the epidermis is an important protecting factor against UV-induced inflammation. Keratinocyte proliferation increases during the first 2 days after UV exposure, accompanied by increased DNA, RNA and protein synthesis. A single UVB exposure results in a twofold thickening of the epidermis lasting for about 6 weeks, whereas a single UVA exposure does not increase epidermal thickness (143).

Apoptosis

Shortly after UVB exposure, apoptotic keratinocytes ("sunburn cells") are formed in the epidermis, a process presumably mediated by p53 and dependent on the amount of DNA damage accumulated in the cell (157). Sunburn cell formation is thought to protect the organism from the accumulation of DNA damage and thereby from the induction of skin cancer.

Pigmentation

UV radiation results in increased skin pigmentation, becoming visible ~72 h after UVB exposure (earlier after UVA exposure). This increased pigmentation is caused by increased activity of melanocytes and of melanocyte tyrosinase. The transfer of melanin to keratinocytes also increases. Interestingly, UV-induced pigmentation gives variable protection against sunburn inflammation, depending on the skin type and on the wavelength that induced the tanning (143). UVA-induced pigmentation is less protective, probably because UVA does not induce thickening of the epidermis.

Vitamin D synthesis

The generation of previtamin D_3 from provitamin D_3 is the result of a photochemical reaction with maximum spectral effectiveness from 297 to 302 nm in basal and suprabasal layers of the skin (158). UV radiation above 315 nm is unable to produce previtamin D_3 in human skin. Previtamin D_3 then undergoes thermal isomerization over a few hours generating vitamin D_3 (cholecalciferol). Vitamin D_3 translocates into the circulation, and its sequential activation occurs in the liver and kidney. In addition, several cell species including keratinocytes, macrophages, prostate epithelial cells and osteoblasts are able to convert vitamin D_3 directly to the active $1\alpha,25(OH)_2D_3$ form (158). Skin cells (keratinocytes, epithelial cells of the epidermal appendages, melanocytes, Langerhans cells, macrophages, T-lymphocytes and dermal fibroblasts) also express vitamin D receptor (VDR), they can respond to active $1\alpha,25(OH)_2D_3$ (159). VDR activation results in transcription of genes involved in cellular growth, differentiation, inflammation and wound healing in keratinocytes. $1\alpha,25(OH)_2D_3$ protects primary human keratinocytes against the induction of CPD by UVB (144). Moreover, calcitriol produced in the skin may enhance UV-induced p53 protein expression and suppress nitric oxide (NO) products resulting in increased DNA repair (161). Hereby vitamin D has photoprotective effects. In addition, vitamin D mediates immunmodulatory effects of UV radiation: UV-induced $1\alpha,25(OH)_2D_3$ produced by DC signals T cells to express the chemokine receptor CCR10 on their surface, which enables them to migrate towards CCL27, the skin-specific chemokine produced by keratinocytes (162).

Induction of antimicrobial peptides

UVB radiation induces the expression of antimicrobial peptides LL37 (163), β-defensin-2, -3, ribonuclease 7 and S100A7 in normal human skin (164). Thus, whereas UVB radiation suppresses adaptive immune responses (see below), it induces innate immunity, which might partly explain why bacterial infections are not more frequent after UVB exposure.

In summary, acute effects of UVB in healthy human skin include sunburn inflammation, pigmentation, hyperplasia, apoptosis of cells with accumulated DNA damage, synthesis of vitamin D and induction of antimicrobial peptides (Figure 4). These effects are the result of activation of primary molecular targets of UVB, such as DNA, reactive oxygen species, membrane receptors or urocanic acid (Figure 4).

Immunosuppressive effects of UVB

Effects of UVB on the immune system have been extensively studied in terms of suppression of contact hypersensitivity response, mainly in mice. Two models have been studied in mice: in the low dose (~100 mJ/cm^2) model, contact hypersensitivity response (CHS) is suppressed only at the site of irradiation, whereas in the high dose (~5000 mJ/cm^2) model, CHS cannot be induced even at non-irradiated sites, indicating systemic immunosuppressive effect of UVB. In both models, mice are first irradiated, about 3 days after which a contact sensitizer is applied epicutaneously either at the irradiated site or at a distant site (sensitization). Six days later the same contact sensitizer is applied topically to yet another body site, usually the ear (challenge). 24 h after this decreased ear swelling can be observed in the irradiated mice as compared to non-irradiated control mice (165).

For a normal CHS to occur, Langerhans cells take up the antigen in the skin and present it in the draining lymph node to Th1 and Th17 cells, which are the effector cells of this delayed-type hypersensitivity reaction (166). Upon UVB radiation, DNA damage occurs in Langerhans cells and other skin cells. Langerhans cells carrying DNA damage induce antigen-specific regulatory T cells in the draining lymph nodes (167, 168). These cells are responsible for the attenuated CHS response, as transfer of these cells into non-irradiated mice prevents the CHS response in the recipients. IL-10 is a critical cytokine for the suppression of the CHS response by UVB, as neutralizing antibodies against IL-10 prevent the suppressive effect of UVB (169).

Next to DNA, cis-UCA is also an important chromophore during UVB-induced immunosuppression, as blocking cis-UCA with a monoclonal antibody can reverse delayed-type hypersensitivity reaction suppression by UVB (170), whereas treatment with cis-UCA in the absence of UVB radiation mimics the immunosuppressive effect of UVB in a dose-dependent manner (171). In addition, cis-UCA inhibits the ability of Langerhans cells to present tumor antigens, contributing hereby to the development of UV-induced skin cancer. The immunostimulatory cytokine IL-12 prevents this effect of cis-UCA (172). Interestingly, injection of IL-12 and IL-18 prevents the UV-induced suppression of CHS by induction of the nucleotide excision repair system (173, 174). These results confirm that DNA damage is essential in the UVB-induced suppression of CHS. In these studies on the effect of IL-12 on UV-induced immunosuppression no attention was given to IL-12 subunits, i.e. whether these IL-12 actions are truly specific to IL-12 or are contributed to the IL-12p40 subunit which is shared with IL-23.

Furthermore, supernatant of irradiated keratinocytes can also inhibit CHS in recipient mice (175). Keratinocytes upregulate receptor activator of NF-κB ligand (RANKL) upon UVB irradiation. Transgenic mice with overexpressed RANKL in basal keratinocytes have increased numbers of regulatory T cells, whereas overexpression of RANKL suppresses CHS, indicating an involvement of RANKL in UVB induced systemic immunosuppression (176).

The CHS response is also suppressed by UVB in humans (117). A single exposure to 4 MED or repetitive daily exposures to 0.75 to 2 MED for four consecutive days suppressed the induction of CHS in humans when the antigen was applied locally to the site of irradiation. A single dose of 4 MED also inhibited the systemic induction of immunity (177).

The murine CHS model shows that UVB radiation inhibits antigen-specific adaptive immune responses, of which the effectors are Th1 and Th17 cells. These T-cell subsets are also critical in the pathogenesis of psoriasis, although the antigen specificity is unknown. The function of regulatory T cells is defective in psoriasis, although their numbers do not differ from that of normal controls (178). It is possible that induction of regulatory T cells plays a role in the effectiveness of UVB therapy in psoriasis, although this effect was not demonstrated in *in vivo* studies. Serum levels of IL-10 do not change during phototherapy (179), but PBMC isolated after phototherapy produce more IL-10 upon *in vitro* stimulation than PBMC from untreated patients (180). Migration of Langerhans cells is defective in psoriasis (43), and it was not investigated whether UVB phototherapy affects this property.

In conclusion, suppressive effects of UVB on adaptive immunity might partly account for the efficacy of UVB treatment in psoriasis, but it was never formally shown in patients undergoing phototherapy. As in psoriasis innate immunity might be more important than adaptive immunity, UVB effects on the CHS response would not give sufficient explanation for the efficacy of this treatment in psoriasis. In addition, NB-UVB did not suppress the CHS response in mice (136), whereas it is highly efficacious in psoriasis, which would argue for distinct mechanisms in the immunosuppressive and anti-psoriatic effects of UVB.

Mode of action of (NB-)UVB in psoriasis

Primary molecular targets of UVB phototherapy in psoriasis

From the primary UVB target molecules that were discussed above, the involvement of urocanic acid (181) and DNA damage (182, 183) was studied during the phototherapy of psoriasis. Furthermore, expression of cytoprotective enzymes was assessed (184). The most important conclusions of these studies are discussed below.

The epidermal cis-UCA concentration was found to be increased by heliotherapy of psoriasis, from a mean initial value of 0.2 nmol/cm^2 to a mean final value of 2.9 nmol/cm^2. Clinical response of psoriasis to heliotherapy, however, seemed to be independent of UCA isomer levels (181).

The amount of DNA mutations during phototherapy was assessed in order to evaluate its carcinogenic potential and compare it with that of BB-UVB (182, 183). NB-UVB induced equal amount of DNA photoproducts as BB-UVB at equally erythemogen doses, indicating that NB-UVB is not more carcinogenic than BB-UVB (182). The amount of CPD increased in the psoriatic skin during the first three treatment sessions, but not hereafter. Clinical improvement is usually only noticable after several weeks of treatment (183).

Expression of many drug metabolizing and cytoprotective enzymes that play a role in the cellular defense against reactive oxygen species is constitutively higher in psoriatic plaques. The role of this high scavenger enzyme expression in psoriatic lesions is unknown. Interestingly, in non-lesional skin of psoriatic patients scavenger enzyme expression is reduced compared to the skin of healthy subjects, and is increased by UV radiation (184).

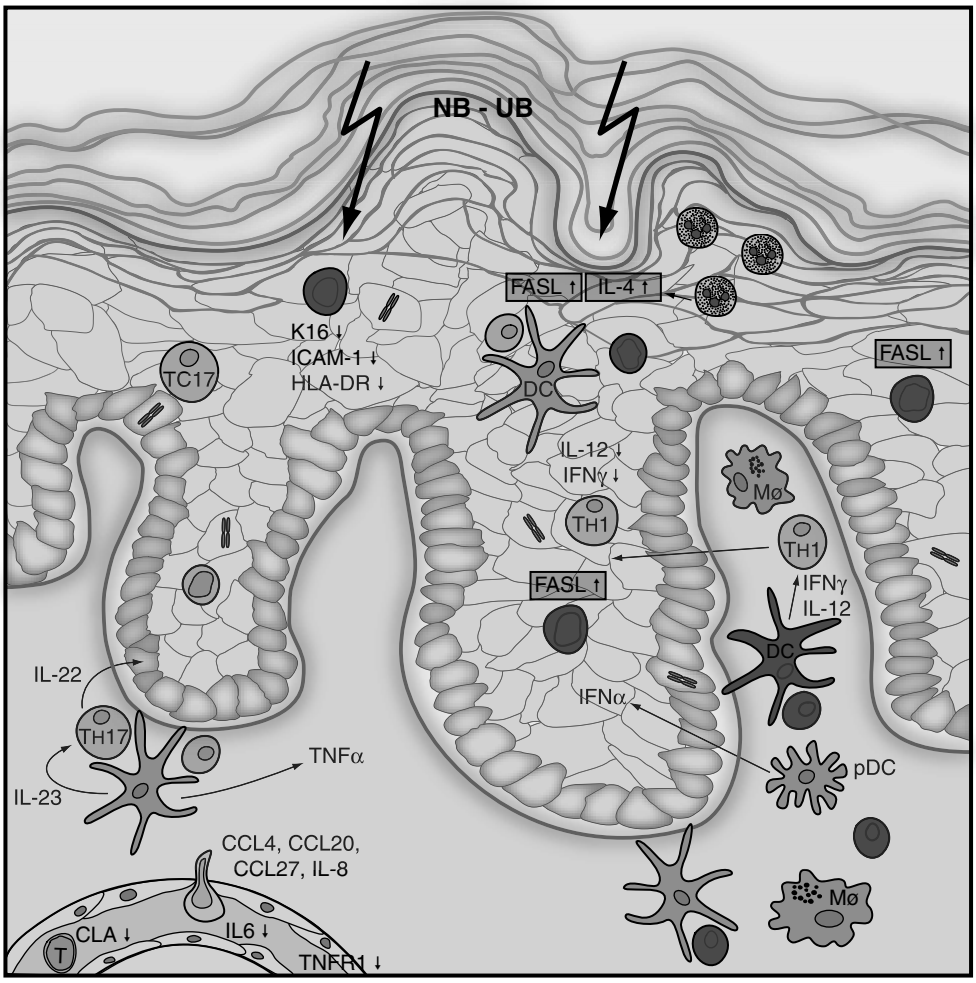

Figure 5. Local effects of UVB in psoriatic plaques.
UVB-induced alterations are marked with dark coloring. Upon UVB phototherapy the number of T lymphocytes decreases in the epidermis and the dermis due to apoptosis. UVB-induced increased expression of FasL on keratinocytes contributes to the apoptosis of intraepidermal T lymphocytes. T lymphocytes that remain in the lesion produce less IFN-γ. Neutrophil granulocytes are the source of enhanced IL-4 production upon UVB irradiation. Keratinocyte proliferation decreases during UVB phototherapy, as well as the expression of adhesion molecules and other activation markers by keratinocytes.

Local effects of UVB in psoriatic skin

Locally, NB-UVB phototherapy reverses several pathologic alterations in psoriasis. Keratinocyte proliferation decreases (185, 186). The number of T lymphocytes in the epidermis and dermis decreases, very probably due to apoptosis (187, 188) (Figure 5). The decrease in epidermal T cells correlated well with the clinical improvement, which was not true for the change in dermal T cell numbers (185). In addition, decrease in epidermal

T cell numbers also correlated with long-lasting remission after treatment (186). Interestingly, early relaps was seen in this study in all patients with remaining keratin 16 staining at the end of NB-UVB treatment (186). Epidermal and dermal T cell numbers were significantly more reduced by NB-UVB than by BB-UVB (187). It was shown *in vitro* that T lymphocytes are 10-fold more sensitive to the cytotoxic effects of UVB than keratinocytes, which explains their depletion from the epidermis upon UVB phototherapy (185). In addition, whereas hyperplastic keratinocytes in untreated psoriatic plaques do not express CD95L/FasL on their plasma membrane, after NB-UVB treatment there is strong and diffuse keratinocyte CD95L/FasL expression that coincided in a temporal fashion with depletion of intraepidermal T cells, indicating a role for FasL in epidermal T cell apoptosis (189).

T cells that remain in the lesions after 4 weeks of NB-UVB treatment produce less IFN-γ and IL-12 and more IL-4 (188, 190, 191) (Figure 5). Already one single dose of broadband UVB radiation resulted in decreased IFN-γ production and increased IL-4 production in psoriatic skin, and interestingly, neutrophils were found to be the source of the increased IL-4 production (192).

The number of Langerhans cells is decreased after NB-UVB phototherapy in non-lesional psoriatic skin (193). It is not known whether this is the case in the lesion as well.

Systemic effects of UVB in psoriasis

Cellular effects

Expression of adhesion molecules on the surface of circulating cells decreases during NB-UVB phototherapy. On the surface of PBMC of psoriatic patients, the skin homing cutaneous lymphocyte-associated antigen (CLA) decreased gradually during NB-UVB phototherapy, parallel with the PASI reduction (194). The same was observed for very late antigen (VLA)-4α, but not for ICAM-1 on PBMC. Accordingly, binding of psoriatic PBMC to psoriatic plaque tissue was completely inhibited after 14 days of daily NB-UVB treatment (195). This inhibition also correlated with decrease in ICAM-1 and E-selectin expression in the plaque tissue after NB-UVB treatment, demonstrating that UVB effects in the skin are also important for the reduced skin homing of circulating cells.

Cytokine secretion of *in vitro* stimulated PBMC isolated from psoriatic patients before and during UVB phototherapy was also investigated by several groups. In an early study, IL-2 production by peripheral blood lymphocytes upon stimulation with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA) was slightly decreased in patients with psoriasis as compared to healthy volunteers (196). In lymphocytes isolated after UVB phototherapy (15 UV treatments), IL-2 synthesis could not be induced by addition of PHA^+PMA. In addition, T helper cell activity of $CD4^+$ lymphocytes of psoriatic patients at the end of phototherapy was decreased as compared to that of cells from healthy controls, as measured by graft versus host response induced by these cells (196).

When PBMC of psoriatic patients, isolated before NB-UVB therapy and weekly hereafter for two weeks, were stimulated with superantigen *in vitro*, reduced production of IL-1β, IL-2, IL-5 and IL-6 and increased production of IL-10 was detected (180).

Furthermore, NB-UVB treatment (as well as treatment with PUVA and BB-UVB) resulted in reduced cytotoxic activity of circulating NK cells (197), whereas the number of circulating

NK cells remained unchanged (198). In contrast, local coal tar treatment did not affect the cytotoxic activity of NK cells (197).

Effects on soluble factors

IL-1α level is elevated 4 h after UVB radiation of psoriatic patients, but this effect is gone after 24 h (199). Serum IL-6 level is shown to be an early marker of clinical response to UVB phototherapy (200). Interestingly, serum levels of immunosuppressive IL-10 and proinflammatory TNF-α did not change during phototherapy (179), whereas serum soluble TNF-R1 levels decreased (201).

After phototherapy, vitamin D levels were dramatically increased by UVB (but not by PUVA) in psoriatic patients as well as in controls (202). Serum vitamin D levels in psoriasis patients increased less with NB-UVB than with broadband UVB phototherapy (203). This difference between BB-UVB and NB-UVB on vitamin D synthesis would indicate that vitamin D synthesis does not correlate with the clinical efficacy, as NB-UVB is at least as effective to treat psoriasis as BB-UVB.

In conclusion, psoriatic plaques differ from normal skin in their content of chromophores for UVB and in scavenging enzymes for ROS. Initiating molecular events of UVB-efficacy in psoriasis are not well defined. Depletion of epidermal lymphocytes, and decrease in adhesion molecules on PBMC correlated with the clinical improvement, confirming a critical role for T lymphocytes in psoriasis. UVB-induced expression of FasL on keratinocytes plays a role in this epidermal T cell apoptosis (Figure 5). In addition, cytokine expression of skin resident and circulating cells is altered upon UVB phototherapy.

GENOME-WIDE EXPRESSION STUDIES

Genome-wide expression studies in psoriasis

Several genome-wide expression studies have been published on the expression profile of lesional psoriatic skin. Some of them compared lesional skin with non-lesional skin (204-211), others compare psoriatic lesional skin with other inflammatory skin disorders (74, 212-215), and yet another group of studies followed patients receiving different treatments for psoriasis and compared gene expression profiles at different time points during treatment (87, 103, 216-218). These studies described the genomic signature of psoriasis lesions, helping thereby the better understanding of the molecular pathophysiology of the disease (Figure 6).

Studies comparing lesional psoriatic skin with non-lesional skin and normal skin confirmed increased proliferation, altered epidermal differentiation and barrier function, and an activated immune response in psoriatic skin, and provided new insights into the pathogenesis, such as a large contribution of CD11c+ myeloid dendritic cells, as well as an activation of interferon- and Wnt-signaling pathways (204-209). A study with basic importance for all other investigations showed that the psoriasis transcriptome does not depend on body site location, and correlates well with disease severity (210).

The psoriatic transcriptome was compared to cell-specific gene expression profiles to identify the contribution of different cellular subsets to the pathogenesis. With this approach

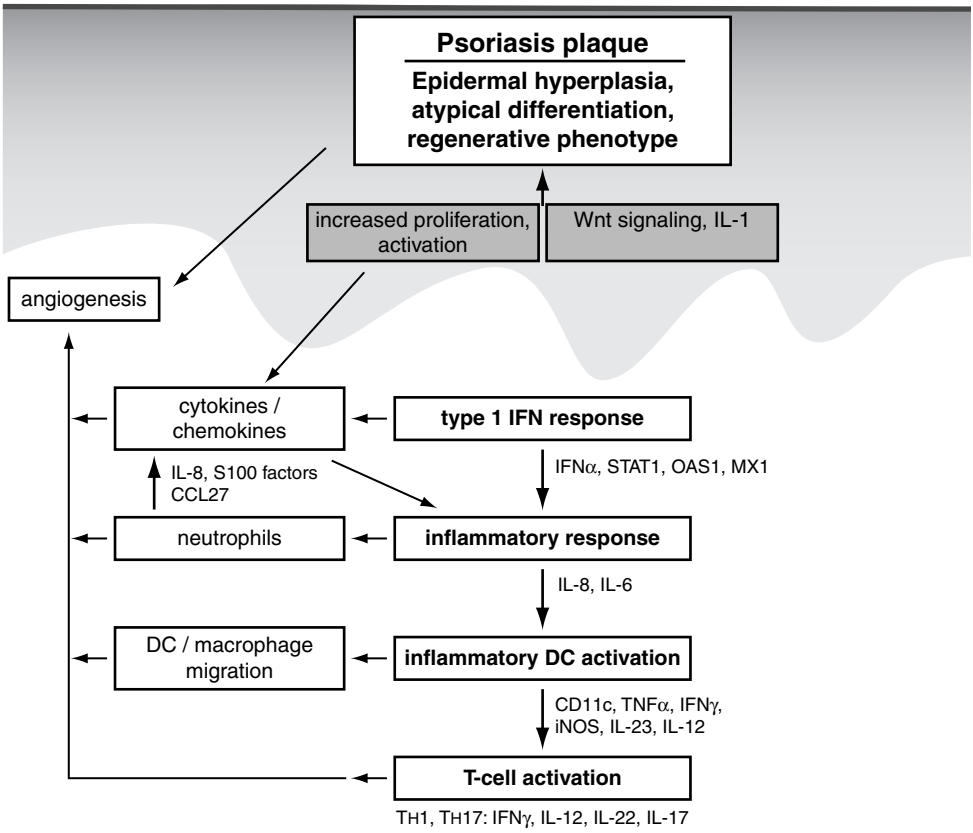

Figure 6. Scheme of the vicious circle of chronic psoriatic skin inflammation.
Modified from Kulski *et al.* (207). The activated epidermis produces chemokines, cytokines and angiogenic factors. In the dermis, pDC produce IFN-α, which triggers an adaptive immune response. Inflammatory myeloid DC activate T cells to produce proinflammatory cytokines, e.g. IL-22 and IFN-γ, which in turn stimulate the regenerative phenotype of epidermal keratinocytes. Neutrophils are attracted to the epidermis by keratinocyte-derived chemokines, and are themselves a source of chemoattractant molecules.

a surprisingly large contribution of myeloid DC to the psoriatic transcriptome was found (212, 219). Interestingly, gene expression profiling of IL-1α-stimulated keratinocytes identified a set of psoriasis genes, among them mainly antimicrobial peptides and other components of the epidermal barrier, emphasizing anomalies of innate immunity in psoriasis (220, 221).

Comparison of gene expression profiles of psoriasis and atopic dermatitis was performed by several research groups (74, 212-215). Advantage of this approach is that molecules that are secondary to the chronic inflammatory and hyperproliferative state will not be found, but only genes that are truly pathogenic (214). A major difference between psoriasis and atopic dermatitis is the high expression of antimicrobial peptides in psoriasis (214). Dermal myeloid DC produce different inflammatory mediators in psoriasis and atopic dermatitis, TNF-α and iNOS being dominant inflammatory mediators in psoriasis (212). Chemokine expression

also differentiates psoriasis from atopic dermatitis. Chemokines with high expression in psoriasis are CCL4, CCL20, CXCL2, CXCL8 and the chemokine receptor CXCR2 74). This comparative approach applied for lichen planus, atopic dermatitis and psoriasis also identified the type I IFN signature as being specific to psoriasis (215).

Psoriasis was also compared with squamous cell carcinoma using the microarray technology (222). Hereby a set of common upregulated genes was identified, including, surprisingly, molecules with immunoregulatory roles, such as IFI27, IL8RB, MX1, OAS1, OASL and STAT1 (222).

Genome-wide expression analysis gave new information on affected molecular pathways of established effective therapies, constructing thereby genomic signatures of successful treatment of psoriasis to guide development of new therapies. Cyclosporin A modulated the expression of Th1 and Th17 pathway genes, and reduced the expression of the DC-products TNF-α and iNOS in lesional psoriatic skin (87). Phototherapy (NB-UVB and Dead Sea treatment) suppressed S100A proteins, TNF-α and NF-kB target genes, and induced the expression of the antiproliferative molecule IGFBP7 in non-lesional epidermis (216). In PBMC of patients, successful dithranol treatment downregulated 18 genes, amongst them IL-8, TNFAIP6, COX2 and desmocollin 2 (217), while experimental IL-10 treatment downregulated a large set of chemokines and cytokines (218). Alefacept initially stimulated proinflammatory genes in PBMC of the treated patients (103). Furthermore, microarray technology was used to investigate the validity of an experimental treatment, cyclopamine, an inhibitor of the hedgehog signaling pathway. Hedgehog signaling was not activated in psoriasis, raising hereby questions by the use of cyclopamine in psoriasis (223).

Genome-wide expression studies in UVB-treated epidermal cells

Gene expression profiling studies described the transcriptomic changes following UVB radiation in cultured human keratinocytes, melanocytes, and healthy human epidermis. The UV-response of human epidermis differed from that of keratinocytes (224). In foreskin-derived primary human keratinocytes, three waves of changes in gene expression were described after irradiation with a single dose of 8 mJ/cm^2 BB-UVB, in a kinetic study (225): within 2 hours, transcription factors (e.g. JunB, JunD, c-fos, EGR1), signal transducing and cytoskeletal proteins are regulated, that change the phenotype of the cells to activated, paused cells. C-Myc expression is suppressed, which results in decreased proliferation. In the second wave, between the 4th and 8th hours after irradiation, cytokines, chemokines and growth factors are secreted to alert the surrounding tissue to the UV damage and attract inflammatory cells to the skin. Later, after 16 hours differentiation-associated genes (e.g. S100A proteins, involucrin, SKALP/elafin and small proline-rich proteins) are regulated, enhancing the epidermal protective covering (225). In another study, foreskin-derived primary human keratinocytes were irradiated with 10 mJ/cm2 BB-UVB, and 6 h later cells were harvested for gene expression profiling (226). At this time point, cytochrome c genes, genes involved in differentiation, transcription and transport were differentially expressed as compared to control cells. In a third publication foreskin-derived primary keratinocytes were irradiated with 10, 20 or 40 mJ/cm2 BB-UVB, and gene expression changes are analysed 4 and 24 h after irradiation (227). Here, too, induction of transcription factors, stress-response and proinflammatory genes were described, as well as a suppression of cell adhesion molecules

(in order to factilitate the migration and enhanced differentiation of the cells) and metabolism-associated genes (227). In addition, DNA repair enzymes and extracellular matrix proteases were induced (227, 228). Expression of cell adhesion molecules was downregulated (227).

In intact human epidermis, the expression profile was geared mainly towards DNA repair upon irradiation with a single dose of 4 MED with a BB-UVB source. In addition, S100A proteins, serine protease inhibitors, cell cycle regulating and apoptosis-related genes, cytokines, chemokines and matrix metalloproteases were induced (224).

In primary epidermal melanocyte cultures, a single irradiation with 25 mJ/cm^2 BB-UVB resulted in the induction of a set of p53-target genes 24 h after irradiation (229).

In conclusion, there are a few, independent studies on expressional changes in keratinocytes, melanocytes or complete epidermis upon BB-UVB irradiation, which describe global gene expression changes, involving several transcription factors, proinflammatory molecules, differentiation-associated genes, DNA repair and cell cycle genes. Effects of NB-UVB on normal epidermal cells were not investigated. However, in 2007 Hochberg *et al.* compared the global gene expression profile before and after NB-UVB therapy or Dead Sea treatment in non-lesional psoriatic epidermis (216). Differentially expressed genes included S100A proteins, dendritic cell markers, TNF-α target genes, matrix metalloproteinases and NF-κB target genes. In addition, insulin-like growth factor binding protein (IGFBP)-7 was identified as an antiproliferative molecule with low expression in non-lesional psoriatic skin, which was induced by NB-UVB therapy, possibly contributing to its antipsoriatic effect.

AIMS OF THE THESIS

The aim of this thesis is to illuminate the local molecular effects of NB-UVB phototherapy in the epidermis in the inflamed, psoriatic environment, and to identify molecular pathways that are responsible for the efficacy of this treatment modality in psoriasis.

In patients with psoriasis, global gene expression profiling was performed of epidermal samples acquired before, during and after NB-UVB phototherapy, in both lesional and non-lesional skin. This microarray study was complemented by *ex vivo* studies using skin of healthy subjects (**Chapter 2**). Based on the results of the gene expression profiling promising NB-UVB target molecules were selected and further investigated *in vitro, ex vivo* and in mice.

Our group previously described activated IFN-α signalling in psoriasis, and upregulation of receptors for double-stranded RNA in psoriasis lesions. This upregulation of dsRNA receptors was mimicked *in vitro* by IFN-α treatment of primary keratinocytes. We asked whether NB-UVB affects this aspect of innate immune activation in psoriasis, investigating dsRNA receptor expression and its response to NB-UVB radiation *in vivo* in psoriatic patients and *in vitro* in primary human keratinocytes (**Chapter 3**).

Recently a novel psoriasis-like skin inflammation mouse model was described by our group. We used this model to validate and explore findings of the microarray study on the transcription factor GATA3 (**Chapter 4**).

Furthermore we had the opportunity to compare the cellular effects of NB-UVB with those of the 585 nm pulsed dye laser in psoriasis, a treatment that selectively destructs dermal blood vessels, and thereby limiting the skin homing of inflammatory cells (**Chapter 5**).

The conclusions of these studies are summarized in **Chapter 6**.

REFERENCES

1. Nijsten, T., *et al.* (2005). Traditional systemic treatments have not fully met the needs of psoriasis patients: results from a national survey. *J Am Acad Dermatol* **52**(3 Pt 1): 434-44.
2. Nestle, F. O., Kaplan, D. H. and Barker, J. (2009). Psoriasis. *N Engl J Med* **361**(5): 496-509.
3. Bowcock, A. M. and Krueger, J. G. (2005). Getting under the skin: the immunogenetics of psoriasis. *Nat Rev Immunol* **5**(9): 699-711.
4. Farber, E. M. and Nall, M. L. (1974). The natural history of psoriasis in 5,600 patients. *Dermatologica* **148**(1): 1-18.
5. Trembath, R. C., *et al.* (1997). Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. *Hum Mol Genet* **6**(5): 813-20.
6. Allen, M. H., *et al.* (1999). A non-HLA gene within the MHC in psoriasis. *Lancet* **353**(9164): 1589-90.
7. Nair, R. P., *et al.* (2006). Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am J Hum Genet* **78**(5): 827-51.
8. Hollox, E. J., *et al.* (2008). Psoriasis is associated with increased beta-defensin genomic copy number. *Nat Genet* **40**(1): 23-5.
9. de Cid, R., *et al.* (2009). Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. *Nat Genet* **41**(2): 211-5.
10. Cargill, M., *et al.* (2007). A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* **80**(2): 273-90.
11. Capon, F., *et al.* (2007). Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. *Hum Genet* **122**(2): 201-6.
12. Nair, R. P., *et al.* (2009). Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* **41**(2): 199-204.
13. Fuchs, E. and Horsley, V. (2008). More than one way to skin. *Genes and Development* **22**(8): 976-85.
14. Gallo, R. L. and Huttner, K. M. (1998). Antimicrobial peptides: an emerging concept in cutaneous biology. *J Invest Dermatol* **111**(5): 739-43.
15. Tschachler, E. (2007). Psoriasis: the epidermal component. *Clin Dermatol* **25**(6): 589-95.
16. Ghadially, R., Reed, J. T. and Elias, P. M. (1996). Stratum corneum structure and function correlates with phenotype in psoriasis. *J Invest Dermatol* **107**(4): 558-64.
17. Thelu, J., Rossio, P. and Favier, B. (2002). Notch signalling is linked to epidermal cell differentiation level in basal cell carcinoma, psoriasis and wound healing. *BMC Dermatol* **2**: 7.
18. Mossner, R., *et al.* (2004). Variations in the genes encoding the peroxisome proliferator-activated receptors alpha and gamma in psoriasis. *Arch Dermatol Res* **296**(1): 1-5.
19. Komine, M., *et al.* (2007). Early inflammatory changes in the “perilesional skin” of psoriatic plaques: is there interaction between dendritic cells and keratinocytes? *J Invest Dermatol* **127**(8): 1915-22.
20. King, L. E., Jr., *et al.* (1990). Epidermal growth factor/transforming growth factor alpha receptors and psoriasis. *J Invest Dermatol* **95**(5 Suppl): 10S-12S.
21. Shen, C. S., *et al.* (2005). The expression of p63 during epidermal remodeling in psoriasis. *J Dermatol* **32**(4): 236-42.
22. Miura, H., *et al.* (2000). Involvement of insulin-like growth factor-I in psoriasis as a paracrine growth factor: dermal fibroblasts play a regulatory role in developing psoriatic lesions. *Arch Dermatol Res* **292**(12): 590-7.
23. Elder, J. T., *et al.* (1990). Growth factor and proto-oncogene expression in psoriasis. *J Invest Dermatol* **95**(5 Suppl): 7S-9S.
24. Steinhoff, M., Brzoska, T. and Luger, T. (2001). Keratinocytes in epidermal immune responses. *Curr Opin Allergy Clin Immunol* **1**: 469-476.
25. Gaspari, A. A. (2006). Innate and adaptive immunity and the pathophysiology of psoriasis. *J Am Acad Dermatol* **54**(3 Suppl 2): S67-80.
26. Bos, J. D., *et al.* (2005). Psoriasis: dysregulation of innate immunity. *Br J Dermatol* **152**(6): 1098-107.
27. Buchau, A. S. and Gallo, R. L. (2007). Innate immunity and antimicrobial defense systems in psoriasis. *Clin Dermatol* **25**(6): 616-24.
28. van der Fits, L., *et al.* (2004). In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *J Invest Dermatol* **122**(1): 51-60.

29. Nestle, F. O., *et al.* (2005). Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* **202**(1): 135-43.
30. Cooper, K. D., *et al.* (1990). Interleukin-1 in human skin: dysregulation in psoriasis. *J Invest Dermatol* **95**(5): 24S-26S.
31. Nickoloff, B. J., *et al.* (2006). Lessons learned from psoriatic plaques concerning mechanisms of tissue repair, remodeling, and inflammation. *J Investig Dermatol Symp Proc* **11**(1): 16-29.
32. Sano, S., *et al.* (1999). Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *Embo J* **18**(17): 4657-68.
33. Dauer, D. J., *et al.* (2005). Stat3 regulates genes common to both wound healing and cancer. *Oncogene* **24**(21): 3397-408.
34. Sano, S., *et al.* (2005). Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* **11**(1): 43-9.
35. Johansen, C., *et al.* (2006). Protein expression of TNF-alpha in psoriatic skin is regulated at a posttranscriptional level by MAPK-activated protein kinase 2. *J Immunol* **176**(3): 1431-8.
36. Funding, A. T., *et al.* (2007). Mitogen- and stress-activated protein kinase 2 and cyclic AMP response element binding protein are activated in lesional psoriatic epidermis. *J Invest Dermatol* **127**(8): 2012-9.
37. Haase, I., *et al.* (2001). A role for mitogen-activated protein kinase activation by integrins in the pathogenesis of psoriasis. *J Clin Invest* **108**(4): 527-36.
38. Otkjaer, K., *et al.* (2007). IL-20 gene expression is induced by IL-1beta through mitogen-activated protein kinase and NF-kappaB-dependent mechanisms. *J Invest Dermatol* **127**(6): 1326-36.
39. Otkjaer, K., *et al.* (2005). The dynamics of gene expression of interleukin-19 and interleukin-20 and their receptors in psoriasis. *Br J Dermatol* **153**(5): 911-8.
40. Wolk, K., *et al.* (2009). IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. *J Mol Med* **87**(5): 523-36.
41. Bos, J., *et al.* (1983). Immunocompetent cells in psoriasis. In situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* **275**(3): 181-9.
42. Zaba, L. C., *et al.* (2009). Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. *J Invest Dermatol* **129**(1): 79-88.
43. Cumberbatch, M., *et al.* (2006). Impaired Langerhans cell migration in psoriasis. *J Exp Med* **203**(4): 953-60.
44. Beurskens, T., *et al.* (1989). Epidermal proliferation and accumulation of polymorphonuclear leukocytes in the psoriatic lesion. *Dermatologica* **178**(2): 67-72.
45. van de Kerkhof, P. C. and Lammers, A. M. (1987). Intraepidermal accumulation of polymorphonuclear leukocytes in chronic stable plaque psoriasis. *Dermatologica* **174**(5): 224-7.
46. Wolk, K., *et al.* (2004). IL-22 increases the innate immunity of tissues. *Immunity* **21**(2): 241-54.
47. Uyemura, K., *et al.* (1993). The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. *J Invest Dermatol.* **101**(5): 701-5.
48. Lee, E., *et al.* (2004). Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* **199**(1): 125-30.
49. Johansen, C., *et al.* (2009). Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. *Br J Dermatol* **160**(2): 319-24.
50. Zaba, L. C., Krueger, J. G. and Lowes, M. A. (2009). Resident and "inflammatory" dendritic cells in human skin. *J Invest Dermatol* **129**(2): 302-8.
51. Wollenberg, A., *et al.* (2002). Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. *J Invest Dermatol* **119**(5): 1096-102.
52. Downs, A. M. and Dunnill, M. G. (2000). Exacerbation of psoriasis by interferon-alpha therapy for hepatitis C. *Clin Exp Dermatol* **25**(4): 351-2.
53. Funk, J., *et al.* (1991). Psoriasis induced by interferon-alpha. *Br J Dermatol* **125**(5): 463-5.
54. Ketikoglou, I., *et al.* (2005). Extensive psoriasis induced by pegylated interferon alpha-2b treatment for chronic hepatitis B. *Eur J Dermatol* **15**(2): 107-9.
55. Pauluzzi, P., *et al.* (1993). Psoriasis exacerbation induced by interferon-alpha. Report of two cases. *Acta Derm Venereol* **73**(5): 395.
56. Lande, R., *et al.* (2007). Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**(7162): 564-9.

57. Zaba, L. C., *et al.* (2007). Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* **204**(13): 3183-94.
58. Lowes, M. A., *et al.* (2005). Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* **102**(52): 19057-62.
59. Wang, F., *et al.* (2006). Prominent production of IL-20 by CD68+/CD11c+ myeloid-derived cells in psoriasis: Gene regulation and cellular effects. *J Invest Dermatol* **126**(7): 1590-9.
60. Clark, R., *et al.* (2006). The vast majority of CLA+ T cells are resident in normal skin. *J Immunol* **176**(7): 4431-9.
61. Albanesi, C., De Pita, O. and Girolomoni, G. (2007). Resident skin cells in psoriasis: a special look at the pathogenetic functions of keratinocytes. *Clin Dermatol* **25**(6): 581-8.
62. Nair, R. P., *et al.* (2009). Psoriasis bench to bedside: genetics meets immunology. *Arch Dermatol* **145**(4): 462-4.
63. Prens, E. P., *et al.* (1991). The autologous mixed epidermal cell-T lymphocyte reaction is elevated in psoriasis: a crucial role for epidermal HLA-DR+/CD1a- antigen-presenting cells. *J Invest Dermatol* **96**(6): 880-7.
64. Lowes, M. A., *et al.* (2008). Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* **128**(5): 1207-11.
65. Li, Y. Y., Zollner, T. M. and Schon, M. P. (2008). Targeting leukocyte recruitment in the treatment of psoriasis. *Clin Dermatol* **26**(5): 527-38.
66. Eedy, D. J., *et al.* (1990). Clearance of severe psoriasis after allogenic bone marrow transplantation. *Bmj* **300**(6729): 908.
67. Gardembas-Pain, M., *et al.* (1990). Psoriasis after allogeneic bone marrow transplantation. *Arch Dermatol* **126**(11): 1523.
68. Boehncke, W., *et al.* (1995). A subset of macrophages located along the basement membrane ("lining cells") is a characteristic histopathological feature of psoriasis. *Am J Dermatopathol.* **17**(2): 139-44.
69. Stratis, A., *et al.* (2006). Pathogenic role for skin macrophages in a mouse model of keratinocyte-induced psoriasis-like skin inflammation. *J Clin Invest* **116**(8): 2094-104.
70. Wang, H., *et al.* (2006). Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasiform skin inflammation. *J Clin Invest* **116**(8): 2105-14.
71. Lowes, M. A., Bowcock, A. M. and Krueger, J. G. (2007). Pathogenesis and therapy of psoriasis. *Nature* **445**(7130): 866-73.
72. Homey, B. and Meller, S. (2008). Chemokines and other mediators as therapeutic targets in psoriasis vulgaris. *Clin Dermatol* **26**(5): 539-45.
73. Homey, B., *et al.* (2002). CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med* **8**(2): 157-65.
74. Nomura, I., *et al.* (2003). Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol.* **112**(6): 1195-202.
75. Caruso, R., *et al.* (2009). Involvement of interleukin-21 in the epidermal hyperplasia of psoriasis. *Nat Med* **15**(9): 1013-1015.
76. Wolk, K., *et al.* (2006). IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur J Immunol* **36**(5): 1309-23.
77. Sa, S. M., *et al.* (2007). The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol* **178**(4): 2229-40.
78. Nograles, K. E., *et al.* (2008). Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *Br J Dermatol* **159**(5): 1092-102.
79. Wei, L., *et al.* (1999). IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. *J Pathol* **187**(3): 358-64.
80. Zheng, Y., *et al.* (2007). Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* **445**(7128): 648-51.
81. Blauvelt, A. (2008). T-helper 17 cells in psoriatic plaques and additional genetic links between IL-23 and psoriasis. *J Invest Dermatol* **128**(5): 1064-7.

82. Piskin, G., *et al.* (2006). In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. *J Immunol* **176**(3): 1908-15.
83. Kryczek, I., *et al.* (2008). Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. *J Immunol* **181**(7): 4733-41.
84. Nair, R. P., *et al.* (2008). Polymorphisms of the IL12B and IL23R genes are associated with psoriasis. *J Invest Dermatol* **128**(7): 1653-61.
85. Krueger, G. G., *et al.* (2007). A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* **356**(6): 580-92.
86. van der Fits, L., *et al.* (2009). Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* **182**(9): 5836-45.
87. Haider, A. S., *et al.* (2008). Identification of cellular pathways of "type 1," Th17 T cells, and TNF- and inducible nitric oxide synthase-producing dendritic cells in autoimmune inflammation through pharmacogenomic study of cyclosporine A in psoriasis. *J Immunol* **180**(3): 1913-20.
88. Haider, A. S., *et al.* (2008). Insights into gene modulation by therapeutic TNF and IFNgamma antibodies: TNF regulates IFNgamma production by T cells and TNF-regulated genes linked to psoriasis transcriptome. *J Invest Dermatol*. **128**(3): 655-66.
89. Tracey, D., *et al.* (2008). Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* **117**(2): 244-79.
90. Costa, C., Incio, J. and Soares, R. (2007). Angiogenesis and chronic inflammation: cause or consequence? *Angiogenesis* **10**(3): 149-66.
91. Detmar, M. (2004). Evidence for vascular endothelial growth factor (VEGF) as a modifier gene in psoriasis. *J Invest Dermatol* **122**(1): xiv-xv.
92. Xia, Y. P., *et al.* (2003). Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. *Blood* **102**(1): 161-8.
93. Katugampola, G. A., Rees, A. M. and Lanigan, S. W. (1995). Laser treatment of psoriasis. *Br J Dermatol* **133**(6): 909-13.
94. Ros, A. M., *et al.* (1996). Psoriasis response to the pulsed dye laser. *Lasers Surg Med* **19**(3): 331-5.
95. Hern, S., *et al.* (2001). Immunohistochemical evaluation of psoriatic plaques following selective photothermolysis of the superficial capillaries. *Br J Dermatol* **145**(1): 45-53.
96. Zelickson, B. D., *et al.* (1996). Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. *J Am Acad Dermatol* **35**(1): 64-8.
97. Ehrlich, A., *et al.* (2004). Micellar paclitaxel improves severe psoriasis in a prospective phase II pilot study. *J Am Acad Dermatol* **50**(4): 533-40.
98. Sauder, D., *et al.* (2002). Neovastat (AE-941), an inhibitor of angiogenesis: Randomized phase I/II clinical trial results in patients with plaque psoriasis. *J Am Acad Dermatol* **47**(4): 535-41.
99. Halin, C., *et al.* (2008). Inhibition of chronic and acute skin inflammation by treatment with a vascular endothelial growth factor receptor tyrosine kinase inhibitor. *Am J Pathol* **173**(1): 265-77.
100. Stojadinovic, O., *et al.* (2007). Novel genomic effects of glucocorticoids in epidermal keratinocytes: inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation. *J Biol Chem* **282**(6): 4021-34.
101. Schon, M. P. and Boehncke, W. H. (2005). Psoriasis. *N Engl J Med* **352**(18): 1899-912.
102. Chamian, F., *et al.* (2005). Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc Natl Acad Sci U S A* **102**(6): 2075-80.
103. Haider, A. S., *et al.* (2007). Novel insight into the agonistic mechanism of alefacept *in vivo*: differentially expressed genes may serve as biomarkers of response in psoriasis patients. *J Immunol* **178**(11): 7442-9.
104. Haider, A. S., *et al.* (2007). Effects of etanercept are distinct from infliximab in modulating proinflammatory genes in activated human leukocytes. *J Investig Dermatol Symp Proc* **12**(1): 9-15.
105. Malaviya, R., *et al.* (2006). Etanercept induces apoptosis of dermal dendritic cells in psoriatic plaques of responding patients. *J Am Acad Dermatol* **55**(4): 590-7.
106. Rozenblit, M. and Lebwohl, M. (2009). New biologics for psoriasis and psoriatic arthritis. *Dermatol Ther* **22**(1): 56-60.

107. Papp, K. A., *et al.* (2008). Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* **371**(9625): 1675-84.
108. Leonardi, C. L., *et al.* (2008). Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* **371**(9625): 1665-74.
109. Reich, K., Yasothan, U. and Kirkpatrick, P. (2009). Ustekinumab. *Nat Rev Drug Discov* **8**(5): 355-6.
110. Kimball, A. B., *et al.* (2008). Safety and efficacy of ABT-874, a fully human interleukin 12/23 monoclonal antibody, in the treatment of moderate to severe chronic plaque psoriasis: results of a randomized, placebo-controlled, phase 2 trial. *Arch Dermatol* **144**(2): 200-7.
111. Schmitt, J., *et al.* (2008). Efficacy and tolerability of biologic and nonbiologic systemic treatments for moderate-to-severe psoriasis: meta-analysis of randomized controlled trials. *Br J Dermatol* **159**(3): 513-26.
112. Robertshaw, H. and Friedmann, P. S. (2005). Pioglitazone: a promising therapy for psoriasis. *Br J Dermatol* **152**(1): 189-91.
113. Mittal, R., *et al.* (2009). Efficacy and safety of combination Acitretin and Pioglitazone therapy in patients with moderate to severe chronic plaque-type psoriasis: a randomized, double-blind, placebo-controlled clinical trial. *Arch Dermatol* **145**(4): 387-93.
114. Murase, J. E., *et al.* (2005). Hormonal effect on psoriasis in pregnancy and post partum. *Arch Dermatol* **141**(5): 601-6.
115. Bolognia, J., Jorizzo, J. and Rapini, R. (2003). *Dermatology*. Mosby, Edinburgh, London, New York, Oxford Philadelphia, St Louis, Sidney, Toronto.
116. Stern, R. (2007). Psoralen and ultraviolet a light therapy for psoriasis. *N Engl J Med* **357**(7): 682-90.
117. Halliday, G. M. and Rana, S. (2008). Waveband and dose dependency of sunlight-induced immunomodulation and cellular changes. *Photochem Photobiol* **84**(1): 35-46.
118. Halverstam, C. and Lebwohl, M. (2008). Nonstandard and off-label therapies for psoriasis. *Clin Dermatol* **26**(5): 546-53.
119. Bonis, B., *et al.* (1997). 308 nm UVB excimer laser for psoriasis. *Lancet* **350**(9090): 1522.
120. Gerber, W., *et al.* (2003). Ultraviolet B 308-nm excimer laser treatment of psoriasis: a new phototherapeutic approach. *Br J Dermatol* **149**(6): 1250-8.
121. Fisher, T., Alsins, J. and Berne, B. (1984). Ultraviolet-action spectrum and evaluation of ultraviolet lamps for psoriasis healing. . *Int J Dermatol* **23**: 633-637.
122. Parrish, J. A. and Jaenicke, K. F. (1981). Action spectrum for phototherapy of psoriasis. *J Invest Dermatol* **76**(5): 359-62.
123. Coven, T. R., *et al.* (1997). Narrowband UV-B produces superior clinical and histopathological resolution of moderate-to-severe psoriasis in patients compared with broadband UV-B. *Arch Dermatol* **133**(12): 1514-22.
124. Walters, I. B., *et al.* (1999). Suberythemogenic narrow-band UVB is markedly more effective than conventional UVB in treatment of psoriasis vulgaris. *J Am Acad Dermatol* **40**(6 Pt 1): 893-900.
125. Picot, E., *et al.* (1992). Treatment of psoriasis with a 311-nm UVB lamp. *Br J Dermatol* **127**(5): 509-12.
126. Storbeck, K., *et al.* (1993). Narrow-band UVB (311 nm) versus conventional broad-band UVB with and without dithranol in phototherapy for psoriasis. *J Am Acad Dermatol* **28**(2 Pt 1): 227-31.
127. Green, C., *et al.* (1988). 311 nm UVB phototherapy--an effective treatment for psoriasis. *Br J Dermatol* **119**(6): 691-6.
128. Larko, O. (1989). Treatment of psoriasis with a new UVB-lamp. *Acta Derm Venereol* **69**(4): 357-9.
129. Van Weelden, H., *et al.* (1988). A new development in UVB phototherapy of psoriasis. *Br J Dermatol* **119**: 11-19.
130. Johnson, B., *et al.* (1988). Ultraviolet radiation phototherapy for psoriasis; the use of a newnarrow band UVB fluorescent lamp. In *Light in biology and medicine* (Douglas, R., Moan, J. and F, D. A. eds.), pp. 173-179. Plenum, Oxford.
131. Karvonen, J., Kokkonen, L.-E. and Routsalainen, E. (1989). 311 nm UVB lamps in the treatment of psoriasis with the Ingram regimen. . *Acta Derm Venereol (Stockholm)* **69**: 82-85.
132. Srinivas, C. (2002). Minimal erythema dose (Med) to narrow band ultraviolet-B (NB-UVB) broad band ultraviolet-B (BB-UVB)--a pilot study. *Indian J Dermatol Venereol Leprol.* **68**(2): 63-4.

133. Tejasvi, T., Sharma, V. and Kaur, J. (2007). Determination of minimal erythemal dose for narrow band-ultraviolet B radiation in north Indian patients: comparison of visual and Dermaspectrometer readings. *Indian J Dermatol Venereol Leprol* **73**(2): 97-9.
134. Youn, J., *et al.* (2003). Assessment of the usefulness of skin phototype and skin color as the parameter of cutaneous narrow band UVB sensitivity in psoriasis patients. *Photodermatol Photoimmunol Photomed* **19**(5): 261-4.
135. Morita, A., *et al.* (2009). Feasibility and accuracy of a newly developed hand-held device with a flat-type fluorescent lamp for measuring the minimal erythema dose for narrow-band UVB therapy. *Photodermatol Photoimmunol Photomed. 2009 Feb;25(1):41-4.* **25**(1): 41-44.
136. el-Ghorr, A. A. and Norval, M. (1997). Biological effects of narrow-band (311 nm TL01) UVB irradiation: a review. *J Photochem Photobiol B* **38**(2-3): 99-106.
137. Gibbs, N., *et al.* (1995). The phototumorigenic potential of broad-band (270-350 nm) and narrow-band (311-313 nm) phototherapy sources cannot be predicted by their edematogenic potential in hairless mouse skin. *J Invest Dermatol* **104**: 359-363.
138. Weischer, M., *et al.* (2004). No evidence for increased skin cancer risk in psoriasis patients treated with broadband or narrowband UVB phototherapy: a first retrospective study. *Acta Derm Venereol* **84**(5): 370-4.
139. Hearn, R., *et al.* (2008). Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. *Br J Dermatol* **159**: 931-935.
140. Man, I., *et al.* (2005). The photocarcinogenic risk of narrowband UVB (TL-01) phototherapy: early follow-up data. *Br J Dermatol.* **152**(4): 755-7.
141. Black, R. J. and Gavin, A. T. (2006). Photocarcinogenic risk of narrowband ultraviolet B (TL-01) phototherapy: early follow-up data. *Br J Dermatol* **154**(3): 566-7.
142. Choi, C. P., *et al.* (2007). The effect of narrowband ultraviolet B on the expression of matrix metalloproteinase-1, transforming growth factor-beta1 and type I collagen in human skin fibroblasts. *Clin Exp Dermatol* **32**(2): 180-5.
143. McGregor, J. and Hawk, J. (2003). Acute effects of ultraviolet radiation on the skin. In *Fitzpatrick's Dermatology in General Medicine* (Freedberg, I., *et al.* eds.), The McGraw-Hill Companies, Inc.
144. Young, A. R., *et al.* (1998). The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *J Invest Dermatol* **111**(6): 982-8.
145. Maccubbin, A. E., *et al.* (1995). DNA damage in UVB-irradiated keratinocytes. *Carcinogenesis* **16**(7): 1659-60.
146. Jans, J., *et al.* (2006). Differential role of basal keratinocytes in UV-induced immunosuppression and skin cancer. *Mol Cell Biol* **26**(22): 8515-26.
147. Schade, N., Esser, C. and Krutmann, J. (2005). Ultraviolet B radiation-induced immunosuppression: molecular mechanisms and cellular alterations. *Photochem Photobiol Sci* **4**(9): 699-708.
148. Rosette, C. and Karin, M. (1996). Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* **274**(5290): 1194-7.
149. Aragane, Y., *et al.* (1998). Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. *J Cell Biol* **140**(1): 171-82.
150. McLoone, P., *et al.* (2005). An action spectrum for the production of cis-urocanic acid in human skin *in vivo*. *J Invest Dermatol* **124**(5): 1071-4.
151. De Fabo, E. C. and Noonan, F. P. (1983). Mechanism of immune suppression by ultraviolet irradiation *in vivo*. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. *J Exp Med* **158**(1): 84-98.
152. Kammeyer, A., *et al.* (1997). Prolonged increase of cis-urocanic acid levels in human skin and urine after single total-body ultraviolet exposures. *Photochem Photobiol* **65**(3): 593-8.
153. Walterscheid, J., *et al.* (2006). Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor. *Proc Natl Acad Sci U S A.* **103**(46): 17420-5.
154. Kaneko, K., *et al.* (2009). cis-Urocanic Acid Stimulates Primary Human Keratinocytes Independently of Serotonin or Platelet-Activating Factor Receptors. *J Invest Dermatol.* **Epub ahead of print**.
155. Ullrich, S. E. (1995). The role of epidermal cytokines in the generation of cutaneous immune reactions and ultraviolet radiation-induced immune suppression. *Photochem Photobiol* **62**(3): 389-401.

156. Feldmeyer, L., *et al.* (2007). The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. *Curr Biol* **17**(13): 1140-5.
157. Murphy, G., *et al.* (2001). The molecular determinants of sunburn cell formation. *Exp Dermatol* **10**(3): 155-60.
158. Lehmann, B. (2009). Role of the vitamin D3 pathway in healthy and diseased skin--facts, contradictions and hypotheses. *Exp Dermatol* **18**(2): 97-108.
159. Milde, P., *et al.* (1991). Expression of 1,25-dihydroxyvitamin D3 receptors in normal and psoriatic skin. *J Invest Dermatol* **97**(2): 230-9.
160. De Haes, P., *et al.* (2005). 1,25-Dihydroxyvitamin D3 and analogues protect primary human keratinocytes against UVB-induced DNA damage. *J Photochem Photobiol B* **78**(2): 141-8.
161. Gupta, R., *et al.* (2007). Photoprotection by 1,25 dihydroxyvitamin D3 is associated with an increase in p53 and a decrease in nitric oxide products. *J Invest Dermatol* **127**(3): 707-15.
162. Sigmundsdottir, H., *et al.* (2007). DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. *Nat Immunol* **8**(3): 285-93.
163. Mallbris, L., *et al.* (2005). UVB upregulates the antimicrobial protein hCAP18 mRNA in human skin. *J Invest Dermatol. 2005 Nov;125(5):1072-4.* **125**(5): 1072-1074.
164. Glaser, R., *et al.* (2009). UV-B radiation induces the expression of antimicrobial peptides in human keratinocytes in vitro and *in vivo*. *J Allergy Clin Immunol* **123**(5): 1117-23.
165. Granstein, R. (2003). Photoimmunology. In *Fitzpatrick's Dermatology in General Medicine* (Freedberg, I., *et al.* eds.), The McGraw-Hill Companies, Inc.
166. Murphy, K., Travers, P. and Walport, M. (2005). *Janeway's Immunobiology*. Garland Science Publishing.
167. Schwarz, T. (2005). Regulatory T cells induced by ultraviolet radiation. *Int Arch Allergy Immunol* **137**(3): 187-93.
168. Schwarz, A., *et al.* (2005). Prevention of UV radiation-induced immunosuppression by IL-12 is dependent on DNA repair. *J Exp Med* **201**(2): 173-9.
169. Schwarz, A., *et al.* (2000). Evidence for functional relevance of CTLA-4 in ultraviolet-radiation-induced tolerance. *J Immunol* **165**(4): 1824-31.
170. el-Ghorr, A. A. and Norval, M. (1995). A monoclonal antibody to cis-urocanic acid prevents the ultraviolet-induced changes in Langerhans cells and delayed hypersensitivity responses in mice, although not preventing dendritic cell accumulation in lymph nodes draining the site of irradiation and contact hypersensitivity responses. *J Invest Dermatol* **105**(2): 264-8.
171. Norval, M., *et al.* (1989). Urocanic acid analogues and the suppression of the delayed type hypersensitivity response to Herpes simplex virus. *Photochem Photobiol* **49**(5): 633-9.
172. Beissert, S., *et al.* (2001). IL-12 prevents the inhibitory effects of cis-urocanic acid on tumor antigen presentation by Langerhans cells: implications for photocarcinogenesis. *J Immunol* **167**(11): 6232-8.
173. Schwarz, A., *et al.* (2002). Interleukin-12 suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair. *Nat Cell Biol* **4**(1): 26-31.
174. Schwarz, A., *et al.* (2006). IL-18 reduces ultraviolet radiation-induced DNA damage and thereby affects photoimmunosuppression. *J Immunol* **176**(5): 2896-901.
175. Schwarz, T., *et al.* (1986). Inhibition of the induction of contact hypersensitivity by a UV-mediated epidermal cytokine. *J Invest Dermatol* **87**(2): 289-91.
176. Loser, K., *et al.* (2006). Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. *Nat Med* **12**(12): 1372-9.
177. Cooper, K. D., *et al.* (1992). UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, CD1a-DR+ epidermal macrophage induction, and Langerhans cell depletion. *Proc Natl Acad Sci U S A* **89**(18): 8497-501.
178. Sugiyama, H., *et al.* (2005). Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. *J Immunol* **174**(1): 164-73.
179. McLoone, P., *et al.* (2004). Whole-body UVB (TL-01) or UVA-1 irradiation does not alter the levels of immunomodulatory cytokines in the serum of human volunteers. *Photodermatol Photoimmunol Photomed* **20**(2): 76-80.

180. Sigmundsdottir, H., *et al.* (2005). Narrowband-UVB irradiation decreases the production of pro-inflammatory cytokines by stimulated T cells. *Arch Dermatol Res*. **297**(1):39-42.
181. Snellman, E., *et al.* (1992). Effect of psoriasis heliotherapy on epidermal urocanic acid isomer concentrations. *Acta Derm Venereol* **72**(3): 231-3.
182. Snellman, E., *et al.* (2003). Effect of the spectral range of a UV lamp on the production of cyclobutane pyrimidine dimers in human skin in situ. *Photodermatol Photoimmunol Photomed.* **19**(6): 281-6.
183. Bataille, V., *et al.* (2000). Photoadaptation to ultraviolet (UV) radiation *in vivo*: photoproducts in epidermal cells following UVB therapy for psoriasis. *Br J Dermatol* **143**(3): 477-83.
184. Smith, G., *et al.* (2003). Quantitative real-time reverse transcription-polymerase chain reaction analysis of drug metabolizing and cytoprotective genes in psoriasis and regulation by ultraviolet radiation. *J Invest Dermatol* **121**(2): 390-8.
185. Krueger, J. G., *et al.* (1995). Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. *J Exp Med* **182**(6): 2057-68.
186. Carrascosa, J., *et al.* (2006). Effects of narrowband UV-B on pharmacodynamic markers of response to therapy: an immunohistochemical study over sequential samples. *J Cutan Pathol* **34**(10): 769-76.
187. Ozawa, M., *et al.* (1999). 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. *J Exp Med* **189**(4): 711-8.
188. Walters, I. B., *et al.* (2003). Narrowband (312-nm) UV-B suppresses interferon gamma and interleukin (IL) 12 and increases IL-4 transcripts: differential regulation of cytokines at the single-cell level. *Arch Dermatol* **139**(2): 155-61.
189. Gutierrez-Steil, C., *et al.* (1998). Sunlight-induced basal cell carcinoma tumor cells and ultraviolet-B-irradiated psoriatic plaques express Fas ligand (CD95L). *J Clin Invest* **101**(1): 33-9.
190. Piskin, G., *et al.* (2003). Ultraviolet-B irradiation decreases IFN-gamma and increases IL-4 expression in psoriatic lesional skin in situ and in cultured dermal T cells derived from these lesions. *Exp Dermatol* **12**(2): 172-80.
191. Piskin, G., *et al.* (2004). T cells in psoriatic lesional skin that survive conventional therapy with NB-UVB radiation display reduced IFN-gamma expression. *Arch Dermatol Res* **295**(12): 509-16.
192. Piskin, G., *et al.* (2003). IL-4 expression by neutrophils in psoriasis lesional skin upon high-dose UVB exposure. *Dermatology* **207**(1): 51-3.
193. DeSilva, B., *et al.* (2008). Local effects of TL01 phototherapy in psoriasis. *Photodermatol Photoimmunol Photomed* **24**(5): 268-9.
194. Sigmundsdottir, H., Gudjonsson, J. E. and Valdimarsson, H. (2003). The effects of ultraviolet B treatment on the expression of adhesion molecules by circulating T lymphocytes in psoriasis. *Br J Dermatol* **148**(5): 996-1000.
195. Cai, J. P., *et al.* (1996). UVB therapy decreases the adhesive interaction between peripheral blood mononuclear cells and dermal microvascular endothelium, and regulates the differential expression of CD54, VCAM-1, and E-selectin in psoriatic plaques. *Br J Dermatol* **134**(1): 7-16.
196. Shohat, B., *et al.* (1993). Effect of UVB and AS101 on interleukin-2 production and helper activity in psoriatic patients. *Nat Immun* **12**(1): 50-5.
197. Gilmour, J. W., *et al.* (1993). Effect of phototherapy and urocanic acid isomers on natural killer cell function. *J Invest Dermatol* **101**(2): 169-74.
198. Tobin, A. M., *et al.* (2009). The effects of phototherapy on the numbers of circulating natural killer cells and T lymphocytes in psoriasis. *Photodermatol Photoimmunol Photomed* **25**(2): 109-10.
199. Konnikov, N., Pincus, S. H. and Dinarello, C. A. (1989). Elevated plasma interleukin-1 levels in humans following ultraviolet light therapy for psoriasis. *J Invest Dermatol* **92**(2): 235-9.
200. Bonifati, C., *et al.* (1994). Serum interleukin-6 levels as an early marker of therapeutic response to UVB radiation and topical steroids in psoriatic patients. *Int J Clin Lab Res* **24**(2): 122-3.
201. Serwin, A. B., Sokolowska, M. and Chodynicka, B. (2005). Soluble tumor necrosis factor alpha receptor type 1 in psoriasis patients treated with narrowband ultraviolet B. *Photodermatol Photoimmunol Photomed* **21**(4): 210-1.
202. Guilhou, J., *et al.* (1990). Vitamin D metabolism in psoriasis before and after phototherapy. *Acta Derm Venereol* **70**(4): 351-4.
203. Osmancevic, A., *et al.* (2009). Vitamin D production in psoriasis patients increases less with narrowband than with broadband ultraviolet B phototherapy. *Photodermatol Photoimmunol Photomed* **25**(3): 119-23.

204. Bowcock, A. M., *et al.* (2001). Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* **10**(17): 1793-805.
205. Oestreicher, J. L., *et al.* (2001). Molecular classification of psoriasis disease-associated genes through pharmacogenomic expression profiling. *Pharmacogenomics J* **1**(4): 272-87.
206. Zhou, X., *et al.* (2003). Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* **13**(1): 69-78.
207. Kulski, J. K., *et al.* (2005). Gene expression profiling of Japanese psoriatic skin reveals an increased activity in molecular stress and immune response signals. *J Mol Med* **83**(12): 964-75.
208. Reischl, J., *et al.* (2007). Increased expression of Wnt5a in psoriatic plaques. *J Invest Dermatol* **127**(1): 163-9.
209. Yao, Y., *et al.* (2008). Type I interferon: potential therapeutic target for psoriasis? *PLoS ONE* **3**(7): e2737.
210. Quekenborn-Trinquet, V., *et al.* (2005). Gene expression profiles in psoriasis: analysis of impact of body site location and clinical severity. *Br J Dermatol* **152**(3): 489-504.
211. Gudjonsson, J. E., *et al.* (2009). Global gene expression analysis reveals evidence for decreased lipid biosynthesis and increased innate immunity in uninvolved psoriatic skin. *J Invest Dermatol*. **Epub ahead of print.**
212. Guttman-Yassky, E., *et al.* (2007). Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis. *J Allergy Clin Immunol* **119**(5): 1210-7.
213. Ito, M., *et al.* (2004). Gene expression of enzymes for tryptophan degradation pathway is upregulated in the skin lesions of patients with atopic dermatitis or psoriasis. *J Dermatol Sci* **36**(3): 157-64.
214. de Jongh, G. J., *et al.* (2005). High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis. *J Invest Dermatol* **125**(6): 1163-73.
215. Wenzel, J., *et al.* (2008). Gene expression profiling of lichen planus reflects CXCL9+-mediated inflammation and distinguishes this disease from atopic dermatitis and psoriasis. *J Invest Dermatol* **128**(1): 67-78.
216. Hochberg, M., *et al.* (2007). Genomic-scale analysis of psoriatic skin reveals differentially expressed insulin-like growth factor-binding protein-7 after phototherapy. *Br J Dermatol* **156**(2): 289-300.
217. Koczan, D., *et al.* (2005). Gene expression profiling of peripheral blood mononuclear leukocytes from psoriasis patients identifies new immune regulatory molecules. *Eur J Dermatol* **15**(4): 251-7.
218. Jung, M., *et al.* (2004). Expression profiling of IL-10-regulated genes in human monocytes and peripheral blood mononuclear cells from psoriatic patients during IL-10 therapy. *Eur J Immunol* **34**(2): 481-93.
219. Haider, A. S., *et al.* (2008). Cellular Genomic Maps Help Dissect Pathology in Human Skin Disease. *J Invest Dermatol*. **128**(3):606-15.
220. Mee, J. B., *et al.* (2006). Interleukin-1: a key inflammatory mediator in psoriasis? *Cytokine* **33**(2): 72-8.
221. Mee, J. B., *et al.* (2007). The psoriatic transcriptome closely resembles that induced by interleukin-1 in cultured keratinocytes: dominance of innate immune responses in psoriasis. *Am J Pathol* **171**(1): 32-42.
222. Haider, A. S., *et al.* (2006). Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. *J Invest Dermatol* **126**(4): 869-81.
223. Gudjonsson, J. E., *et al.* (2009). Lack of evidence for activation of the hedgehog pathway in psoriasis. *J Invest Dermatol* **129**(3): 635-40.
224. Enk, C. D., *et al.* (2006). The UVB-induced gene expression profile of human epidermis *in vivo* is different from that of cultured keratinocytes. *Oncogene* **25**(18): 2601-14.
225. Li, D., *et al.* (2001). Rays and arrays: the transcriptional program in the response of human epidermal keratinocytes to UVB illumination. *FASEB J* **15**(13): 2533-5.
226. Takao, J., *et al.* (2002). Genomic scale analysis of the human keratinocyte response to broad-band ultraviolet-B irradiation. *Photodermatol Photoimmunol Photomed.* **18**(1): 5-13.
227. Sesto, A., *et al.* (2002). Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *Proc Natl Acad Sci U S A* **99**(5): 2965-70.
228. Dazard JE, G. H., Amariglio N, Rechavi G, Domany E, Givol D. (2003). Genome-wide comparison of human keratinocyte and squamous cell carcinoma responses to UVB irradiation: implications for skin and epithelial cancer. *Oncogene* **22**(19): 2993-3006.
229. Yang, G., *et al.* (2006). Expression profiling of UVB response in melanocytes identifies a set of p53-target genes. *J Invest Dermatol* **126**(11): 2490-506.

2

Narrow-band UVB phototherapy inhibits epidermal interferon and Th17 pathways in psoriasis

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ABSTRACT

Narrow-band ultraviolet-B (NB-UVB) phototherapy is an effective treatment for psoriasis. The molecular mechanisms underlying its efficacy are incompletely understood. We aimed to identify NB-UVB-induced molecular pathways that may account for the anti-inflammatory efficacy of the treatment.

Skin biopsies were obtained from psoriatic patients undergoing NB-UVB phototherapy from lesional and non-lesional skin before, during and after therapy. Gene expression profiling and pathway analysis was performed using epidermal RNA. Additionally, skin biopsies from healthy individuals were cultured with IL-22 and effects of UVB irradiation on the Th17 signalling pathway were assessed.

NB-UVB phototherapy downregulated Th17 and interferon signalling pathways in psoriatic epidermis. Direct 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. In addition, UVB affected well-known therapeutic targets in psoriasis, such as the glucocorticoid, vitamin D, peroxisome proliferator-activated receptor and IL-4 signalling pathways.

In conclusion, clinical improvement of psoriasis by NB-UVB is associated with downregulation of Th17 and type I and II interferon signalling pathways, which are critical in the pathogenesis of the disease. Our data provide novel insights into the molecular mechanisms underlying successful NB-UVB therapy for psoriasis.

INTRODUCTION

Narrow-band ultraviolet-B (NB-UVB) phototherapy, referred to as NB-UVB therapy hereafter, is a standard and safe treatment for moderate to severe psoriasis. Its mechanism of action is incompletely understood and has mainly been studied *in vitro* or in mice.

NB-UVB therapy has both local effects in the skin, and systemic effects. Locally, NB-UVB therapy reverses several pathologic alterations in psoriasis: the number of epidermal T lymphocytes and dendritic cells (DC) decrease during phototherapy, very probably due to UVB-induced apoptosis (1-4). Moreover, T cells that persist in UVB-treated skin lesions exhibit a functional shift towards less IFN-γ and more IL-4-production (5, 6). In addition, keratinocyte proliferation decreases during NB-UVB therapy (4). It is not known via which molecular mechanisms or pathways NB-UVB achieves these cellular changes. Because most of the UVB radiation is absorbed by the epidermis, this is presumably the primary site of UVB action.

In this study microarray technology was used to analyse kinetic changes in epidermal total genome expression profile induced by NB-UVB therapy, which is instrumental to identify novel pathways and mechanisms that are involved in its anti-inflammatory mode of action.

Microarray technology was previously used to better understand the mode of action of other established therapies in psoriasis such as alefacept, infliximab and cyclosporin A. Alefacept, an LFA3-Ig fusion protein that binds to CD2 and inhibits T cell activation, was found to induce leukocyte activation genes which might explain the inefficacy of this treatment in approximately 50% of psoriasis patients (7). Infliximab, a therapeutic antibody against TNF-α was shown to additionally inhibit IFN-γ-induced genes, thereby targeting a major pathway of the adaptive immune response in psoriasis (8). Cyclosporine A was shown to suppress Th1 and Th17 pathways, TNF-α and nitric oxide synthase-producing DCs in the skin of patients with psoriasis (9).

Transcriptomic effects of NB-UVB therapy had to date only been examined in non-lesional skin samples of 3 patients with psoriasis (10). We analyzed epidermal gene expression profiles in lesional and non-lesional skin of 10 patients with psoriasis taken before and after NB-UVB treatment. Additionally, in the same patients, short-term effects of NB-UVB and immediate targets of phototherapy were investigated in lesional and non-lesional skin 6 h after the first treatment session.

RESULTS

Clinical response to NB-UVB therapy

The mean PASI reduction after standard NB-UVB therapy in all patients included in this study was 81%. (range 58 – 100%). The mean PASI score decreased from 12.7 (range 10.0 – 23.2) to 2.4 (range 0 – 8.4). For the microarray analysis, patient samples were pooled in 2 groups, as described in the Methods section. The mean PASI reduction was similar in these patient groups.

Correlation of gene expression profiles

The degree of similarity between global gene expression profiles was assessed using the OmniViz package. Expression profiles of the two different pools of patient samples were similar to each other for all conditions, demonstrating valid duplicate measurements.

Two clusters of samples were identified on the basis of strong similarities in their gene expression profiles (Figure 1). One smaller cluster was formed by lesional skin biopsies that were taken on the first day of NB-UVB therapy, immediately before and 6 h after irradiation. The strong correlation between these samples shows that NB-UVB induced relatively small changes in gene expression in lesional skin 6 h after irradiation. Gene expression profiles in lesional biopsies taken after three months of NB-UVB therapy cluster together with the non-lesional skin biopsies taken before and after NB-UVB therapy.

Differences between lesional and non-lesional epidermis before therapy

The gene expression profiles in untreated psoriasis lesions were compared to untreated non-lesional skin. In lesional skin, 251 genes were upregulated and 383 genes were downregulated compared to non-lesional skin. There was great similarity between the gene expression profiles found in this study and previously reported profiles in psoriatic lesional and non-lesional skin without NB-UVB intervention (11-18), although these studies extracted RNA from whole skin biopsies (epidermis and dermis together) whereas we exclusively used the epidermis. Genes upregulated in lesional skin included members of the interferon signaling pathway (e.g. IFI27, MX1, STAT1, OAS1), Th17 pathway (STAT3, β-defensin 2, CCL20, keratin 16, IL20R, S100A7, S100A12), other immune response genes (IL8, IL8RB, CXCR4, CD24), epidermal differentiation-associated genes (keratinocyte transglutaminase, SKALP/Elafin, keratin 6, keratin 17), and various proteinases (kallikrein 6, kallikrein 13, cathepsin C, tissue plasminogen activator, kynunerinase). Genes higher expressed in non-lesional skin had functions such as cell proliferation (betacellulin, CRIP1, INSIG1), cell cycle progression (CCND1) and the epidermal differentiation complex (corneodesmosin, KRT15, sciellin, filaggrin, LCE1B).

Genes regulated by NB-UVB phototherapy in the lesional and non-lesional epidermis

When gene expression profiles in skin samples taken before and after NB-UVB therapy were compared, 183 genes were downregulated and 329 genes were upregulated by NB-UVB in lesional skin (Supplemental Tables 1 and 2). In non-lesional skin, 17 genes were downregulated and 1 gene upregulated after NB-UVB therapy (Supplemental Table 3). There was a marginal overlap between genes regulated in lesional and non-lesional skin, represented by 6 genes (S100A9, keratin 6, alpha-actinin, STEAP4, ets homologous factor, betacellulin).

Examination of the lists of differentially expressed genes in psoriasis lesions showed that NB-UVB had a strong suppressive effect on genes regulated by the Th17 and interferon signalling pathways and members of the epidermal differentiation complex (Table 1).

Functional annotation of the differentially expressed genes in lesional skin before vs. after NB-UVB therapy is shown in Supplemental Figure 1. Immune responses, response to virus and response to stress were suppressed by NB-UVB therapy, whereas epidermis development/differentiation, extracellular matrix components and negative regulators of biological processes were induced.

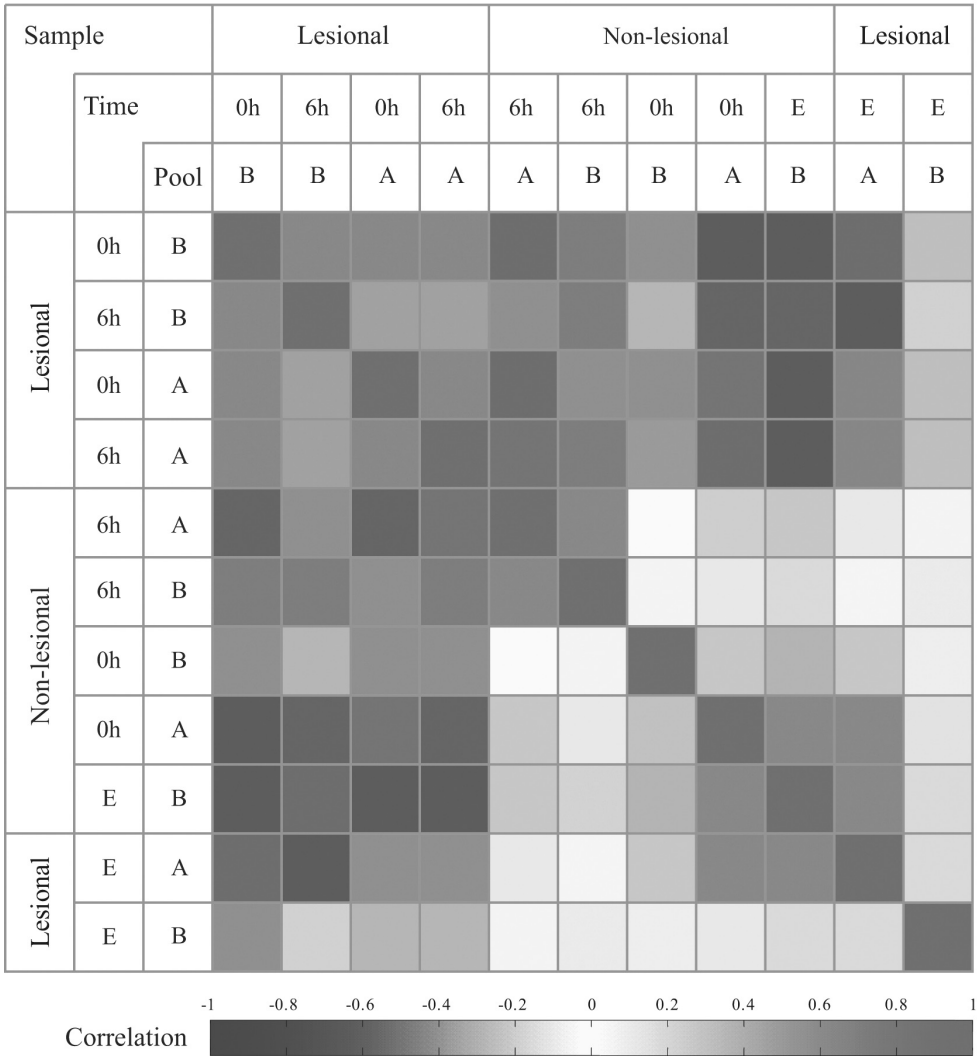

Figure 1. Correlation view of expression profiles of the different RNA pools.
Red squares indicate positive pairwise correlations and blue squares indicate negative pairwise correlations. L: lesional samples, N: non-lesional samples; 0 h: sample taken before the first irradiation, 6 h: sample taken 6 h after the first irradiation, E: sample taken after completion of NB-UVB therapy; A, B: patient pools. See page 150 for a full-color representation of this figure.

NB-UVB downregulates the pathogenic Th17 pathway in psoriasis skin lesions

Amongst the genes that were most suppressed by NB-UVB phototherapy in lesional and non-lesional skin, there were many known members of the Th17 pathway (Figure 2A). From this group of genes, S100A9 and β-defensin 2 were selected to validate the microarray

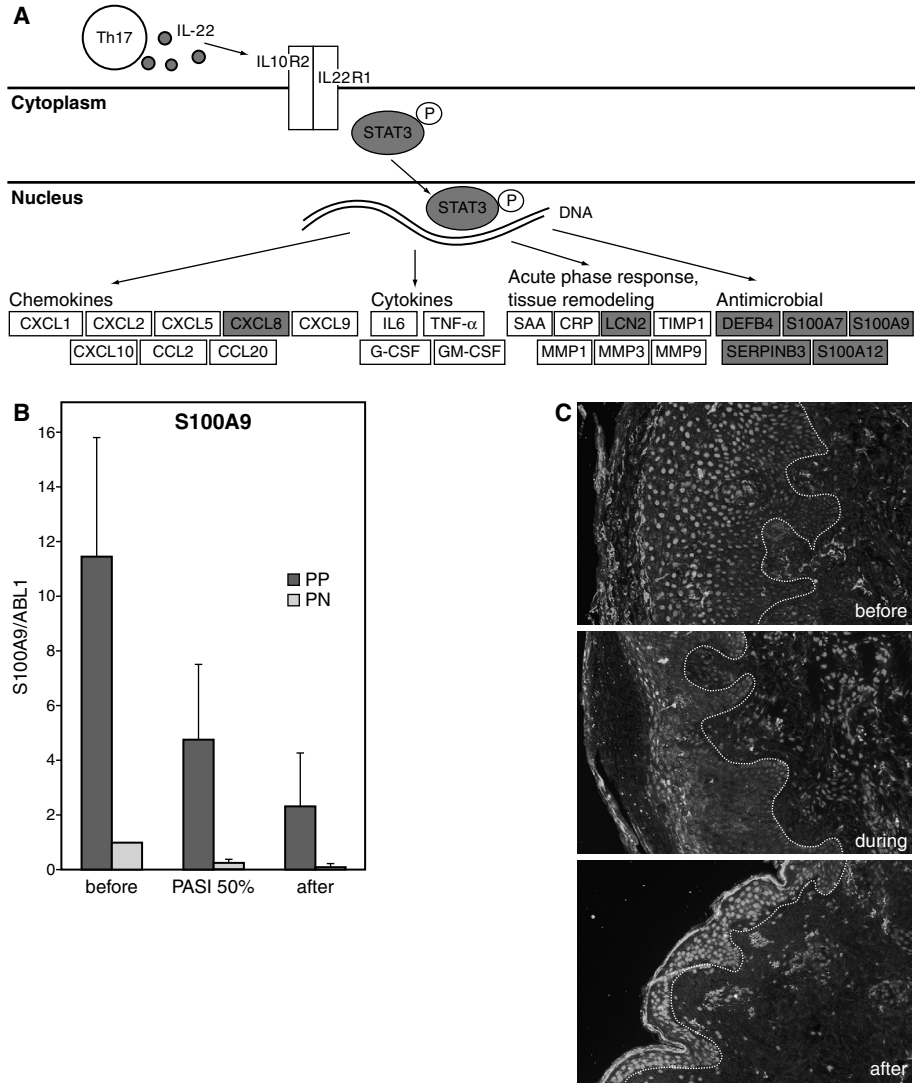

Figure 2. NB-UVB therapy suppresses the Th17 pathway.
A. Th17 pathway. Grey indicates gene downregulation. **B.** S100A9 expression measured by RT-PCR. PP: lesional, PN: non-lesional samples; 50%: sample taken at 50% PASI score reduction. Error bars indicate SEM, n=4 representative patients. **C.** β-defensin-2 in psoriatic skin samples during NB-UVB therapy. G-CSF: granulocyte colony-stimulating factor, GM-CSF: granulocyte macrophage colony-stimulating factor, SAA: serum amyloid A, CRP: C-reactive protein, LCN2: lipocalin 2, TIMP1: TIMP metallopeptidase inhibitor 1, MMP1: matrix metalloprotease 1, DEFB4: β-defensin 2, SERPINB3: serine proteinase inhibitor, clade B, member 3. See page 151 for a full-color representation of this figure.

data at the mRNA and protein levels. Expression of S100A9 was significantly downregulated in lesional and non-lesional epidermis after NB-UVB phototherapy on the basis of the

microarray data. Using mRNA isolated from individual patients and quantitative RT-PCR we found a similar downregulation (Figure 2B). Expression of β-defensin 2, assayed by immunofluorescent staining, was clearly suppressed by NB-UVB phototherapy in the lesional skin, confirming hereby the downregulation of the Th17 pathway at the protein level (Figure 2C).

The Th17 cytokine IL-22 was previously shown to induce a psoriasis-like phenotype in reconstituted human epidermis, including induction of acanthosis, antimicrobial proteins such as β-defensins, and activation of STAT3 (19). We assessed the effect of NB-UVB radiation on these IL-22-driven functions in an *ex vivo* skin culture system. IL-22 stimulation increased the number of cells expressing phospho-STAT3, whereas this was inhibited by subsequent NB-UVB irradiation (Figure 3A, B). Expression of β-defensin 2 in the epidermis is induced by IL-22 (19, 20), and this effect was drastically inhibited by NB-UVB irradiation (Figure 3C).

Collectively, our data demonstrate that NB-UVB suppresses the Th17 pathway in psoriatic skin, both *in vivo* during NB-UVB therapy as well as in irradiated *ex vivo* cultured skin explants.

NB-UVB downregulates the IFN signaling pathway in lesional psoriatic epidermis

In psoriasis lesions, NB-UVB therapy suppressed members of the IFN signalling pathway and many IFN-inducible molecules (Figure 4A). Interestingly, this signalling pathway was not affected by NB-UVB therapy in non-lesional skin. We selected the IFN-inducible cytoplasmic RNA receptor IFIH1/MDA5 to validate the microarray data using mRNA of individual

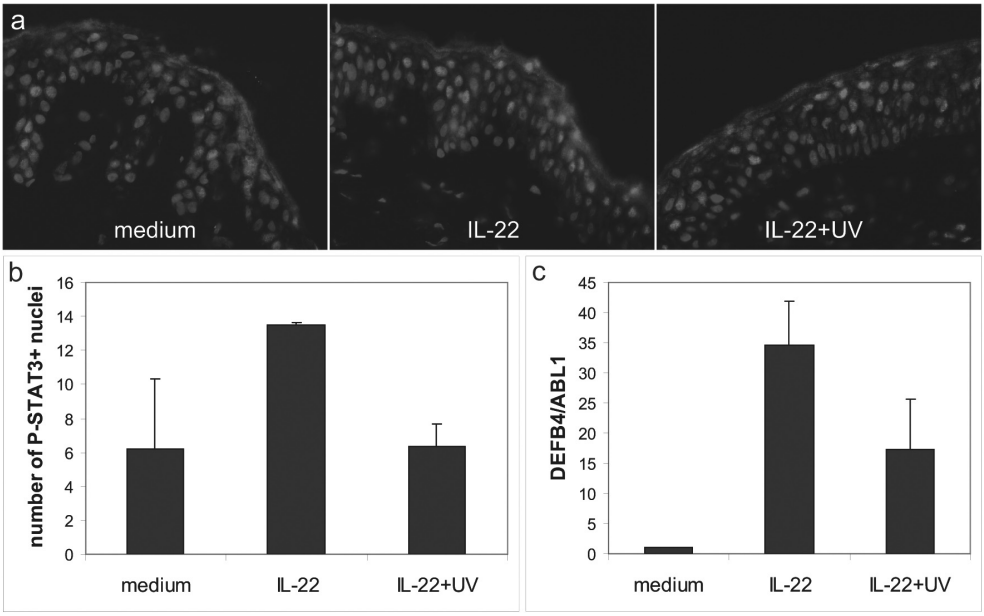

Figure 3. NB-UVB inhibits epidermal STAT3 activation and β-defensin 2 production.
A. Phosphorylated STAT3 in skin biopsies of healthy controls, treated *in vitro* with IL-22 +/- NB-UVB. **B.** Counts of phospho-STAT3+ nuclei in the epidermis. Error bars indicate SEM, n=4 subjects. **C.** β-defensin 2 mRNA expression in the epidermis of healthy controls. Error bars indicate SEM, n=4 subjects. See page 152 for a full-color representation of this figure.

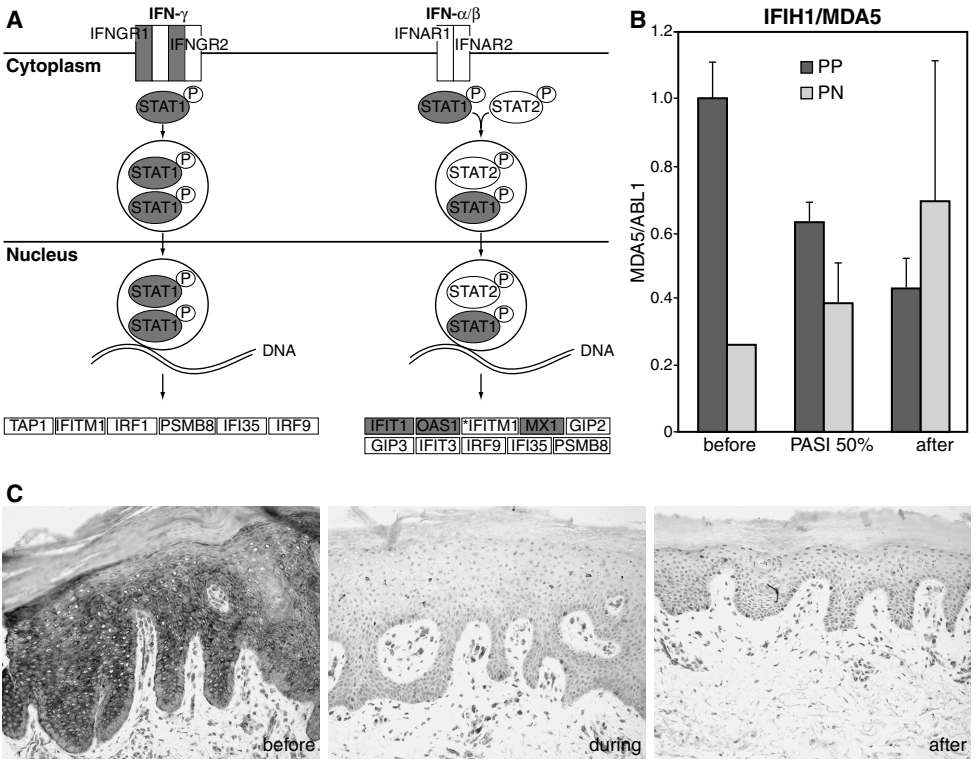

Figure 4. NB-UVB therapy suppresses IFN signalling pathways.
A. IFN signalling pathways. Grey colour indicates gene downregulation. **B.** IFIH1/MDA5 expression measured by RT-PCR. PP: lesional, PN: non-lesional samples; 50%: sample taken at 50% PASI score reduction. Error bars indicate SEM, n=5 patients. **C.** MxA expression during NB-UVB therapy. IFNGR: IFN-γ receptor, IFNAR: IFN-α receptor, TAP1: transporter 1, ATP-binding cassette, B, IFITM1: interferon induced transmembrane protein 1, IRF1: interferon regulatory factor 1, PSMB8: proteasome beta 8, IFI35: interferon-induced protein 35, IRF9:interferon regulatory factor 9. See page 153 for a full-color representation of this figure.

patients, including skin samples taken when the PASI scores were reduced by 50%. IFIH1/MDA5 mRNA was suppressed in lesional skin and remained unchanged in non-lesional skin, confirming the microarray data (Figure 4B). Additionally, to verify the downregulation of the interferon signaling pathway by NB-UVB at the protein level, the expression of MxA protein, a well-established marker of type I IFN-signaling, was studied using immunostaining. The expression of MxA was markedly decreased in lesional psoriatic skin treated with NB-UVB (Figure 4C).

NB-UVB therapy affects the terminal epidermal differentiation

Our microarray results show that many genes that are linked to terminal epidermal differentiation were affected by NB-UVB therapy in both lesional and non-lesional skin

(Table 1). NB-UVB also induced some selective effects in lesional versus non-lesional skin. For example, corneodesmosin expression in psoriasis lesions was decreased when compared to non-lesional skin before therapy, whereas NB-UVB therapy increased the expression in

Table 1. IFN-signalling, Th17 pathway and epidermal differentiation are affected by NB-UVB phototherapy in the lesional epidermis.

Name	Symbol	Fold change
Th17 pathway		
S100 calcium binding protein A7	S100A7	36.5 ↓
defensin, beta 2	DEFB4	36.2 ↓
peptidase inhibitor 3, skin-derived (SKALP)	SKALP	24.7 ↓
S100 calcium binding protein A9	S100A9	17.7 ↓
S100 calcium binding protein A12	S100A12	15.3 ↓
Keratin 16	KRT16	12.9 ↓
lipocalin 2 (oncogene 24p3)	LCN2	11.6 ↓
serpin peptidase inhibitor, clade B (ovalbumin), member 3	SERPINB3	10.4 ↓
interleukin 8	IL8	5.0 ↓
signal transducer and activator of transcription 3	STAT3	2.5 ↓
secretory leukocyte peptidase inhibitor	SLPI	1.7 ↓
interleukin 20 receptor beta	IL20RB	1.9 ↓
IFN signaling		
keratin 17	KRT17	9.5 ↓
interferon, alpha-inducible protein 6	IFI6	4.6 ↓
myxovirus resistance 1, interferon-inducible protein p78 (mouse)	MX1	4.3 ↓
interferon-induced protein 44-like	IFI44L	4.1 ↓
signal transducer and activator of transcription 1	STAT1	3.7 ↓
2'-5'-oligoadenylate synthetase 2, 69/71kDa	OAS2	3.4 ↓
interferon gamma receptor 1	IFNGR	2.1 ↓
interferon induced with helicase C domain 1 (MDA5)	IFIH1/MDA5	1.8 ↓
Epidermal differentiation		
keratin 6	KRT6	9.7 ↓
small proline-rich protein 2C	SPRR2C	9.6 ↓
gap junction protein, beta 2, 26kDa	GJB2	6.7 ↓
desmocollin 2	DSC2	5.4 ↓
keratin 15	KRT15	3.7 ↑
GATA binding protein 3	GATA3	2.9 ↑
transglutaminase K	TGM1	2.4 ↓
corneodesmosin	CDSN	2.1 ↑

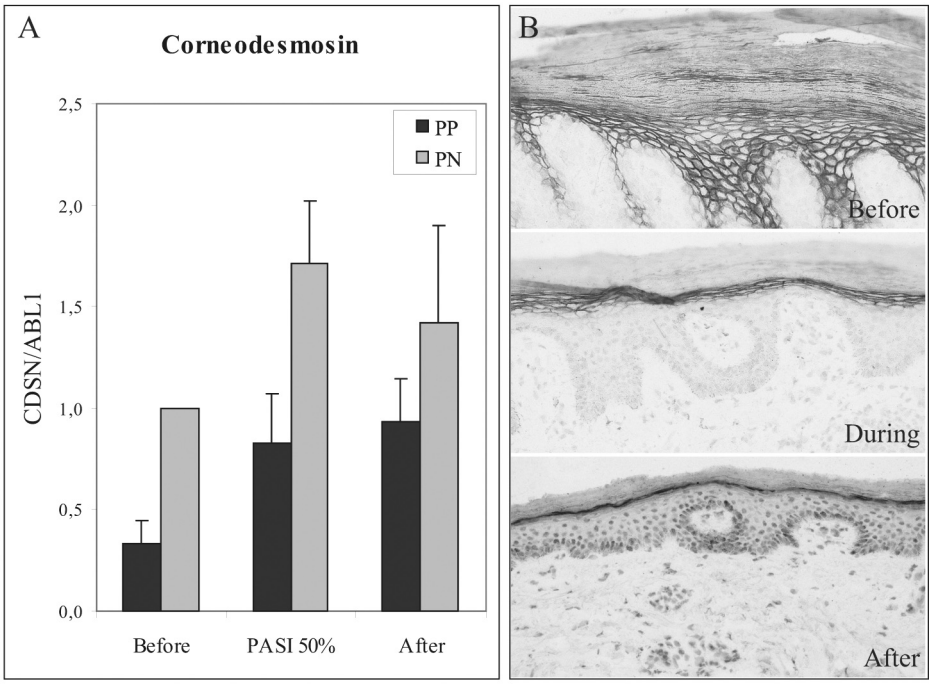

Figure 5. NB-UVB therapy suppresses epidermal differentiation.
A. Corneodesmosin (CDSN) expression measured by RT-PCR. PP: lesional, PN: non-lesional samples; 50%: sample taken at 50% PASI score reduction. Error bars indicate SEM, n=5 patients. **B.** Transglutaminase K expression during NB-UVB therapy. See page 154 for a full-color representation of this figure.

lesional, but not in non-lesional skin. These results were validated by RT-PCR using mRNA from individual patients (Figure 5A). Validation of this functional cluster at the protein level was done using immunostaining that clearly showed the downregulation of keratinocyte transglutaminase expression (downregulated 2.4-fold by NB-UVB therapy in the lesional epidermis in the microarray data) (Figure 5B).

NB-UVB therapy affects known therapeutic targets of psoriasis

Ingenuity Pathway Analysis software was additionally used to identify signalling pathways affected by NB-UVB therapy in psoriasis lesions. Interestingly, NB-UVB therapy regulated several pathways that are known therapeutic targets in psoriasis, such as glucocorticoid receptor and vitamin D receptor signalling (Table 2). Furthermore, NB-UVB therapy regulated some pathways that were previously identified as potential therapeutic targets in psoriasis, such as PPAR, IL-4 and IL-10 signalling (21-25). Pathways described as targets of UV radiation in normal skin and that are involved in the pathogenesis of psoriasis, such as NF-κB, MAPK, p53 and IGF-1 signalling were also affected by NB-UVB therapy in lesional psoriatic skin (Table 2).

Table 2. Effect of NB-UVB phototherapy on pathways known to be therapeutic target in psoriasis, or target of UVB radiation.

	Pathway	*p*-value	Molecules affected by NB-UVB within these pathways	
			Upregulated	Downregulated
Therapeutic target in psoriasis	Glucocorticoid receptor signalling	1.1E-03	PIK3R1, PIK3C2G, HSPA2, BCL2, IL1R2, TGFBR2, TSC22D3, KAT2B, AKT3, NCOR2, ADRB2, UBE2I	IL8, STAT3, STAT1
	VDR/RXR activation	4.2E-03	IGFBP5, NCOR2, CST6	DEFB4, SERPINB1, KLK6, HSD17B2
	NF-κB signalling	2.3E-02	IL1R2, CSNK2A2, IL18, PIK3R1, PIK3C2G, AKT3, IL1F7	IL1F9
Induced by UV in normal skin	p53 signalling	2.1E-03	KAT2B, TP53INP1, PIK3R1, PIK3C2G, AKT3, CCND1, BCL2	SCO2
	p38 MAPK signalling	3.5E-03	IL1R2, TGFBR2, IL18, H3F3B, IL1F7, EEF2K	IL1F9, STAT1
	EGF signalling	5.4E-03	CSNK2A2, PIK3R1, PIK3C2G	STAT3, STAT1
	IGF-1 signalling	2.4E-02	CSNK2A2, PIK3R1, PIK3C2G, AKT3, IGFBP5, IGFBP7	
Proposed as therapeutic target in psoriasis	LXR/RXR activation	2.5E-04	IL1R2, APOE, IL18, IL1F7, NCOR2	IL1F9, LDLR, CCL7
	IL-10 signalling	4.2E-03	IL1R2, IL18, IL1F7	IL4R, IL1F9, STAT3
	PPAR signalling	1.8E-02	IL1R2, IL18, IL1F7, NCOR2, PDGFC	IL1F9
	IL-4 signalling	2.2E-02	PIK3R1, PIK3C2G, AKT3	IL4R, IL13RA1
Other	GM-CSF signalling	1.5E-04	PIK3R1, PIK3C2G, AKT3, CCND1	CSF2RB, LYN, STAT3, STAT1
	Acute phase response signalling	3.2E-04	PIK3R1, SOCS6, IL1F7, IL18, AKT3	C1S, SERPINA3, STAT3, C1R, IL1F9, SOD2, CRABP2, CFB
	Interferon signalling	6.3E-04		IFIT1, OAS1, IFNGR1, MX1, STAT1
	PDGF signalling	2.1E-03	CSNK2A2, PIK3R1, CAV1, PIK3C2G, PDGFC	STAT3, STAT1
	JAK/Stat signalling	4.2E-03	PIK3R1, SOCS6, PIK3C2G, AKT3	STAT3, STAT1
	IL-6 signalling	7.2E-03	IL1R2, CSNK2A2, IL18, IL1F7	IL8, IL1F9, STAT3
	Complement system	9.5E-03	CD59	C1R, C1S, CFB
	LPS/IL-1 mediated inhibition of RXR function	1.9E-02	IL1R2, GSTA3, APOE, ALDH3A2, CAT, HS3ST6, ABCC4	ALDH1A3, PAPSS2, HS3ST3A1
	VEGF signalling	1.9E-02	PIK3R1, PIK3C2G, AKT3, BCL2	HIF1A, ACTN1
	Wnt/β-catenin signalling	2.2E-02	TGFBR2, CSNK2A2, FRZB, DKK3, SOX10, AKT3, CCND1	SOX9, FZD5
	Xenobiotic metabolism signalling	3.2E-02	GSTA3, ALDH3A2, PIK3R1, MAF, CAT, PIK3C2G, HS3ST6, NCOR2	ALDH1A3, UGT1A9, HS3ST3A1
	Fc epsilon RI signalling	3.6E-02	FYN, VAV3, PIK3R1, PIK3C2G, AKT3	LYN
	IL-2 signalling	4.3E-02	CSNK2A2, PIK3R1, PIK3C2G, AKT3	

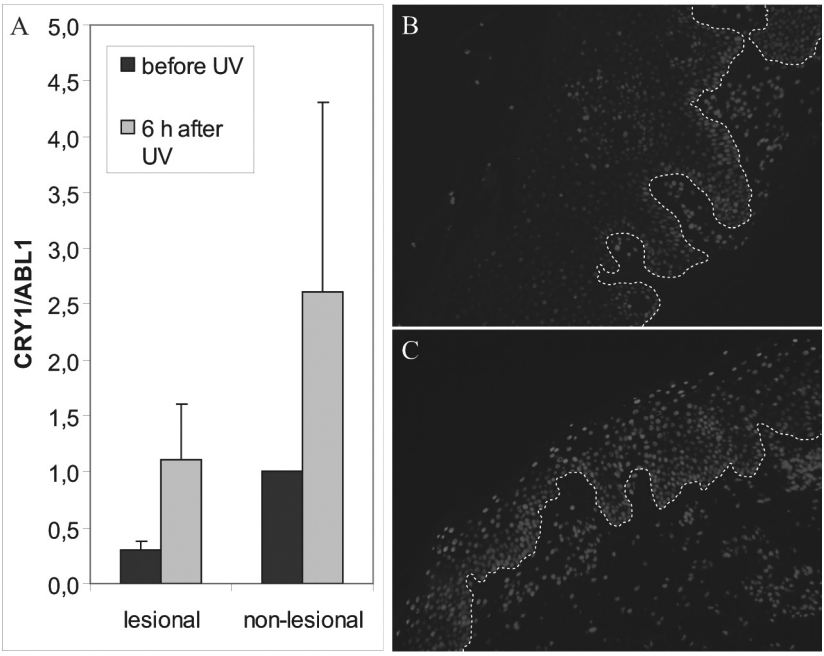

Figure 6. Immediate epidermal effects of NB-UVB.
A. mRNA expression of cryptochrome 1 (CRY1) immediately before and 6 h after the first irradiation, relative to ABL1. Error bars indicate SEM, n=6 patients. **B-C.** Immunohistochemical staining for CPDs in lesional skin before (**B**) and 15 min after (**C**) irradiation with 70% MED. See page 155 for a full-color representation of this figure.

The clinical starting NB-UVB dose has minor effects in lesional skin after 6 h, while in non-lesional skin marked gene expression changes are observed

To study the short-term effects of NB-UVB in psoriatic skin, gene expression in skin biopsies taken before and 6 h after the first irradiation was compared. In lesional skin only 3 genes (CYP1B1, periostin and cryptochrome 1) were significantly (>2-fold) upregulated 6 h after the first irradiation. Of these genes, periostin and cryptochrome 1 were also upregulated in lesional skin after three months of treatment. Assessment of cryptochrome 1 expression by RT-PCR using individual RNA samples confirmed the microarray results (Figure 6A).

In contrast, in non-lesional skin, NB-UVB affected the expression of 272 genes more than 2-fold, 6 h 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 due to incomplete penetration of NB-UVB light into the thickened psoriatic epidermis, immunohistochemical staining for cyclobutane pyrimidine dimers (CPD) as marker of UV damage was performed on lesional and non-lesional skin samples taken before and 15 min after the first UV irradiation (70% MED). Robust CPD positivity was observed in almost all epidermal layers of the psoriatic plaque (Figure 6B, C). Thus, the relatively small number of genes affected by a single NB-UVB dose in lesional skin is not due to lack of penetration of NB-UVB in the lesional epidermis.

DISCUSSION

Our results show that clinically effective NB-UVB therapy is associated with suppression of type I and type II interferon signalling and downregulation of the Th17 pathway in the lesional epidermis.

IL-23 and the Th17 cytokines IL-17, IL-22 and IL-21 recently emerged as key cytokines in the pathogenesis of psoriasis (26). IL-23 is overproduced in psoriasis lesions by dendritic cells and keratinocytes, and this cytokine stimulates Th17 cells within the dermis to produce Th17 cytokines (27-29). 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 in the pathogenesis of psoriasis (19, 30, 31). The Th17 pathway is also rapidly inhibited in psoriatic skin during treatment with Cyclosporine A and etanercept (TNF receptor – immunoglobulin fusion protein) (32). Cyclosporine A modulation is observed within 2 weeks after the start of the treatment and correlates well with the clinical improvement of the disease (9). The importance of the IL-17/IL-23 immunological pathway is further underlined by the facts that polymorphisms in genes encoding IL23R and IL12/IL23p40 are associated with susceptibility to psoriasis (33, 34), and that monoclonal antibodies against IL12/IL23p40 are highly effective in psoriasis (35). We have recently shown that psoriasis-like dermatitis in mice induced by the TLR7/TLR8 agonist imiquimod is critically dependent on the IL-23/IL-17 axis (36).

In our study, expression of Th17 pathway genes was suppressed in both lesional and non-lesional skin by NB-UVB therapy. Interestingly, Th17 autoinflammatory responses are inhibited by Vitamin D3 (37). Vitamin D3 is known to be induced by NB-UVB and we have found this signalling pathway to be affected by NB-UVB therapy. Inhibition of the Th17 pathway was confirmed by demonstrating that in skin explants, NB-UVB inhibited the IL-22-induced phosphorylation of STAT3 and the induction of β-defensin-2, already 6 h after irradiation. This clearly demonstrates a rapid effect of NB-UVB radiation on activation of 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 (38). Interestingly, STAT3 phosphorylation is inhibited by a reactive oxygen species (ROS)-inducer (manumycin) in cancer cells (39), indicating that induction of ROS might also be responsible for STAT3 phosphorylation inhibition by NB-UVB.

Type I IFNs are produced mainly by plasmacytoid dendritic cells in autoimmune diseases such as systemic lupus erythematodes (40), and in response to viral infections as part of the innate immune response. Type I IFNs are critical in the pathogenesis of psoriasis (41-45). We previously showed that the type I IFN pathway is activated in psoriasis skin lesions (41). Furthermore, treatment of patients with pre-existing psoriasis or a familial predisposition for psoriasis with IFN-α can induce or exacerbate the disease (42-45). Blocking of IFN-α signalling in a xenograft murine model of psoriasis prevented the T cell-dependent development of psoriatic lesions in non-lesional skin transplanted onto immunocompromised mice (46).

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 (47).

NB-UVB is known to deplete haemopoietic cells such as T lymphocytes and Langerhans cells from the skin (1, 48). Is conceivable that the observed inhibition of both type I and II IFN signalling pathways are due to depletion of lymphocytes and plasmacytoid dendritic cells from psoriasis lesions by NB-UVB.

Psoriatic lesions show distinctive signs of altered epidermal differentiation clinically and histologically (49). 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 (50, 51) and around the corneodesmosin gene on chromosome 6p21 (52) are closely associated with psoriasis. NB-UVB phototherapy modulated the expression of epidermal differentiation-associated genes in the psoriatic lesions towards the expression observed in normal skin.

A surprising finding in our study was that 6 h after the first UV-irradiation many more genes were differentially expressed in non-lesional skin than in lesional skin, with only 3 genes differentially expressed in lesional skin. This phenomenon has not been reported before since previous microarray studies on the genomic effects of NB-UVB in the skin used normal skin or cultured keratinocytes (53-56), or non-lesional skin from patients with psoriasis (10). We excluded the possibility of incomplete UV penetration in lesional skin by demonstrating that CPDs occur throughout the lesional epidermis already 15 min after a single UV irradiation.

The resistance of psoriasis lesions to UVB may be due to powerful inflammatory stimuli from the infiltrating immune cells, maintaining high transcription levels for many genes. Thus, a limitation of our *in vivo* kinetic study during total body NB-UVB therapy is that the results were obtained from psoriasis lesions exposed to 70% MED. Low starting doses during NB-UVB therapy are important to maintain the safety of the treatment, and the first irradiation (70% MED) is too low to have therapeutically relevant effects. Our results also imply that effects of UVB in non-inflamed skin cannot simply be extrapolated to inflamed skin.

In this study we only used the epidermal compartment of the skin because most UVB radiation is absorbed there. Interactions between the epidermis and (products from) immunocytes in the dermis are essential for the chain of cellular and molecular events resulting in full blown psoriasis.

In conclusion, our results show that clinically effective NB-UVB therapy is associated with downregulation of the critical Th17 and interferon signaling pathways and induction of ortho-differentiation in the skin. In addition several anti-inflammatory pathways, such as glucocorticoid, vitamin D, peroxisome proliferator-activated receptor and IL-4 signaling pathways 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 inflammation.

MATERIALS AND METHODS

Patients and treatments

Ten patients with psoriasis were recruited after informed consent (METC registration number 234.237/2003/210). Patients (nine men, one woman, age range 20-73) had Psoriasis Area and Severity Index (PASI) scores of at least 10 (Supplemental Table 4) and had not

received systemic therapy for at least one month or topical therapy for at least two weeks prior to the start of the study. Patients were treated with standard NB-UVB phototherapy until total clearance of psoriasis was reached, or for a maximum of three months. NB-UVB treatment was applied three times weekly using a Waldmann 7001 UVB cabinet equipped with Philips TL-01 bulbs. Starting UVB dose was 0.1-0.3 J/cm^2 (depending on the skin type of the patient); the mean cumulative NB-UVB dose was 42.0 J/cm^2 (range 30-60 J/cm^2). During the course of UVB treatment PASI scores were evaluated every two weeks.

Biopsy samples and RNA extraction

Three-mm biopsies were taken from psoriasis lesional and non-lesional skin before the start of NB-UVB therapy and after the last treatment session. To investigate the short-term effects of UVB radiation in psoriatic skin, biopsies were taken 6 h after the first irradiation. When PASI scores reached a reduction of 50% from the baseline score, additional biopsies from lesional and non-lesional skin were taken before and 6 h after UVB irradiation. For cyclobutane pyrimidine dimer (CPD) immunohistochemistry, three-mm skin biopsies were taken from lesional and non-lesional skin of three patients before and 15 min after the first treatment session of NB-UVB phototherapy.

The epidermis was separated from the dermis after incubation in 1 mg/ml protease X (Sigma Aldrich, Zwijndrecht, the Netherlands) for 90 min at 37°C, and stored in RNA lysis buffer at –80°C until further processing. Total messenger RNA was isolated from the epidermis only, using GenElute Mammalian Total RNA Miniprep kit (Sigma Aldrich). RNA purity and integrity was verified by scanning with an Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip.

Array hybridization and analysis

For hybridization on gene expression arrays, RNA samples of four individual patients per array (250 ng mRNA per patient) were pooled. Patients were divided into two groups in order to have duplicate arrays for each time point and condition (Supplemental Table 5). This pooling of RNA samples is a validated method in microarray studies (57, 58). The advantage of pooling RNA samples is that the results are less influenced by patient-specific variations in gene expression, and rather reflect the gene expression profile specific to the studied condition that is common between all patients i.e. the effect of NB-UVB. Biotinylated target RNA was prepared from the pooled (1 μg) total RNA, and hybridized on GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA). Array hybridization and scanning was performed as described previously (59). The data were read and robust multichip analysis (RMA) (60) was used to remove the background and normalize the data across arrays (61). These values were $\log_2$-transformed for further analysis, yielding numbers between 0 and 16. A two-way ANOVA with factors “probe” and “condition” was used for each probeset to calculate both average expression levels per condition (62) and a *p*-value for the difference between conditions. The resulting *p*-values were adjusted for multiple testing using Šidák step-up adjustment (63).Genes were considered differentially expressed when *p*-values were < 0.05 and the absolute fold change was greater than 2.

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, www.ingenuity.com) 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) (64).

Confirmation of gene expression patterns at the mRNA and protein levels

To confirm the gene expression patterns of selected genes as identified by gene expression microarray, RNA of individual patients was transcribed into cDNA, and RT-PCR was performed as described previously (65). ABL1 was used as a housekeeping control gene. Sequences of newly designed primers and probe numbers of the Exiqon probe library system (Exiqon, Vedbaek, Denmark) are listed in Table 3.

To validate the microarray results at the protein level, immunostatinings were performed on biopsies from psoriasis lesions of patients. Immunostaining procedures with anti-MxA antibody (1:100; mouse monoclonal anti-Mx antibody, M143, Otto Haller, Freiburg, Germany) and anti-human keratinocyte transglutaminase (1:200; Biomedical Technologies Inc, Stoughton, MA) were applied as described previously (41).

For immunofluorescent staining, cryosections were fixed for 10 min in 4% PFA in PBS. Before staining with anti-cyclobutane pyrimidine dimer (CPD) antibody, DNA was denatured using 0.07 M NaOH in 70 % ethanol, and slides were preincubated with 5% normal rabbit serum in PBS with 0.1% BSA. Primary antibodies used for immunofluorescent stainings included anti-CPD (1:4000; Kamiya Biomedical Company, Seattle, WA), anti-β-defensin 2 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-phosphorylated STAT3 (1:50; Cell Signalling Technology Inc, Danvers, MA). Relevant Cy5- or TxR-conjugated antibodies (1:100, Abcam) were used to detect primary antibodies. All fluorescent images were made with an Axio Imager (Zeiss) fluorescence microscope.

Skin organ culture

Normal skin biopsies (3 mm diameter) were obtained from healthy volunteers undergoing breast reduction in the Department of Plastic Surgery of the Sint Franciscus Gasthuis, Rotterdam, the Netherlands, after informed consent. Biopsies were cultured as described previously (66). Recombinant human IL-22 (50 ng/ml) (R&D Systems, Abingdon, UK) was added to the culture medium and 16 h later biopsies were irradiated with NB-UVB using a small Waldmann irradiation device equipped with TL-01 UV 236-01 lamps (Waldmann Medizintechnik, Villingen-Schwenningen, Germany). In all experiments a single NB-UVB dose of 600 mJ/cm^2 was used, representing a dose that is usually reached within 3 to 9 clinical UVB-therapy sessions (1-3 weeks of clinical treatment). The cultured biopsies were collected 6 h after UV-irradiation. One of the 4 biopsies was snap-frozen, while the epidermis of the 3 other biopsies was separated from the dermis and total messenger RNA was isolated as described above.

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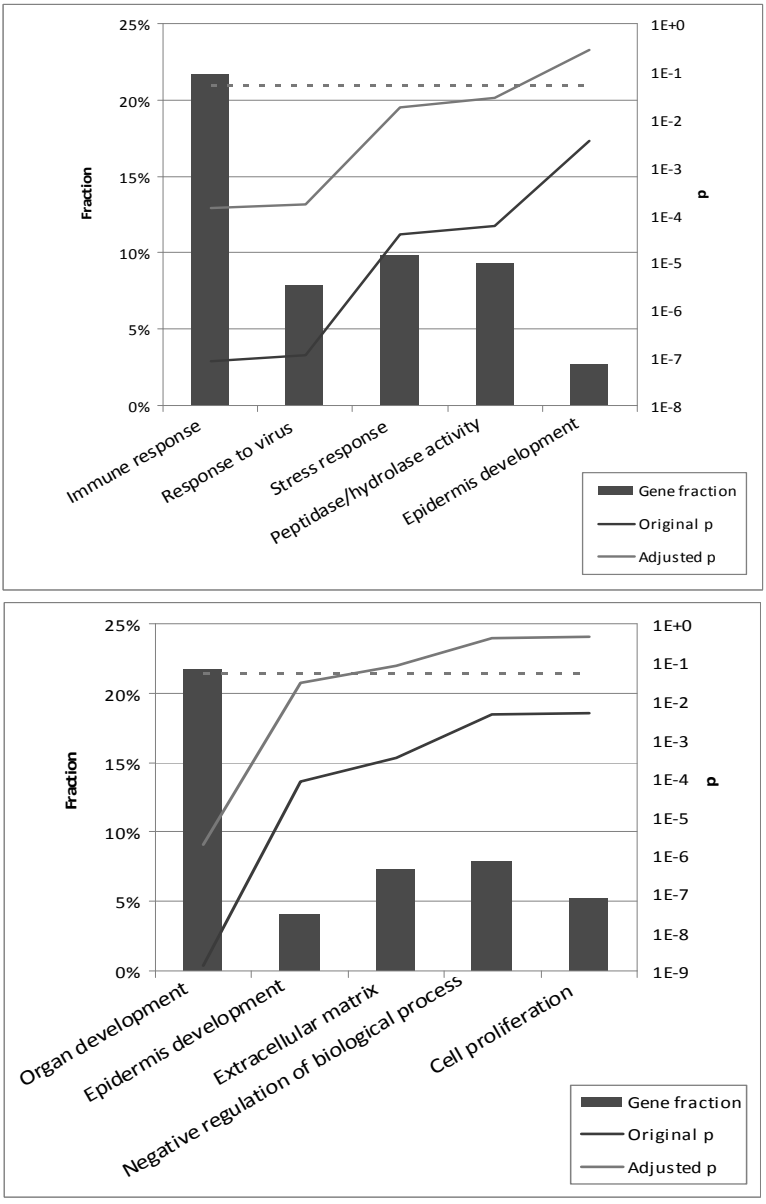
REFERENCES

1. Ozawa, M., K. Ferenczi, T. Kikuchi, I. Cardinale, L. M. Austin, T. R. Coven, L. H. Burack, and J. G. Krueger. 1999. 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. *J Exp Med* **189**:711-718.
2. Walters, I. B., M. Ozawa, I. Cardinale, P. Gilleaudeau, W. L. Trepicchio, J. Bliss, and J. G. Krueger. 2003. Narrowband (312-nm) UV-B suppresses interferon gamma and interleukin (IL) 12 and increases IL-4 transcripts: differential regulation of cytokines at the single-cell level. *Arch Dermatol* **139**:155-161.
3. Carrascosa, J. M., G. Tapia, I. Bielsa, M. J. Fuente, and C. Ferrandiz. 2007. Effects of narrowband UV-B on pharmacodynamic markers of response to therapy: an immunohistochemical study over sequential samples. *J Cutan Pathol* **34**:769-776.
4. Erkin, G., Y. Ugur, C. K. Gurer, E. Asan, P. Korkusuz, S. Sahin, and F. Kolemen. 2007. Effect of PUVA, narrow-band UVB and cyclosporin on inflammatory cells of the psoriatic plaque. *J Cutan Pathol* **34**:213-219.
5. Piskin, G., R. M. Sylva-Steenland, J. D. Bos, and M. B. Teunissen. 2004. T cells in psoriatic lesional skin that survive conventional therapy with NB-UVB radiation display reduced IFN-gamma expression. *Arch Dermatol Res* **295**:509-516.
6. Piskin, G., C. W. Koomen, D. Picavet, J. D. Bos, and M. B. Teunissen. 2003. Ultraviolet-B irradiation decreases IFN-gamma and increases IL-4 expression in psoriatic lesional skin *in situ* and in cultured dermal T cells derived from these lesions. *Exp Dermatol* **12**:172-180.
7. Haider, A. S., M. A. Lowes, H. Gardner, R. Bandaru, K. Darabi, F. Chamian, T. Kikuchi, P. Gilleaudeau, M. S. Whalen, I. Cardinale, I. Novitskaya, and J. G. Krueger. 2007. Novel insight into the agonistic mechanism of alefacept *in vivo*: differentially expressed genes may serve as biomarkers of response in psoriasis patients. *J Immunol* **178**:7442-7449.
8. Haider, A. S., J. Cohen, J. Fei, L. C. Zaba, I. Cardinale, K. Toyoko, J. Ott, and J. G. Krueger. 2008. Insights into gene modulation by therapeutic TNF and IFN gamma antibodies: TNF regulates IFN gamma production by T cells and TNF-regulated genes linked to psoriasis transcriptome. *J Invest Dermatol* **128**:655-666.
9. Haider, A. S., M. A. Lowes, M. Suarez-Farinas, L. C. Zaba, I. Cardinale, A. Khatcherian, I. Novitskaya, K. M. Wittkowski, and J. G. Krueger. 2008. Identification of cellular pathways of "type 1," Th17 T cells, and TNF- and inducible nitric oxide synthase-producing dendritic cells in autoimmune inflammation through pharmacogenomic study of cyclosporine A in psoriasis. *J Immunol* **180**:1913-1920.
10. Hochberg, M., S. Zeligson, N. Amariglio, G. Rechavi, A. Ingber, and C. D. Enk. 2007. Genomic-scale analysis of psoriatic skin reveals differentially expressed insulin-like growth factor-binding protein-7 after phototherapy. *Br J Dermatol* **156**:289-300.
11. Zhou, X., J. G. Krueger, M. C. Kao, E. Lee, F. Du, A. Menter, W. H. Wong, and A. M. Bowcock. 2003. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63, 100-element oligonucleotide array. *Physiol Genomics* **13**:69-78.
12. Kulski, J. K., W. Kenworthy, M. Bellgard, R. Taplin, K. Okamoto, A. Oka, T. Mabuchi, A. Ozawa, G. Tamiya, and H. Inoko. 2005. Gene expression profiling of Japanese psoriatic skin reveals an increased activity in molecular stress and immune response signals. *J Mol Med* **83**:964-975.
13. Reischl, J., S. Schwenke, J. M. Beekman, U. Mrowietz, S. Sturzebecher, and J. F. Heubach. 2007. Increased expression of Wnt5a in psoriatic plaques. *J Invest Dermatol* **127**:163-169.
14. Quekenborn-Trinquet, V., P. Fogel, O. Aldana-Jammayrac, P. Ancian, M. Demarchez, P. Rossio, H. L. Richards, B. Kirby, C. Nguyen, J. J. Voegel, and C. E. Griffiths. 2005. Gene expression profiles in psoriasis: analysis of impact of body site location and clinical severity. *Br J Dermatol* **152**:489-504.

15. Yao, Y., L. Richman, C. Morehouse, M. de los Reyes, B. W. Higgs, A. Boutrin, B. White, A. Coyle, J. Krueger, P. A. Kiener, and B. Jallal. 2008. Type I interferon: potential therapeutic target for psoriasis? *PLoS ONE* **3**:e2737.
16. Oestreicher, J. L., I. B. Walters, T. Kikuchi, P. Gilleaudeau, J. Surette, U. Schwertschlag, A. J. Dorner, J. G. Krueger, and W. L. Trepicchio. 2001. Molecular classification of psoriasis disease-associated genes through pharmacogenomic expression profiling. *Pharmacogenomics J* **1**:272-287.
17. Mee, J. B., C. M. Johnson, N. Morar, F. Burslem, and R. W. Groves. 2007. The psoriatic transcriptome closely resembles that induced by interleukin-1 in cultured keratinocytes: dominance of innate immune responses in psoriasis. *Am J Pathol* **171**:32-42.
18. Bowcock, A. M., W. Shannon, F. Du, J. Duncan, K. Cao, K. Aftergut, J. Catier, M. A. Fernandez-Vina, and A. Menter. 2001. Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* **10**:1793-1805.
19. Sa, S. M., P. A. Valdez, J. Wu, K. Jung, F. Zhong, L. Hall, I. Kasman, J. Winer, Z. Modrusan, D. M. Danilenko, and W. Ouyang. 2007. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol* **178**:2229-2240.
20. Liang, S. C., X. Y. Tan, D. P. Luxenberg, R. Karim, K. Dunussi-Joannopoulos, M. Collins, and L. A. Fouser. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* **203**:2271-2279.
21. Sertznig, P., M. Seifert, W. Tilgen, and J. Reichrath. 2008. Peroxisome proliferator-activated receptors (PPARs) and the human skin: importance of PPARs in skin physiology and dermatologic diseases. *Am J Clin Dermatol* **9**:15-31.
22. Robertshaw, H., and P. S. Friedmann. 2005. Pioglitazone: a promising therapy for psoriasis. *Br J Dermatol* **152**:189-191.
23. Martin, R. 2003. Interleukin 4 treatment of psoriasis: are pleiotropic cytokines suitable therapies for autoimmune diseases? *Trends Pharmacol Sci* **24**:613-616.
24. Asadullah, K., W. Sterry, and H. D. Volk. 2003. Interleukin-10 therapy-review of a new approach. *Pharmacological reviews* **55**:241-269.
25. Docke, W. D., K. Asadullah, G. Belbe, M. Ebeling, C. Hoflich, M. Friedrich, W. Sterry, and H. D. Volk. 2009. Comprehensive biomarker monitoring in cytokine therapy: heterogeneous, time-dependent, and persisting immune effects of interleukin-10 application in psoriasis. *J Leukoc Biol* **85**:582-593.
26. Blauvelt, A. 2008. T-helper 17 cells in psoriatic plaques and additional genetic links between IL-23 and psoriasis. *J Invest Dermatol* **128**:1064-1067.
27. Lee, E., W. L. Trepicchio, J. L. Oestreicher, D. Pittman, F. Wang, F. Chamian, M. Dhodapkar, and J. G. Krueger. 2004. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* **199**:125-130.
28. Piskin, G., R. M. Sylva-Steenland, J. D. Bos, and M. B. Teunissen. 2006. *In vitro* and *in situ* expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. *J Immunol* **176**:1908-1915.
29. Wilson, N. J., K. Boniface, J. R. Chan, B. S. McKenzie, W. M. Blumenschein, J. D. Mattson, B. Basham, K. Smith, T. Chen, F. Morel, J. C. Lecron, R. A. Kastelein, D. J. Cua, T. K. McClanahan, E. P. Bowman, and R. de Waal Malefyt. 2007. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* **8**:950-957.
30. Wolk, K., E. Witte, E. Wallace, W. D. Docke, S. Kunz, K. Asadullah, H. D. Volk, W. Sterry, and R. Sabat. 2006. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur J Immunol* **36**:1309-1323.
31. Nograles, K. E., L. C. Zaba, E. Guttman-Yassky, J. Fuentes-Duculan, M. Suarez-Farinas, I. Cardinale, A. Khatcherian, J. Gonzalez, K. C. Pierson, T. R. White, C. Pensabene, I. Coats, I. Novitskaya, M. A. Lowes, and J. G. Krueger. 2008. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *Br J Dermatol* **159**:1092-1102.
32. Zaba, L. C., I. Cardinale, P. Gilleaudeau, M. Sullivan-Whalen, M. Suarez-Farinas, J. Fuentes-Duculan, I. Novitskaya, A. Khatcherian, M. J. Bluth, M. A. Lowes, and J. G. Krueger. 2007. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* **204**:3183-3194.

33. Cargill, M., S. J. Schrodi, M. Chang, V. E. Garcia, R. Brandon, K. P. Callis, N. Matsunami, K. G. Ardlie, D. Civello, J. J. Catanese, D. U. Leong, J. M. Panko, L. B. McAllister, C. B. Hansen, J. Papenfuss, S. M. Prescott, T. J. White, M. F. Leppert, G. G. Krueger, and A. B. Begovich. 2007. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* **80**:273-290.
34. Nair, R. P., A. Ruether, P. E. Stuart, S. Jenisch, T. Tejasvi, R. Hiremagalore, S. Schreiber, D. Kabelitz, H. W. Lim, J. J. Voorhees, E. Christophers, J. T. Elder, and M. Weichenthal. 2008. Polymorphisms of the IL12B and IL23R genes are associated with psoriasis. *J Invest Dermatol* **128**:1653-1661.
35. Krueger, G. G., R. G. Langley, C. Leonardi, N. Yeilding, C. Guzzo, Y. Wang, L. T. Dooley, and M. Lebwohl. 2007. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* **356**:580-592.
36. Van der Fits, L., S. Mourits, J. Voerman, M. Kant, L. Boon, J. Laman, F. Cornelissen, A.-M. Mus, E. Florencia, E. Prens, and E. Lubberts. 2009. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* in press.
37. Tang, J., R. Zhou, D. Luger, W. Zhu, P. B. Silver, R. S. Grajewski, S. B. Su, C. C. Chan, L. Adorini, and R. R. Caspi. 2009. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. *J Immunol* **182**:4624-4632.
38. Sano, S., K. S. Chan, M. Kira, K. Kataoka, S. Takagi, M. Tarutani, S. Itami, K. Kiguchi, M. Yokoi, K. Sugasawa, T. Mori, F. Hanaoka, J. Takeda, and J. DiGiovanni. 2005. Signal transducer and activator of transcription 3 is a key regulator of keratinocyte survival and proliferation following UV irradiation. *Cancer Res* **65**:5720-5729.
39. Dixit, D., V. Sharma, S. Ghosh, N. Koul, P. Mishra, and E. Sen. 2009. Manumycin inhibits STAT3, telomerase activity, and growth of glioma cells by elevating intracellular reactive oxygen species generation. *Free Radic Biol Med.* Epub ahead of print.
40. Banchereau, J., and V. Pascual. 2006. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity* **25**:383-392.
41. van der Fits, L., L. I. van der Wel, J. D. Laman, E. P. Prens, and M. C. Verschuren. 2004. In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *J Invest Dermatol* **122**:51-60.
42. Downs, A. M., and M. G. Dunnill. 2000. Exacerbation of psoriasis by interferon-alpha therapy for hepatitis C. *Clin Exp Dermatol* **25**:351-352.
43. Funk, J., T. Langeland, E. Schrumpf, and L. E. Hanssen. 1991. Psoriasis induced by interferon-alpha. *Br J Dermatol* **125**:463-465.
44. Ketikoglou, I., S. Karatapanis, I. Elefsiniotis, G. Kafiri, and A. Moulakakis. 2005. Extensive psoriasis induced by pegylated interferon alpha-2b treatment for chronic hepatitis B. *Eur J Dermatol* **15**:107-109.
45. Pauluzzi, P., F. Kokelj, V. Perkan, G. Pozzato, and M. Moretti. 1993. Psoriasis exacerbation induced by interferon-alpha. Report of two cases. *Acta Derm Venereol* **73**:395.
46. Nestle, F. O., C. Conrad, A. Tun-Kyi, B. Homey, M. Gombert, O. Boyman, G. Burg, Y. J. Liu, and M. Gilliet. 2005. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* **202**:135-143.
47. Wei, L., R. Debets, J. J. Hegmans, R. Benner, and E. P. Prens. 1999. IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. *J Pathol* **187**:358-364.
48. DeSilva, B., R. C. McKenzie, J. A. Hunter, and M. Norval. 2008. Local effects of TL01 phototherapy in psoriasis. *Photodermatol Photoimmunol Photomed* **24**:268-269.
49. Tschachler, E. 2007. Psoriasis: the epidermal component. *Clin Dermatol* **25**:589-595.
50. de Cid, R., E. Riveira-Munoz, P. L. Zeeuwen, J. Robarge, W. Liao, E. N. Dannhauser, E. Giardina, P. E. Stuart, R. Nair, C. Helms, G. Escaramis, E. Ballana, G. Martin-Ezquerra, M. den Heijer, M. Kamsteeg, I. Joosten, E. E. Eichler, C. Lazaro, R. M. Pujol, L. Armengol, G. Abecasis, J. T. Elder, G. Novelli, J. A. Armour, P. Y. Kwok, A. Bowcock, J. Schalkwijk, and X. Estivill. 2009. Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. *Nat Genet* **41**:211-215.
51. Zhang, X. J., W. Huang, S. Yang, L. D. Sun, F. Y. Zhang, Q. X. Zhu, *et al*. 2009. Psoriasis genome-wide association study identifies susceptibility variants within LCE gene cluster at 1q21. *Nat Genet* **41**:205-210.

52. Orru, S., E. Giuressi, C. Carcassi, M. Casula, and L. Contu. 2005. Mapping of the major psoriasis-susceptibility locus (PSORS1) in a 70-Kb interval around the corneodesmosin gene (CDSN). *Am J Hum Genet* **76**:164-171.
53. Sesto, A., M. Navarro, F. Burslem, and J. Jorcano. 2002. Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *Proc Natl Acad Sci U S A* **99**:2965-2970.
54. Li, D., T. Turi, A. Schuck, I. Freedberg, G. Khitrov, and M. Blumenberg. 2001. Rays and arrays: the transcriptional program in the response of human epidermal keratinocytes to UVB illumination. *FASEB J* **15**:2533-2535.
55. Takao, J., K. Ariizumi, I. Dougherty, and P. J. Cruz. 2002. Genomic scale analysis of the human keratinocyte response to broad-band ultraviolet-B irradiation. *Photodermatol Photoimmunol Photomed.* **18**:5-13.
56. Enk, C. D., J. Jacob-Hirsch, H. Gal, I. Verbovetski, N. Amariglio, D. Mevorach, A. Ingber, D. Givol, G. Rechavi, and M. Hochberg. 2006. The UVB-induced gene expression profile of human epidermis *in vivo* is different from that of cultured keratinocytes. *Oncogene* **25**:2601-2614.
57. Kendziorski, C., R. A. Irizarry, K. S. Chen, J. D. Haag, and M. N. Gould. 2005. On the utility of pooling biological samples in microarray experiments. *Proc Natl Acad Sci U S A* **102**:4252-4257.
58. Glass, A., J. Henning, T. Karopka, T. Scheel, S. Bansemer, D. Koczan, L. Gierl, A. Rolfs, and U. Gimsa. 2005. Representation of individual gene expression in completely pooled mRNA samples. *Biosci Biotechnol Biochem* **69**:1098-1103.
59. Staal, F. J., F. Weerkamp, M. R. Baert, C. M. van den Burg, M. van Noort, E. F. de Haas, and J. J. van Dongen. 2004. Wnt target genes identified by DNA microarrays in immature CD34+ thymocytes regulate proliferation and cell adhesion. *J Immunol* **172**:1099-1108.
60. Irizarry, R. A., B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis, U. Scherf, and T. P. Speed. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**:249-264.
61. Bolstad, B. M., R. A. Irizarry, M. Astrand, and T. P. Speed. 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**:185-193.
62. Dik, W. A., K. Pike-Overzet, F. Weerkamp, D. de Ridder, E. F. de Haas, M. R. Baert, P. van der Spek, E. E. Koster, M. J. Reinders, J. J. van Dongen, A. W. Langerak, and F. J. Staal. 2005. New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. *J Exp Med* **201**:1715-1723.
63. Ge, U., S. Dudoit, and T. Speed. 2003. Resampling-based multiple testing for microarray data analysis. *TEST* **12**:1-44.
64. Dennis, G., Jr., B. T. Sherman, D. A. Hosack, J. Yang, W. Gao, H. C. Lane, and R. A. Lempicki. 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* **4**:P3.
65. van der Fits, L., L. I. van der Wel, J. D. Laman, E. P. Prens, and M. C. Verschuren. 2003. Psoriatic lesional skin exhibits an aberrant expression pattern of interferon regulatory factor-2 (IRF-2). *J Pathol* **199**:107-114.
66. Companjen, A. R., L. I. van der Wel, L. Wei, J. D. Laman, and E. P. Prens. 2001. A modified *ex vivo* skin organ culture system for functional studies. *Arch Dermatol Res* **293**:184-190.



Supplemental Figure 1. Top 5 GO annotation clusters as found by DAVID (Database for Annotation, Visualization, and Integrated Discovery) in the list of genes downregulated (a) or upregulated (b) in the lesional epidermis before vs. after NB-UVB therapy.

Each cluster contains multiple GO terms. Shown are the average fraction of genes assigned to the terms in the cluster and the geometric means of the original and Benjamini-Hochberg adjusted *p*-values of the terms in the cluster, respectively. The dashed line indicates the significance threshold (adjusted *p*-value = 0.05). See page 156 for a full-color representation of this figure.

Supplemental Table 1. Genes that are downregulated by NB-UVB phototherapy in lesional skin.

Name	Symbol	Fold change
Epidermal differentiation		
serpin peptidase inhibitor, clade B (ovalbumin), member 4	SERPINB4	68.37
peptidase inhibitor 3, skin-derived (SKALP)	PI3	23.60
keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	KRT16	12.94
keratin 6A / keratin 6C	KRT6A /C	9.70
small proline-rich protein 2C	SPRR2C	9.61
serpin peptidase inhibitor, clade B (ovalbumin), member 3	SERPINB3	7.50
gap junction protein, beta 2, 26kDa	GJB2	6.66
keratin 17	KRT17	5.18
keratin 6B	KRT6B	5.14
desmocollin 2	DSC2	3.46
Inflammation, IFN signaling		
lactotransferrin / similar to lactotransferrin	LTF	21.19
interleukin 1 family, member 9	IL1F9	5.08
interferon, alpha-inducible protein 6	IFI6	4.57
myxovirus resistance 1, interferon-inducible protein p78 (mouse)	MX1	4.32
interferon-induced protein 44-like	IFI44L	4.12
interferon-induced protein with tetratricopeptide repeats 3	IFIT3	3.38
interferon-induced protein 44	IFI44	3.32
interferon-induced protein with tetratricopeptide repeats 1	IFIT1	3.26
Antimicrobial		
S100 calcium binding protein A7A	S100A7A	36.50
defensin, beta 2 / defensin, beta 2	DEFB4	36.21
S100 calcium binding protein A12	S100A12	15.32
Proteases, protein modification		
plasminogen activator, tissue	PLAT	14.01
transmembrane protease, serine 11D	TMPRSS11D	3.98
ISG15 ubiquitin-like modifier	ISG15	3.77
kallikrein-related peptidase 6	KLK6	3.40
heparanase	HPSE	3.21
Stress response		
glutathione peroxidase 3 (plasma)	GPX3	5.81
calcium regulated heat stable protein 1, 24kDa	CARHSP1	3.62
superoxide dismutase 2, mitochondrial	SOD2	3.35
Other		
transcobalamin I (vitamin B12 binding protein, R binder family)	TCN1	35.57
lipocalin 2 (oncogene 24p3)	LCN2	11.60
aldo-keto reductase family 1, member B10 (aldose reductase)	AKR1B10	8.54
Rh family, C glycoprotein	RHCG	8.24
cytochrome P450 4Z2 pseudogene	CYP4Z2P	5.75
hypothetical protein LOC129607	LOC129607	5.64
zinc finger CCCH-type containing 12A	ZC3H12A	4.33
aldehyde dehydrogenase 1 family, member A3	ALDH1A3	4.15
cellular retinoic acid binding protein 2	CRABP2	3.77
nestin	NES	3.60
hect domain and RLD 6	HERC6	3.55
fibroblast growth factor binding protein 1	FGFBP1	3.43
non-metastatic cells 7, protein expressed in	NME7	3.34
ATPase, H^+/K^+ transporting, nongastric, alpha polypeptide	ATP12A	3.27
hyaluronan synthase 3	HAS3	3.26
pancreatic lipase-related protein 3	PNLIPRP3	3.23
epithelial stromal interaction 1 (breast)	EPSTI1	3.20

Supplemental Table 2. Genes that are upregulated by NB-UVB phototherapy in lesional skin.

Name	Symbol	Fold change
Epidermal differentiation		
serpin peptidase inhibitor, clade A, member 12	SERPINA12	6.75
keratin 77	KRT77	6.38
cystatin E/M	CST6	4.24
keratin 15	KRT15	3.70
tubulin polymerization promoting protein	TPPP	3.50
sciellin	SCEL	3.39
laminin, beta 4	LAMB4	3.28
Cell adhesion		
periostin, osteoblast specific factor	POSTN	50.13
cell adhesion molecule with homology to L1CAM	CHL1	3.69
Immune response		
interleukin 1 family, member 7 (zeta)	IL1F7	6.74
chemokine (C-C motif) ligand 27	CCL27	5.09
transforming growth factor, beta-induced, 68kDa	TGFBI	3.91
Wnt signaling		
solute carrier family 1, member 6	SLC1A6	4.98
solute carrier family 46, member 2	SLC46A2	3.81
v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	IL1F7	3.80
heat shock 70kDa protein 2	HSPA2	3.70
protocadherin 7	PCDH7	3.65
protocadherin 21	PCDH21	3.62
Notch signaling		
ligand of numb-protein X 1	LNX1	4.47
mastermind-like 2 (Drosophila)	MAML2	3.21
Transcription, translation		
Inhibitor of DNA binding 4	ID4	8.27
eukaryotic elongation factor-2 kinase	EEF2K	4.31
Apoptosis		
calpain 3, (p94)	CAPN3	3.34
Other		
similar to AVLV472	MGC23985	9.46
microseminoprotein, beta-	MSMB	8.68
chromosome 1 open reading frame 68	C1orf68	5.87
abhydrolase domain containing 12B	ABHD12B	5.38
cysteine-rich protein 1 (intestinal)	CRIP1	5.22
cytochrome b reductase 1	CYBRD1	5.10
betacellulin	BTC	4.98
calcineurin B homologous protein 2	CHP2	4.84
syntaxin binding protein 6 (amisyn)	STXBP6	4.77
asporin	ASPN	4.50
proteolipid protein 1	PLP1	4.22
cytidine monophosphate-N-acetylneuraminic acid hydroxylase	CMAH	4.15
integral membrane protein 2A	ITM2A	4.07
insulin-like growth factor binding protein 5	IGFBP5	4.04
transmembrane protein 116	TMEM116	4.04
Hypothetical protein LOC283666	LOC283666	3.87
ribonuclease, RNase A family, 4	RNASE4	3.82
transmembrane protein 99	TMEM99	3.55
platelet derived growth factor C	PDGFC	3.53
EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	3.43
protein tyrosine phosphatase, non-receptor type 21	PTPN21	3.43
heparan sulfate (glucosamine) 3-O-sulfotransferase 6	HS3ST6	3.41
carboxypeptidase E	CPE	3.38
glycoprotein M6B	GPM6B	3.29
dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2)	DCT	3.27
retinoic acid induced 14	RAI14	3.27
development and differentiation enhancing factor 2	DDEF2	3.26

Supplemental Table 3. Genes affected by NB-UVB therapy in non-lesional skin.

Name	Symbol	Fold change	Up/ Down	Change in lesional skin
S100 calcium binding protein A8	S100A8	24.8	Down	Unchanged
S100 calcium binding protein A9	S100A9	18.1	Down	17.66 Down
S100 calcium binding protein A7	S100A7	17.6	Down	Unchanged
small proline-rich protein 2B	SPRR2B	10.9	Down	Unchanged
keratin 6A, keratin 6C	KRT6A, KRT6C	7.9	Down	9.7 Down
small proline-rich protein 2G	SPRR2G	6.1	Down	Unchanged
chitinase 3-like 2	CHI3L2	4.2	Down	Unchanged
C-type lectin domain family 7, member A	CLEC7A	3.0	Down	Unchanged
chemokine (C-C motif) ligand 20	CCL20	2.5	Down	Unchanged
S100 calcium binding protein P	S100P	2.3	Down	Unchanged
STEAP family member 4	STEAP4	2.1	Down	3.8 Down
actinin, alpha 1	ACTN1	2.1	Down	3.2 Down
chromosome 15 open reading frame 48	C15orf48	2.1	Down	Unchanged
UDP glucuronosyltransferase 1 family, polypeptide A1-10	UGT1A1-10	2.1	Down	Unchanged
Ets homologous factor	EHF	2.0	Down	2.0 Down
cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	CDKN3	2.0	Down	Unchanged
hypothetical protein LOC202134	LOC202134	2.0	Down	Unchanged
betacellulin	BTC	2.3	Up	5.0 Up

Supplemental Table 4. Patient characteristics.

Patient nr.	M/F	Age	PASI before	PASI after	ΔPASI (%)
1	M	55	13.8	1.6	88.4
2	M	33	23.2	8.4	63.8
3	M	51	10.3	0	100.0
4	M	26	10.0	0	100.0
5	M	63	10.4	1.6	84.6
6	M	52	11.4	1.5	86.8
7	M	65	13.2	5.4	59.1
8	M	74	14.3	1.0	93.0
9	M	39	10.6	0	100.0
10	F	21	10.0	4.2	58.0

Supplemental Table 5. Pooling of RNA samples.

Condition	Time point	Pool A (patient numbers[1])	Pool B (patient numbers[1])
Lesional samples	**Before therapy**	1+2+3+4	5+6+7+8
	6 h after first irradiation	1+2+3+4	5+6+7+8
	After therapy	1+2+3+4	5+6+8+9
Non-lesional samples	**Before therapy**	1+2+3+4	5+6+7+10
	6 h after first irradiation	1+2+3+4	5+6+7+10
	After therapy		5+6+7+10

[1] Each number corresponds to one patient number as shown in Supplementary Table I.

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Narrow-band UVB strongly inhibits expression of activating innate cytosolic RNA receptors in keratinocytes *in vitro* and in psoriatic skin

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ABSTRACT

The mode of action of narrow-band UVB (NB-UVB) therapy in clearing psoriasis is incompletely understood and has been investigated only to a limited extent at the molecular level in patients. We previously demonstrated increased expression of double-stranded RNA (dsRNA) receptors in psoriasis lesions, resulting in enhanced sensitivity to innate triggering, thereby putatively contributing to the maintenance of psoriatic inflammation.

We hypothesized that suppression of dsRNA receptor expression by NB-UBV might add to the efficacy of this therapy. Analysis of epidermal samples of patients with psoriasis undergoing NB-UVB treatment demonstrated significant downregulation of mRNA expression of the major RIG-I-like helicase (RLH) family members MDA-5 and RIG-I during NB-UVB treatment, whereas the negative regulator LGP2 and the other activating receptors TLR3 and PKR were not affected. *In vitro* stimulation of primary human keratinocytes with IFN-α or IFN-γ, critical cytokines in psoriasis, resulted in upregulation of all activating dsRNA receptors. This induction was completely blocked by NB-UVB irradiation, resulting in an attenuated inflammatory cytokine response to dsRNA (expression of IFN-β, RANTES/CCL5 and IL-6).

Thus, NB-UVB irradiation cripples the local innate inflammatory response induced by dsRNA. These findings suggest a new mechanism contributing to the clinical efficacy of NB-UVB phototherapy in psoriasis.

INTRODUCTION

Psoriasis, a chronic inflammatory skin disease, is nowadays considered as a genetically determined dysregulation of innate and adaptive immunity (1). A dysregulation of the innate detection and response system can lead to both the epidermal phenotype and the abnormal T cell function that characterize the disease (2). Examples of abnormalities in the innate response system in psoriasis are the increased expression of antimicrobial peptides, such as β-defensins and LL-37 (3) and activation of the type I interferon (IFN) system (4, 5).

We have previously shown that the cytoplasmic receptors for double-stranded RNA (dsRNA) retinoic acid-inducible gene-I (RIG-I), melanoma differentiation associated gene 5 (MDA-5) and double-stranded RNA-dependent protein kinase (PKR) are upregulated in the epidermis of patients with psoriasis (6). These pattern recognition receptors (PRRs), together with Toll-like receptors (TLRs), are part of the innate detection system. Activation of these receptors induces the initial anti-microbial responses by upregulating antimicrobial peptides, inflammation and chemotaxis of leukocytes.

DsRNA, a virus-derived molecular pattern can be recognized by the intravesicular transmembrane receptor TLR3, as well as by four different intracytoplasmic receptors. RIG-I and MDA-5 are part of the RIG-I-like helicase (RLH) family together with laboratory of genetics and physiology-2 (LGP2) (7). LGP2 has been suggested to function as a negative regulator of RIG-I/MDA-5 signaling (8). Activation of PKR, also an intracellular detector of viral dsRNA, leads to the inhibition of translation of the host cell, which represents a unique contribution to anti-viral immunity (9).

Not only microbial components but also endogenous molecules have been shown to activate TLRs and other PRRs. Therefore innate antigen receptors have been implicated in the pathogenesis of different autoimmune diseases: in systemic lupus erythematosus, activation of TLR7 and TLR9 by nucleic acids from dead or dying cells is implicated, while in rheumatoid arthritis TLR3 is activated by RNA from necrotic cells (10, 11). Recently it was shown that in psoriasis, activation of TLR9 in plasmacytoid dendritic cells (pDC) by self-DNA complexed with the antimicrobial peptide LL37 plays a pathogenic role, by strongly inducing IFN-α production (12). Endogenous ligands have been described for TLR3 and RIG-I. RNA from necrotic cells worsens septic peritonitis and ischemic gut injury in mice, mediated via TLR3 (13). In addition, uptake of apoptotic material containing RNA with 5'-triphosphate groups by dendritic cells or macrophages leads to the engagement of RIG-I (14).

In vitro IFN-α-treatment of primary human epidermal keratinocytes induced the expression of TLR3, RIG-I, MDA-5 and PKR, resulting in an increased proinflammatory response to dsRNA (6). Elevated IFN-α-levels in psoriasis skin might partly be responsible for the higher expression of dsRNA-receptors, which in turn might increase the sensitivity of psoriatic epidermal cells to innate triggering, thereby contributing to the vicious cycle of chronic inflammation. Therefore, functional blockade or downregulation of the dsRNA receptors would be a potential therapeutic option in psoriatic skin inflammation.

Narrow-band UVB (NB-UVB) therapy is a standard treatment for moderate to severe psoriasis. It is safe in terms of minimal carcinogenicity, and has high efficacy scores in a large proportion of patients (15-19). The mode of action of NB-UVB treatment in psoriasis is incompletely understood and much of current thinking is based on *in vitro* studies and

studies in mice. In general, the efficacy of NB-UVB is thought to be mediated via inhibition of keratinocyte proliferation, induction of apoptosis in intraepidermal bone marrow-derived cells (dendritic cells, (natural killer) T cells, pDCs), skewing of T cell differentiation from Th1 and Th17 towards the Th2 phenotype. However, it has become clear that the skin immune system of the mouse and the mouse genome differ more from man than previously anticipated, underscoring the need for studies in patients with active disease. Molecular analysis of the impact of NB-UVB on innate inflammatory pathways may generate more insight into the mode of action of NB-UVB in the pathophysiology of psoriasis and to define potential therapeutic target molecules.

Based on our observations on dsRNA receptor expression in psoriasis, we investigated whether NB-UVB treatment affects the expression and function of these molecules in psoriatic skin. To address this question, a dual clinical and *in vitro* approach was followed. Collectively, our data show strong inhibition of the expression and function of innate antigen receptors for dsRNA, both *in vivo* in patients with psoriasis undergoing NB-UVB therapy, as well as *in vitro* in irradiated keratinocytes.

RESULTS

Inhibition of RIG-I and MDA-5 gene expression in patients undergoing NB-UVB therapy

Affymetrix gene expression arrays were used to detect differences in epidermal mRNA expression in patients with plaque-type psoriasis undergoing conventional NB-UVB therapy. The PASI scores showed an improvement from a mean of 13.3 (range 10.0 - 23.2) to a mean of 2.4 (range 0 and 8.4) after 12 weeks; thus, the mean PASI reduction was 84.5%. For the microarray analysis, patient samples were pooled in 2 groups, as described in the Materials and methods section and shown in Table 1. The mean PASI reduction did not differ significantly between these two groups.

All five dsRNA receptors were detected on the gene expression arrays. In lesional skin, expression levels of RIG-I and MDA-5 were significantly reduced following NB-UVB treatment, with a reduction of 1.9-fold and 1.8-fold respectively (Figure 1). Expression of PKR was also decreased, but this change did not reach significance, whereas TLR3 and LGP2

Table 1. Patient characteristics.

Patient nr.	M/F	Age	PASI before	PASI after	ΔPASI (%)	Pool nr.
1	M	55	13.8	1.6	88.4	1
2	M	33	23.2	8.4	63.8	1
3	M	51	10.3	0	100.0	1
4	M	26	10.0	0	100.0	1
5	M	63	10.4	1.6	84.6	2
6	M	52	11.4	1.5	86.8	2
7	M	65	13.2	5.4	59.1	2
8	M	74	14.3	1.0	93.0	2

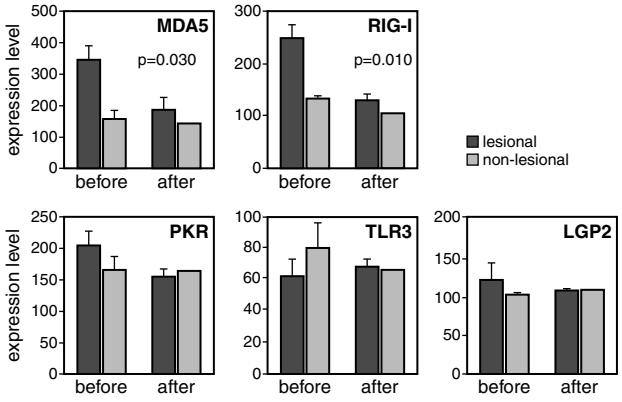

Figure 1. NB-UVB inhibits mRNA expression of dsRNA receptors in psoriatic lesional epidermis.
Lesional and non-lesional skin biopsy samples of 8 patients with psoriasis were collected before and after conventional NB-UVB treatment. Epidermal RNA of 4 patients was pooled for each gene expression array in order to have duplicate arrays for each time point, as described in the Materials and methods section. Probe sets for dsRNA receptor genes were selected from the array results. Bars show the mRNA expression levels of two pools (mean +/- SD). *p*-values calculated by a two-way ANOVA are indicated in the figure if < 0.05.

expression remained unchanged. In non-lesional skin, expression of none of these receptors changed significantly after NB-UVB therapy (Figure 1).

Microarray results were validated using quantitative RT-PCR on RNA samples from 5 individual patients, including skin samples taken at a PASI reduction of 50%. ABL1 was used as an internal control housekeeping gene. Here, too, on average about two-fold reduction of MDA-5 and RIG-I expression was observed at the end of the treatment (Figure 2). The mean expression of MDA-5 was significantly reduced when PASI scores were at 50% of the baseline score (Figure 2).

NB-UVB suppresses type I and type II IFN-induced expression of dsRNA receptors in primary keratinocytes

To investigate whether the *in vivo* observed downregulation of dsRNA receptors was a direct effect of NB-UVB, or only correlated with the clinical improvement of psoriasis, experiments were conducted using primary human keratinocytes, the primary source of epidermal dsRNA receptor expression (6). In order to mimic the *in vivo* situation in psoriasis, IFN-α and IFN-γ activated primary human keratinocytes were irradiated with a single dose of NB-UVB. IFN-α and IFN-γ are known to induce the expression of all four activating dsRNA receptors (6). Cells were harvested after 0, 2, 4, 8 and 24 h, and mRNA expression of dsRNA receptors was measured by RT-PCR. As expected, IFN-α and IFN-γ induced the expression of MDA-5 (maximal induction approximately 14-fold and 10-fold, respectively with IFN-α and IFN-γ), RIG-I (13-fold and 12-fold), TLR3 (9-fold and 5-fold), and PKR (4-fold and 2-fold). NB-UVB irradiation resulted in a rapid and almost complete inhibition of all dsRNA receptors during the first 8 h of culture. This inhibition was lost after 24 h of culture (Figure 3 and Figure 4). Expression of the negative regulator LGP2 was induced 5-fold by IFN-α and 2-fold by IFN-γ in keratinocytes. The induction of LGP2 mRNA was not affected by NB-UVB irradiation (Figure 3 and Figure 4).

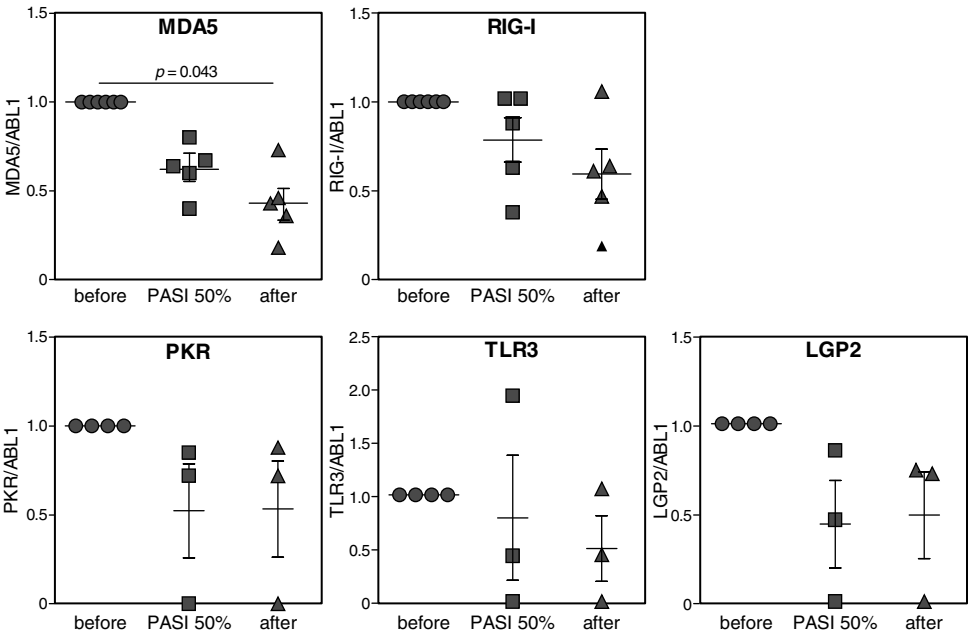

Figure 2. Confirmation of the downregulation of dsRNA receptors after NB-UVB therapy using quantitative RT-PCR.

Lesional skin biopsy samples of 4 patients with psoriasis were collected before and after NB-UVB treatment, and during treatment when PASI scores reached 50% of the baseline scores. Epidermal mRNA levels of dsRNA receptor genes were determined by RT-PCR, and calculated relative to ABL1 mRNA levels. Values for individual patients are depicted. Horizontal lines show the mean and the standard error of the mean.

NB-UVB inhibits keratinocyte activation by the dsRNA analog poly I:C

We next assessed whether the decreased expression of dsRNA receptors in irradiated keratinocytes would affect the functional responsiveness to dsRNA. Upon stimulation with polyriboinosinic-polyribocytidylic acid (poly I:C) normal keratinocytes produce a broad array of pro-inflammatory cytokines and chemokines, as well as adhesion molecules (20), and this response is boosted by pre-incubating the cells with IFN-α (6). Activation of all cytoplasmic dsRNA receptors in keratinocytes by poly I:C was clearly demonstrated previously (21). A few marker molecules of this poly I:C-induced inflammatory response in keratinocytes are IL-6, CCL5/RANTES, and IFN-β. All these markers are expressed at high levels in inflamed skin lesions of psoriasis patients. Keratinocytes were irradiated with a single dose of NB-UVB, incubated with IFN-α or IFN-γ for 6 h and stimulated with 1 μg/ml poly I:C. Both type I and II IFN pre-incubation greatly enhanced the poly I:C-induced expression of IFN-β, RANTES and IL-6. NB-UVB-irradiation of keratinocytes resulted in a strong inhibition of the response to poly I:C (Figure 5 a-c).

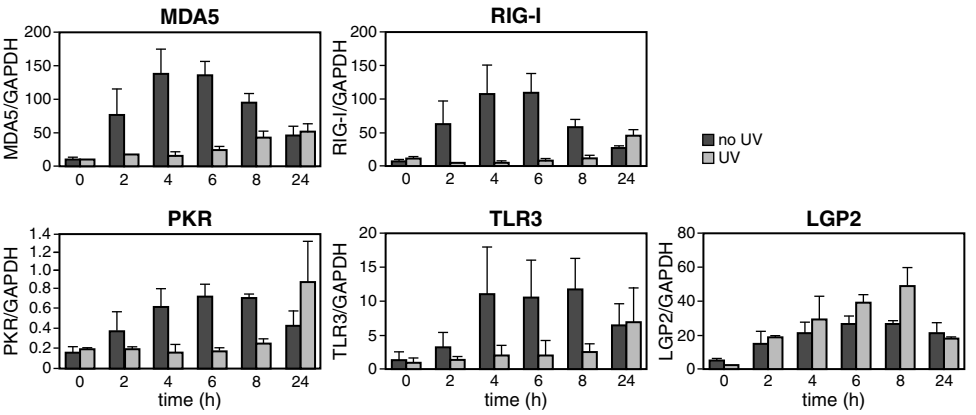

Figure 3. NB-UVB inhibits IFN-α-induced expression of dsRNA-receptors in keratinocytes.
Primary human keratinocytes were irradiated with 600 mJ/cm^2 NB-UVB, or were sham-irradiated. Immediately hereafter IFN-α (500 U/ml) was added to the cell culture. Cells were harvested at the time points indicated and mRNA expression of dsRNA receptors was quantified using RT-PCR, and calculated relative to GAPDH levels. Data are representative of two independent experiments with primary keratinocytes from two different donors.

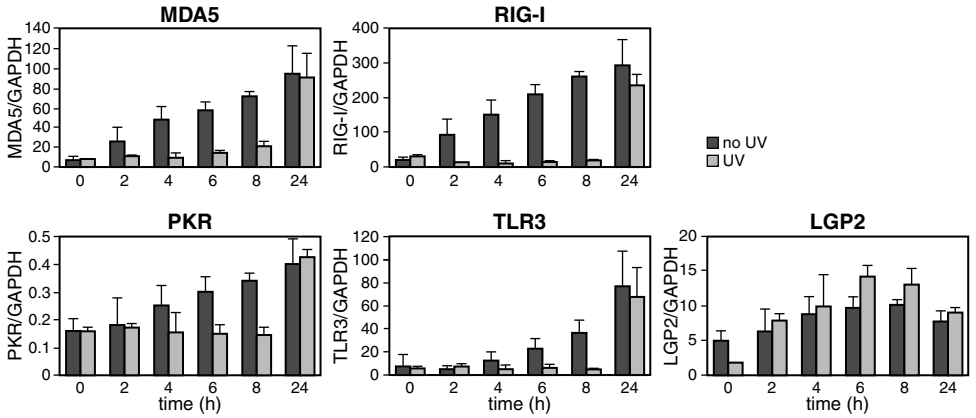

Figure 4. NB-UVB inhibits IFN-γ-induced expression of dsRNA-receptors in keratinocytes.
Primary human keratinocytes were irradiated with 600 mJ/cm^2 NB-UVB, or were sham-irradiated. Immediately hereafter IFN-γ (500 U/ml) was added to the cell culture. Cells were harvested at the time points indicated and mRNA expression of dsRNA receptors was quantified using RT-PCR, and calculated relative to GAPDH levels. Data are shown from two independent experiments with keratinocytes from two different donors.

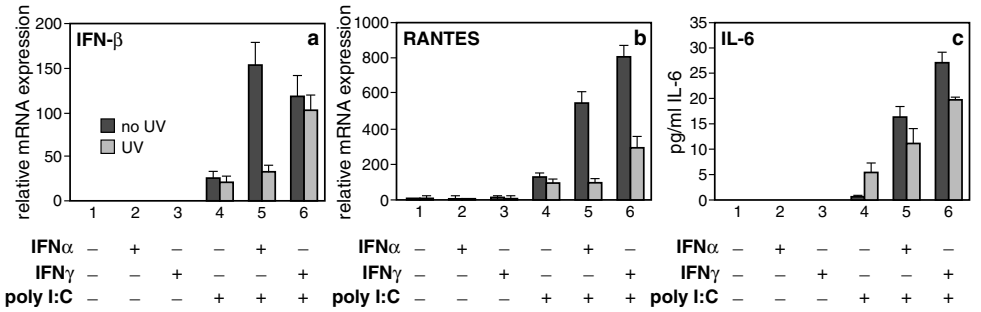

Figure 5. NB-UVB limits poly I:C-activation of keratinocytes pretreated with type I and type II IFNs.
Primary human keratinocytes were irradiated with 600 mJ/cm^2 NB-UVB, or sham-irradiated. Immediately hereafter 500 U/ml IFN-α or IFN-γ was added to the cells. After incubation for 6 h these stimuli were removed and poly I:C (1 μg/ml) was added to the cells for 3 h. mRNA expression of RANTES/CCL5 and IFN-β was determined by quantitative RT-PCR, and calculated relative to GAPDH levels (a-b). IL-6 levels in the supernatants were measured using ELISA (c). Results of a representative experiment are shown.

DISCUSSION

This study shows that *in vivo* NB-UVB treatment inhibits the expression of RIG-I and MDA-5 in lesional epidermis of patients with psoriasis and of all dsRNA receptors in keratinocytes *in vitro*. We have previously shown that these receptors are highly expressed by keratinocytes in lesional psoriatic epidermis (6). Expression of PKR and LGP2 was not suppressed in the patients by NB-UVB therapy. PKR is involved in the control of cell proliferation, differentiation and apoptosis upon virus infection (22). The persistence of PKR and of the negative regulator LGP2 after NB-UVB therapy suggests that immune modulation by UVB occurs in a selective mode, which may explain some of its reported safety.

The *in vitro* suppression of the proinflammatory poly I:C-response in keratinocytes by NB-UVB can be explained by the rapid inhibition of all activating dsRNA receptors while the expression of the negative regulator LGP2 remained unaffected. This may happen by direct short-term effect of UVB on the receptors or their signaling pathways, or by known UVB-activated cellular processes such as DNA damage and repair, formation of reactive oxygen species or cis-urocanic acid. In addition, UVB radiation might interfere with the downstream signaling after dsRNA binding, since both UVB (23) and the dsRNA receptors TLR3 and PKR (21) act partly via activation of NF-κB.

The upregulation of LGP2 by both type I and type II IFNs was not inhibited by NB-UVB. This effect of NB-UVB on the IFN-induced expression of LGP2 also strongly suggests that the inhibitory effect on other receptors is not a consequence of a general toxic effect of UV-radiation.

In both the *in vivo* and the *in vitro* parts of our study narrow-band UVB lamps were used. It is known that at least 5 to 10-fold higher doses of NB-UVB are needed for the induction of erythema than with broad-band (BB-) UVB (24-28). NB-UVB doses required for the induction

of hyperplasia, edema, sunburn cell response and Langerhans cell depletion are 5-7 times higher than equally effective BB-UVB doses (29). In *in vitro* assays, Aufiero *et al* studied the response of keratinocytes to increasing doses of NB-UVB, and found that a dose as high as 1000 mJ/cm^2 was necessary to induce apoptosis in keratinocytes, whereas in T lymphocytes a dose of 750 mJ/cm^2 (but not one of 500 mJ/cm^2) already induced apoptosis (30).

The efficacy of NB-UVB treatment has largely been attributed to its apoptotic effect on inflammatory epidermal T cells, and by its ability to reduce IFN-γ-dependent Th1 differentiation of T cells and promote Th2 differentiation (31-34). These effects on T lymphocyte skewing are considered a central or systemic effect of UVB (35).

The rapid suppression of dsRNA receptors by NB-UVB as demonstrated in primary keratinocytes, is highly suggestive for a direct effect of NB-UVB on specific dsRNA receptors. NB-UVB irradiation interfered with the interaction of poly I:C with these molecules, preventing the activation of keratinocytes as measured by IFN-β and RANTES mRNA, and IL-6 protein expression. These results suggest that in addition to an effect on adaptive immunity, NB-UVB also has a direct short-term effect on innate immunity in psoriasis.

Decrease of dsRNA receptor expression observed in the patients might be due not only to direct downregulation in keratinocytes. Induction of apoptosis by NB-UVB in keratinocytes and other cells together with the depletion of Langerhans cells or pDC, may also play a role (36). Human epidermal Langerhans cells are known to express functional TLR3 (37, 38). MDA5, RIG-I and PKR can be expressed by neutrophils (39), a cell type predominantly present in Munro microabscesses in psoriatic epidermis, structures that are cleared during NB-UVB treatment. Thus, expression of dsRNA receptors by neutrophils and/or Langerhans cells might contribute to the expression levels of these receptors measured in psoriatic epidermal RNA samples. The presence of cell types other than keratinocytes in the plaques might also explain the discrepancies between our *in vivo* and *in vitro* results, i.e. that whereas in primary human keratinocytes, the IFN-induced expression of all four activating dsRNA-receptors was inhibited by UV irradiation, by microarray analysis of epidermal samples only the expression of MDA-5 and RIG-I was significantly reduced. Interestingly, of four dsRNA receptors tested, these two were found to be most differentially expressed in lesional psoriatic epidermis when compared to healthy and non-lesional skin (6).

The ability of UVB to inhibit IFN-γ-induced processes in keratinocytes has previously been demonstrated (40, 41). Our results show that UVB also suppresses the response to IFN-α in keratinocytes. A key mechanism involved may be inhibition of the phosphorylation of STAT1, a signal transduction molecule shared by type I and type II interferons. In mouse keratinocytes, broad-band UVB light has been shown to interfere with the phosphorylation of STAT1, partly by activation of a tyrosine phosphatase (40). Furthermore, interferon-dependent dsRNA responses have previously been shown to depend on STAT1 in human fibrosarcoma-derived cell lines (42).

In keratinocytes, functionality of all dsRNA receptors have been demonstrated (21). It will be worthwhile to investigate whether downregulation of one specific dsRNA receptor (e.g. via silencing with siRNA) would also be sufficient to improve the psoriatic skin pathology. According to our data, MDA-5 and RIG-I pathways are possible candidates to target in order to reduce inflammation in psoriasis, since their expression was also downregulated in *in vivo* irradiated psoriatic skin.

In conclusion, here we show that NB-UVB-induced improvement of skin inflammation in psoriasis is accompanied by a reduction in the mRNA expression of the innate cytosolic dsRNA receptors MDA-5 and RIG-I, but not PKR. In cultured primary keratinocytes, NB-UVB irradiation strongly inhibited the type I and type II IFN-induced expression of all activating dsRNA-receptors but not that of the negative regulator LGP2. This rapid suppression of dsRNA receptors and interference with poly I:C interaction with these molecules by NB-UVB in primary keratinocytes is suggestive for a direct short-term effect on innate immunity in psoriasis. NB-UVB irradiation hereby breaks a positive feedback loop of epidermal inflammation in psoriasis. These results reveal a novel mechanism of action contributing to the efficacy of NB-UVB phototherapy in psoriasis.

MATERIALS AND METHODS

Patients and treatment

Eight patients with plaque type psoriasis were recruited after informed consent and prior approval by the Medical Ethical Committee of the Erasmus MC, University Medical Center (METC 234.237/2003/210). Patients (eight men, age range 26-74) with Psoriasis Activity and Severity Index (PASI) scores of at least 10, did not receive systemic therapy for at least one month or topical therapy (except for emollients) for at least two weeks prior to this study. Patient characteristics are depicted in Table 1. The patients were treated with standard NB-UVB phototherapy three times weekly (increasing doses of NB-UVB) using a Waldmann 7001 UVB cabinet equipped with Philips TL-01 bulbs, until total clearance of psoriasis or for a maximum of three months. Starting UVB dose was 0.1-0.3 J/cm^2; the mean cumulative UVB dose was 45.9 (range 30-60) J/cm^2. The initial NB-UVB dose was 70% of the minimal erythema dose (MED). PASI scores were evaluated every two weeks.

Biopsy samples, RNA extraction, array hybridization, scanning and analysis

Biopsy samples of 3 mm in diameter were taken from lesional and non-lesional skin before the start of NB-UVB treatment and at the end of therapy. Additional biopsies were collected when PASI scores reached 50% of the baseline score. The epidermis was separated from the dermis after incubation in 0.5 mg/ml Thermolysin (Sigma, St Louis, MO) supplemented with 5 μg/ml Actinomycin D (Sigma) for 90 min at 37°C, and stored in RNA lysis buffer at -80°C until further processing. Total messenger RNA was isolated using GenElute Mammalian Total RNA Miniprep kit (Sigma). RNA purity and integrity was determined by scanning with an Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip.

For the microarray analysis, patients were divided into 2 groups, and RNA samples from 4 patients (250 ng mRNA per patient) were pooled, biotinylated and hybridized to each GeneChip Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA). Two pools were formed from the samples taken before therapy (patients 1-4 and 5-8), as well as two pools from samples of the same patient groups, taken after therapy. This pooling of RNA samples is an accepted and validated method in microarray studies (43, 44). The advantage of pooling RNA samples is that the results are less influenced by patient-specific alterations in gene expression, and rather reflect the gene expression profile specific to the condition of

interest, i.e. the effect of NB-UVB in all patients. The influence of patient-specific alterations in gene expression was further reduced by analysis of two independent pools of RNA samples (biological duplicates) for each of the two conditions, "before therapy" and "after therapy".

Array hybridization and scanning was performed as described previously (45). The data were read and robust multichip analysis (RMA) (46) was used to remove the background and normalize the data across arrays (47). These values were $\log_2$-transformed for further analysis, yielding numbers between 0 and 16. A two-way ANOVA with factors "probe" and "condition" was used for each probeset to calculate both average expression levels per condition (48) and a *p*-value for the difference between conditions. The resulting *p*-values were adjusted for multiple testing using Šidák step-up adjustment (49). For this study we looked at the expression levels of probesets for dsRNA receptors.

Quantitative RT-PCR

RNA was transcribed into cDNA, and RT-PCR was performed as described previously (50). Primer and FAM-labeled probe sequences for TLR3, RIG-I, MDA-5 and PKR have been described by Prens *et al.* (6). Other primer and probe sequences are listed in Table 2.

In the microarray results a marked effect of *in vivo* NB-UVB treatment was observed on the expression levels of the housekeeping gene GAPDH. For this reason another widely used and accepted internal control gene, ABL1 was chosen for the validation of the results with RT-PCR. The expression of this gene remained stable throughout all samples.

Culture, stimulation and NB-UVB irradiation of primary keratinocytes

Primary human keratinocytes (Promocell GmbH, Heidelberg, Germany) were cultured in Keratinocyte Growth Medium 2 with SupplementMix (Promocell). Cells were used in passage 2 to 7.

Keratinocytes were stimulated with 500 U/ml clinical grade IFN-α (IntronA IFN-α2b, Schering-Plough, Kenilworth, NJ), 500 U/ml clinical grade IFN-γ (Boehringer Ingelheim, Heidelberg, Germany), with or without the subsequent addition of 1 μg/ml poly I:C (Amersham Biosciences, Piscataway, NJ) as functional mimic of dsRNA.

Table 2. Sequences of primers and probes for real-time PCR.

Gene	Forward primer	Reverse primer	Probe
LGP2	GCCTTGCAAACAGTACAACCT	TCTTCAGCAAGTCCCCAAA	Nr. 71 [1]
RANTES	TGCATCTGCCTCCCCATATT	AGTGGGCGGGCAATGTAG	TCGGACACCACA CCCTGCTGCT
IFN-β	GATTCCTACAAAGAAGCAGCAAT	CATCCTGTCCTTGAGGCAGTATT	AGCCTCCCATTCA ATTGCCACAGGA
ABL1	TGGAGATAACACTCTAAGCATAA CTAAAGGT	GATGTAGTTGCTTGGGACCCA	CCATTTTTGGTTTG GGCTTCACACCATT
GAPDH	TCCACTGGCGTCTTCAC	GGCAGAGATGATGACCCTTTT	Nr. 45 [1]

[1] Probe from the Exiqon probe library system (Exiqon, Vedbaek, Denmark).

Primary cell cultures were irradiated using a Waldmann irradiation device equipped with TL-01 UV 236-01 lamps (Waldmann Medizintechnik, Villingen-Schwenningen, Germany), with main emission between 310-315 nm (peak: 311 nm). In all experiments, a single UV-dose of 600 mJ/cm^2 was used, after rinsing the cells thoroughly with PBS, leaving the cells covered with a thin layer of PBS to protect them from drying out. This UV dose is not apoptotic in human keratinocytes (51), and is higher than the starting dose of clinical NB-UVB treatment, during which this dose is usually reached within 1-3 weeks. Viability of keratinocytes after increasing doses of NB-UVB was measured by methylene blue staining (Supplemental Figure 1). In brief, 24 h after irradiation cell were fixed by 30 min incubation (room temperature) with 20% formaldehyde in 0.09% NaCl. After removal of the fixative, cells were incubated with 1% methylene blue (Boom BV, Meppel, the Netherlands) in 0.01 M H_3BO_3. After washing away this solution, the blue stain was extracted from the cells using a 1:1 mixture of ice cold ethanol and 0.1 M HCl, the extract was transferred to a 96-wells plate and the amount of blue stain was assessed by measuring the absorbance at 650 nm using a Multiskan RC microplate reader (Thermo Labsystems, Finland).

At the time points indicated, total messenger RNA was extracted from the cells using GenElute Mammalian Total RNA Miniprep kit (Sigma). Levels of IL-6 as a prototypic inflammatory cytokine in the culture supernatants were determined with commercial capture ELISA as recommended by the manufacturer and as described elsewhere (6).

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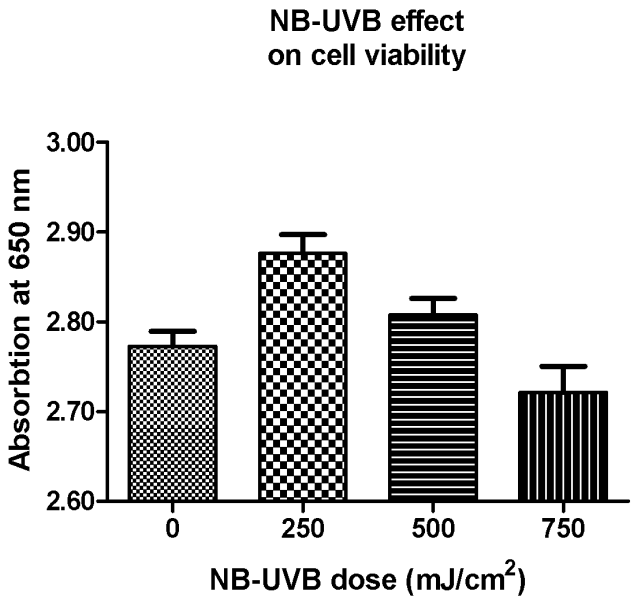
REFERENCES

1. Bos, J. D., M. A. de Rie, M. B. Teunissen, and G. Piskin. 2005. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* **152**:1098-1107.
2. Buchau, A. S., and R. L. Gallo. 2007. Innate immunity and antimicrobial defense systems in psoriasis. *Clin Dermatol* **25**:616-624.
3. Hollox, E. J., U. Huffmeier, P. L. Zeeuwen, R. Palla, J. Lascorz, D. Rodijk-Olthuis, P. C. van de Kerkhof, H. Traupe, G. de Jongh, M. den Heijer, A. Reis, J. A. Armour, and J. Schalkwijk. 2008. Psoriasis is associated with increased beta-defensin genomic copy number. *Nat Genet* **40**:23-25.
4. van der Fits, L., L. I. van der Wel, J. D. Laman, E. P. Prens, and M. C. Verschuren. 2004. In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *J Invest Dermatol* **122**:51-60.
5. Nestle, F. O., C. Conrad, A. Tun-Kyi, B. Homey, M. Gombert, O. Boyman, G. Burg, Y. J. Liu, and M. Gilliet. 2005. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* **202**:135-143.
6. Prens, E. P., M. Kant, G. van Dijk, L. I. van der Wel, S. Mourits, and L. van der Fits. 2008. IFN-alpha enhances poly-IC responses in human keratinocytes by inducing expression of cytosolic innate RNA receptors: relevance for psoriasis. *J Invest Dermatol* **128**:932-938.
7. Takeuchi, O., and S. Akira. 2008. MDA5/RIG-I and virus recognition. *Curr Opin Immunol* **20**:17-22.

8. Rothenfusser, S., N. Goutagny, G. DiPerna, M. Gong, B. G. Monks, A. Schoenemeyer, M. Yamamoto, S. Akira, and K. A. Fitzgerald. 2005. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. *J Immunol* **175**:5260-5268.
9. Balachandran, S., P. C. Roberts, L. E. Brown, H. Truong, A. K. Pattnaik, D. R. Archer, and G. N. Barber. 2000. Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. *Immunity* **1**3:129-141.
10. Barrat, F. J., T. Meeker, J. Gregorio, J. H. Chan, S. Uematsu, S. Akira, B. Chang, O. Duramad, and R. L. Coffman. 2005. Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. *J Exp Med* **202**:1131-1139.
11. Brentano, F., O. Schorr, R. E. Gay, S. Gay, and D. Kyburz. 2005. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via Toll-like receptor 3. *Arthritis Rheum* **52**:2656-2665.
12. Lande, R., J. Gregorio, V. Facchinetti, B. Chatterjee, Y. H. Wang, B. Homey, W. Cao, B. Su, F. O. Nestle, T. Zal, I. Mellman, J. M. Schroder, Y. J. Liu, and M. Gilliet. 2007. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**:564-569.
13. Cavassani, K. A., M. Ishii, H. Wen, M. A. Schaller, P. M. Lincoln, N. W. Lukacs, C. M. Hogaboam, and S. L. Kunkel. 2008. TLR3 is an endogenous sensor of tissue necrosis during acute inflammatory events. *J Exp Med* **205**:2609-2621.
14. Bowie, A. G., and K. A. Fitzgerald. 2007. RIG-I: tri-ing to discriminate between self and non-self RNA. *Trends Immunol* **28**:147-150.
15. Hearn, R. M., A. C. Kerr, K. F. Rahim, J. Ferguson, and R. S. Dawe. 2008. Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. *Br J Dermatol* **159**:931-935.
16. Coven, T. R., L. H. Burack, R. Gilleaudeau, M. Keogh, M. Ozawa, and J. G. Krueger. 1997. Narrowband UV-B produces superior clinical and histopathological resolution of moderate-to-severe psoriasis in patients compared with broadband UV-B. *Arch Dermatol* **133**:1514-1522.
17. Walters, I. B., L. H. Burack, T. R. Coven, P. Gilleaudeau, and J. G. Krueger. 1999. Suberythemogenic narrow-band UVB is markedly more effective than conventional UVB in treatment of psoriasis vulgaris. *J Am Acad Dermatol* **40**:893-900.
18. Ibbotson, S. H., D. Bilsland, N. H. Cox, R. S. Dawe, B. Diffey, C. Edwards, P. M. Farr, J. Ferguson, G. Hart, J. Hawk, J. Lloyd, C. Martin, H. Moseley, K. McKenna, L. E. Rhodes, and D. K. Taylor. 2004. An update and guidance on narrowband ultraviolet B phototherapy: a British Photodermatology Group Workshop Report. *Br J Dermatol* **151**:283-297.
19. Carrascosa, J. M., J. Gardeazabal, A. Perez-Ferriols, A. Alomar, P. Manrique, M. Jones-Caballero, M. Lecha, J. Aguilera, and J. de la Cuadra. 2005. [Consensus document on phototherapy: PUVA therapy and narrow-band UVB therapy]. *Actas Dermosifiliogr* **96**:635-658.
20. Lebre, M. C., A. M. van der Aar, L. van Baarsen, T. M. van Capel, J. H. Schuitemaker, M. L. Kapsenberg, and E. C. de Jong. 2007. Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. *J Invest Dermatol* **127**:331-341.
21. Kalali, B. N., G. Kollisch, J. Mages, T. Muller, S. Bauer, H. Wagner, J. Ring, R. Lang, M. Mempel, and M. Ollert. 2008. Double-stranded RNA induces an antiviral defense status in epidermal keratinocytes through TLR3-, PKR-, and MDA5/RIG-I-mediated differential signaling. *J Immunol* **181**:2694-2704.
22. Garcia, M. A., E. F. Meurs, and M. Esteban. 2007. The dsRNA protein kinase PKR: virus and cell control. *Biochimie* **89**:799-811.
23. Lewis, D. A., and D. F. Spandau. 2008. UVB-induced activation of NF-kappaB is regulated by the IGF-1R and dependent on p38 MAPK. *J Invest Dermatol* **128**:1022-1029.
24. Van Weelden, H., H. Baart de la Faille, E. Young, and J. van der Leun. 1988. A new development in UVB phototherapy of psoriasis. *Br J Dermatol* **119**:11-19.
25. Johnson, B., C. Green, T. Lakshmipathi, and J. Ferguson. 1988. Ultraviolet radiation phototherapy for psoriasis; the use of a newnarrow band UVB fluorescent lamp. In *Light in biology and medicine*. R. Douglas, J. Moan, and D. A. F, eds. Plenum, Oxford. 173-179.
26. Karvonen, J., L.-E. Kokkonen, and E. Routsalainen. 1989. 311 nm UVB lamps in the treatment of psoriasis with the Ingram regimen. . *Acta Derm Venereol (Stockholm)* **69**:82-85.

27. Storbeck, K., E. Holzle, N. Schurer, P. Lehmann, and G. Plewig. 1993. Narrow-band UVB (311 nm) versus conventional broad-band UVB with and without dithranol in phototherapy for psoriasis. *J Am Acad Dermatol* **28**:227-231.
28. Srinivas, C. 2002. Minimal erythema dose (Med) to narrow band ultraviolet-B (NB-UVB) broad band ultraviolet-B (BB-UVB)--a pilot study. *Indian J Dermatol Venereol Leprol.* **68**:63-64.
29. el-Ghorr, A. A., and M. Norval. 1997. Biological effects of narrow-band (311 nm TL01) UVB irradiation: a review. *J Photochem Photobiol B* **38**:99-106.
30. Aufiero, B. M., H. Talwar, C. Young, M. Krishnan, J. S. Hatfield, H. K. Lee, H. K. Wong, I. Hamzavi, and G. J. Murakawa. 2006. Narrow-band UVB induces apoptosis in human keratinocytes. *J Photochem Photobiol B* **82**:132-139.
31. Krueger, J. G., J. T. Wolfe, R. T. Nabeya, V. P. Vallat, P. Gilleaudeau, N. S. Heftler, L. M. Austin, and A. B. Gottlieb. 1995. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. *J Exp Med* **182**:2057-2068.
32. Piskin, G., C. W. Koomen, D. Picavet, J. D. Bos, and M. B. Teunissen. 2003. Ultraviolet-B irradiation decreases IFN-gamma and increases IL-4 expression in psoriatic lesional skin *in situ* and in cultured dermal T cells derived from these lesions. *Exp Dermatol* **12**:172-180.
33. Piskin, G., R. M. Sylva-Steenland, J. D. Bos, and M. B. Teunissen. 2004. T cells in psoriatic lesional skin that survive conventional therapy with NB-UVB radiation display reduced IFN-gamma expression. *Arch Dermatol Res* **295**:509-516.
34. Walters, I. B., M. Ozawa, I. Cardinale, P. Gilleaudeau, W. L. Trepicchio, J. Bliss, and J. G. Krueger. 2003. Narrowband (312-nm) UV-B suppresses interferon gamma and interleukin (IL) 12 and increases IL-4 transcripts: differential regulation of cytokines at the single-cell level. *Arch Dermatol* **139**:155-161.
35. Sleijffers, A., A. Kammeyer, F. R. de Gruijl, G. J. Boland, J. van Hattum, W. A. van Vloten, H. van Loveren, M. B. Teunissen, and J. Garssen. 2003. Epidermal cis-urocanic acid levels correlate with lower specific cellular immune responses after hepatitis B vaccination of ultraviolet B-exposed humans. *Photochem Photobiol* **77**:271-275.
36. DeSilva, B., R. C. McKenzie, J. A. Hunter, and M. Norval. 2008. Local effects of TL01 phototherapy in psoriasis. *Photodermatol Photoimmunol Photomed* **24**:268-269.
37. Flacher, V., M. Bouschbacher, E. Verronese, C. Massacrier, V. Sisirak, O. Berthier-Vergnes, B. de Saint-Vis, C. Caux, C. Dezutter-Dambuyant, S. Lebecque, and J. Valladeau. 2006. Human Langerhans cells express a specific TLR profile and differentially respond to viruses and Gram-positive bacteria. *J Immunol* **177**:7959-7967.
38. Renn, C. N., D. J. Sanchez, M. T. Ochoa, A. J. Legaspi, C. K. Oh, P. T. Liu, S. R. Krutzik, P. A. Sieling, G. Cheng, and R. L. Modlin. 2006. TLR activation of Langerhans cell-like dendritic cells triggers an antiviral immune response. *J Immunol* **177**:298-305.
39. Tamassia, N., V. Le Moigne, M. Rossato, M. Donini, S. McCartney, F. Calzetti, M. Colonna, F. Bazzoni, and M. A. Cassatella. 2008. Activation of an immunoregulatory and antiviral gene expression program in poly(I:C)-transfected human neutrophils. *J Immunol* **181**:6563-6573.
40. Aragane, Y., D. Kulms, T. A. Luger, and T. Schwarz. 1997. Down-regulation of interferon gamma-activated STAT1 by UV light. *Proc Natl Acad Sci U S A* **94**:11490-11495.
41. Aragane, Y., A. Schwarz, T. A. Luger, K. Ariizumi, A. Takashima, and T. Schwarz. 1997. Ultraviolet light suppresses IFN-gamma-induced IL-7 gene expression in murine keratinocytes by interfering with IFN regulatory factors. *J Immunol* **158**:5393-5399.
42. Elco, C. P., and G. C. Sen. 2007. Stat1 required for interferon-inducible but not constitutive responsiveness to extracellular dsRNA. *J Interferon Cytokine Res* **27**:411-424.
43. Kendziorski, C., R. A. Irizarry, K. S. Chen, J. D. Haag, and M. N. Gould. 2005. On the utility of pooling biological samples in microarray experiments. *Proc Natl Acad Sci U S A* **102**:4252-4257.
44. Glass, A., J. Henning, T. Karopka, T. Scheel, S. Bansemer, D. Koczan, L. Gierl, A. Rolfs, and U. Gimsa. 2005. Representation of individual gene expression in completely pooled mRNA samples. *Biosci Biotechnol Biochem* **69**:1098-1103.
45. Staal, F. J., F. Weerkamp, M. R. Baert, C. M. van den Burg, M. van Noort, E. F. de Haas, and J. J. M. van Dongen. 2004. Wnt target genes identified by DNA microarrays in immature CD34+ thymocytes regulate proliferation and cell adhesion. *J Immunol* **172**:1099-1108.

46. Irizarry, R. A., B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis, U. Scherf, and T. P. Speed. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**:249-264.
47. Bolstad, B. M., R. A. Irizarry, M. Astrand, and T. P. Speed. 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**:185-193.
48. Dik, W. A., K. Pike-Overzet, F. Weerkamp, D. de Ridder, E. F. de Haas, M. R. Baert, P. van der Spek, E. E. Koster, M. J. Reinders, J. J. van Dongen, A. W. Langerak, and F. J. Staal. 2005. New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. *J Exp Med* **201**:1715-1723.
49. Ge, U., S. Dudoit, and T. Speed. 2003. Resampling-based multiple testing for microarray data analysis. *TEST* 12:1-44.
50. van der Fits, L., L. I. van der Wel, J. D. Laman, E. P. Prens, and M. C. Verschuren. 2003. Psoriatic lesional skin exhibits an aberrant expression pattern of interferon regulatory factor-2 (IRF-2). *J Pathol* **199**:107-114.
51. Aufiero, B. M., H. Talwar, C. Young, M. Krishnan, J. S. Hatfield, H. K. Lee, H. K. Wong, I. Hamzavi, and G. J. Murakawa. 2006. Narrow-band UVB induces apoptosis in human keratinocytes. *J Photochem Photobiol B* **82**:132-139.



Supplemental Figure 1. The human keratinocyte cell line HaCaT was irradiated with the indicated doses of NB-UVB. 24 h after irradiation the viability of the cells was determined by methylene blue staining. The intensity of blue coloring correlates with the number of viable cells. Bars represent staining intensity. Error bars show standard deviation of duplicate measurements.

4

GATA3 expression is reduced in psoriasis and under conditions of epidermal regeneration, and is induced by narrow-band UVB

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ABSTRACT

Psoriasis is characterized by hyperproliferation of keratinocytes and by infiltration of Th1 and Th17 cells in the (epi)dermis. The GATA3 transcription factor fulfils important roles in both epidermal and T helper cell differentiation. Hence, we investigated the role of GATA3 in psoriatic skin inflammation. Since the tissue response in psoriasis is similar to that of epidermal regeneration, we also studied GATA3 expression under such conditions.

Psoriatic lesional skin showed decreased GATA3 expression compared to non-lesional skin. GATA3 expression was markedly decreased in inflamed skin in the imiquimod-induced psoriasis-like dermatitis mouse model. Tape-stripping of non-lesional skin of patients with psoriasis, a standardized psoriasis-inducing technique resulted in reduced GATA3 expression. Moreover, low GATA3 mRNA and protein expression was detected in skin wounds in mice. Thus, GATA3 expression is downregulated under regenerative and inflammatory skin conditions. GATA3 expression could be re-induced by successful narrow-band UVB treatment of both human psoriasis and imiquimod-induced dermatitis in mice.

Based on these findings we postulate that decreased GATA3 expression is causatively related to keratinocyte hyperproliferation and skin barrier dysfunction in psoriasis. Further investigations are necessary into the downstream targets of GATA3 and its mode of action in regulating keratinocyte proliferation.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease characterized by sharply demarcated, red, thick, scaly plaques. Histologically it is characterized by epidermal acanthosis, papillomatosis and parakeratosis, infiltrating leukocytes and in the papillary dermis a dense mononuclear infiltrate and dilated capillaries.

In psoriasis, altered keratinocyte differentiation is characterized by downregulation of late keratinocyte differentiation markers and upregulation of early differentiation markers (1), while the pool of proliferating keratinocytes is increased. These alterations in keratinocyte proliferation and differentiation lead to impairment of the skin barrier function, and this barrier impairment correlates with the severity of the disease (2).

The factors controlling keratinocyte hyperproliferation and the disturbed keratinocyte differentiation in psoriasis remain poorly understood. Hyperproliferation in psoriasis and proliferation in cancers share many characteristics, such as the induction of the same oncogenes and transcription factors (3). In a previous study (Racz *et al.* submitted) we took advantage of one of the few effective treatments for psoriasis, narrow-band (NB)-UVB therapy, to assess by gene expression microarray which biological pathways are altered. One of the main factors emerging from this gene expression analysis was the transcription factor GATA3.

GATA3 is a transcription factor with two zinc finger motifs that binds to a six-nucleotide consensus sequence (A/T)GATA(A/G) (4). In the skin, GATA3 is expressed in the epidermis and in the inner root sheath of the hair follicle (5) where it serves as a regulator of inner root cell lineage formation of the hair follicle, postnatal hair growth and maintenance (6). In addition, GATA3 is required for correct formation of the epidermal barrier and has a role in regulation of epidermal differentiation (7, 8). Antimicrobial peptides, such as β-defensins and S100A proteins, are upregulated in the skin of epidermis-specific GATA3 -/- mice (8). Recently GATA3 was proposed to regulate desquamation during epidermal differentiation, by activating kallikrein 1, a serine protease that is responsible for the degradation of adhesion molecules in corneodesmosomes (9). In addition, GATA3 is abundantly expressed in the developing nervous system, inner ear, the eye, skin, mammary glands, embryonic kidney and thymic rudiment (6, 7, 10-12). In the hematopoietic system GATA3 expression is confined to the T, NK and NKT cell lineages (13).

During lymphoid cell development, GATA3 is involved in decisive T cell commitment (14). Functional T helper cell subset differentiation to Th2 is induced by GATA3 via a Stat6-dependent route (15-18). Polymorphisms in the Th2 cytokine genes IL-4 and IL-13 have been described in psoriasis (19), demonstrating that not only an excessive Th1/Th17 axis but also a defective Th2 axis can trigger the disease.

We previously compared the gene expression profiles of lesional and non-lesional skin of patients with psoriasis (Racz *et al.*, submitted), and found decreased GATA3 expression in lesional skin. Because epidermal abnormalities resembling human psoriasis occur in mice with an epidermis-specific deletion of GATA3-/- (7, 8), we hypothesized that downregulation of GATA3 was associated with the pathological changes in psoriatic skin. This prompted us to investigate the association between GATA3 expression and skin inflammation in psoriasis and in the murine imiquimod-induced psoriasis-like skin inflammation model (20).

RESULTS

Epidermal GATA3 expression is reduced in human psoriatic lesions and in a psoriasis-like murine skin-inflammation model

GATA3 expression was determined *in situ* at the protein level, using skin biopsies from healthy individuals, and lesional and non-lesional skin biopsies of patients with psoriasis. In healthy control skin GATA3 expression was present in all layers except the basal layer (Figure 1A). In non-lesional psoriatic skin, GATA3 expression was also visible in the basal layer, while in lesional psoriatic skin overall GATA3 expression was reduced being only slightly visible in the lower suprabasal layers (Figure 1A). The mRNA expression of GATA3 was downregulated in lesional psoriatic skin in a global gene expression profiling study (Racz *et al.*, submitted). This finding was validated by RT-PCR using RNA samples of five patients with psoriasis. A 5-fold (range 3-12-fold) lower expression of GATA3 was observed in lesional epidermis compared to non-lesional epidermis (Figure 1B).

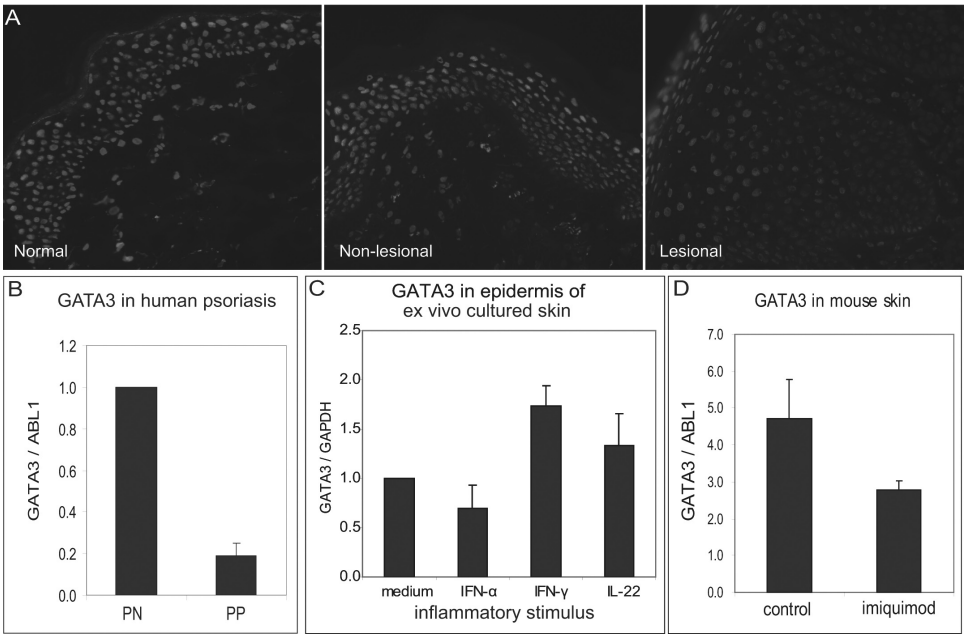

Figure 1. Epidermal GATA3 expression is reduced in psoriatic lesions.
A. GATA3 protein was present in the nuclei of differentiating layers of the epidermis in normal skin. In non-lesional skin expression of GATA3 was also present in the basal layer of the epidermis whereas in lesional skin GATA3 expression was downregulated. **B.** Expression of GATA3 was lower in lesional skin (PP) compared to non-lesional skin (PN). Epidermal GATA3 mRNA expression was determined by RT-PCR using ABL1 as a housekeeping control gene. Bars represent mean +/- SEM (n=5 patients). **C.** 3 mm biopsy samples from normal human skin were cultured in the presence of proinflammatory cytokines for 24 h. The epidermis was separated from the dermis and GATA3 expression was determined by RT-PCR in epidermal RNA, using ABL1 as a housekeeping control gene. Bars represent mean +/- SEM (n=4 healthy donors). **D.** GATA3 mRNA expression in the imiquimod-induced psoriasis-like dermatitis mouse model. GATA3 mRNA expression was determined in imiquimod- or control cream-treated back skin of Balb/C mice. ABL1 was used as a housekeeping control gene. Bars indicate the mean +/- SEM (n=3 mice per group). See page 157 for a full-color representation of this figure.

We then assayed whether low epidermal GATA3 expression in psoriasis is a consequence of the presence of inflammatory cytokines derived from infiltrating immune cells that are present in the inflamed psoriatic skin. Human skin biopsies from healthy volunteers were cultured with IFN-α, a type I IFN produced mainly by plasmacytoid DC that plays an important role in the initiation of psoriatic plaque formation (21), IFN-γ or IL-22, prototypic Th1 and Th17 cytokines, respectively. IFN-γ induced GATA3 expression in the epidermis, whereas IFN-α and IL-22 had no significant effect on GATA3 expression (Figure 1C). These data suggest that the reduced level of epidermal GATA3 in psoriasis is not caused by IFN-α, IFN-γ or IL-22.

Next we assessed the expression of GATA3 in a murine psoriasis-like skin inflammation model. Daily application of the TLR7/8 agonist imiquimod cream (trade name Aldara) on mouse back skin induces skin inflammation that strongly resembles human psoriasis in terms of phenotypic and histological appearance (20). GATA3 mRNA expression in the mouse skin was measured by RT-PCR on the 6th day of daily treatment with imiquimod. GATA3 mRNA expression was 1.7-fold lower in the imiquimod-treated mice than in mice treated with control cream only (Figure 1D).

GATA3 expression in psoriasis is upregulated by effective treatment of the disease

NB-UVB phototherapy is a standard and effective treatment modality for human psoriasis. We evaluated whether NB-UVB treatment of patients with psoriasis or psoriasis-like skin inflammation in mice would restore GATA3 expression in the skin. GATA3 expression was measured by RT-PCR in patients with psoriasis undergoing standard NB-UVB therapy. GATA3 mRNA expression increased gradually during the course of NB-UVB treatment, correlating with the clinical improvement (Figure 2A).

Interestingly, NB-UVB irradation of the mice on the 1st, 3rd and 5th day of imiquimod treatment resulted in less inflammation in the imiquimod-induced psoriasis-like dermatitis model (Figure 2B). In this model, skin inflammation as assessed by erythema, scaling and thickness was less pronounced after NB-UVB irradiation (Figure 2B). Accordingly, histological examination showed decreased epidermal thickness, improved epidermal differentiation and a significantly reduced number of proliferating/BrdU+ cells in imiquimod plus NB-UVB-treated mouse skin as compared to those treated with imiquimod alone (Figure 2C, D). In addition, NB-UVB treatment induced a clear reduction in markers of psoriasis disease activity in mouse skin, such as phosphorylated STAT3, dendritic cell and neutrophil granulocyte infiltrates and endothelial cells (Supplemental Figure 1). Thus, NB-UVB irradiation of imiquimod-treated mouse skin resulted in improvement of psoriasis-like dermatitis, as assayed by clinical, histological and immunohistochemical parameters.

We next assessed whether reduction of imiquimod-induced psoriasis-like inflammation by NB-UVB in mice is accompanied by alterations in GATA3 expression. Therefore GATA3-LacZ knock in mice (22) were treated with imiquimod daily, and were irradiated every other day with NB-UVB. In this mouse expression of the LacZ transgene is driven by the GATA3 promoter, and thus expression of the LacZ product X-Gal depends on GATA3 promoter activity. On the 6th day mice were sacrificed and GATA3 expression was assayed by X-Gal staining of back skin. X-Gal staining was reduced in imiquimod-treated mouse skin, and was clearly re-induced by NB-UVB irradiation (Figure 2E).

In summary, successful treatment of psoriasis and of psoriasis-like skin inflammation restored the low GATA3 expression, which correlated with clinical improvement.

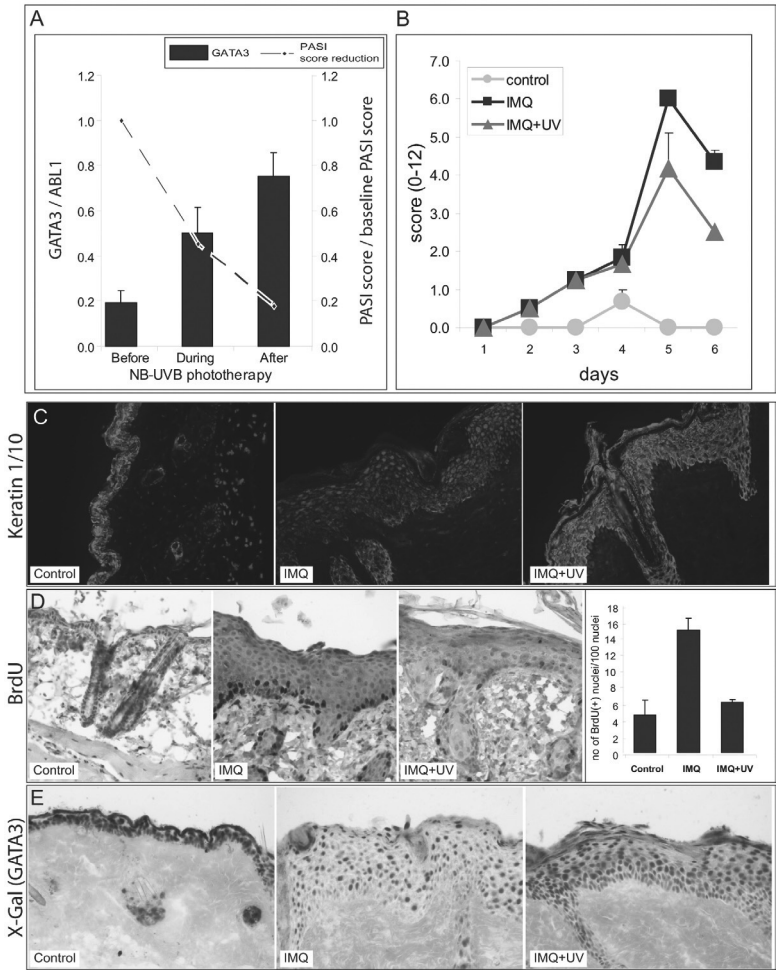

Figure 2. Upregulation of GATA3 expression during NB-UVB phototherapy.
A. GATA3 expression is upregulated during the course of NB-UVB phototherapy in patients with psoriasis. Three-mm biopsy samples were collected from lesional (PP) and non-lesional skin of patients with psoriasis before, during and after NB-UVB phototherapy. Epidermal GATA3 mRNA expression was determined with RT-PCR using ABL1 as a housekeeping control gene. Bars represent mean +/- SEM (n=5 patients). The broken line shows the change in the PASI score, relative to the baseline score. **B**. BALB/c mice were treated daily with imiquimod 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. Erythema, scaling, and the 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. **C.** Mice were sacrificed on day 6. Imiquimod-induced inflammation was studied on sections made from the back skin of the mice. Keratin 1/10 immunofluorescent staining of the back of the mice is shown. **D.** BrdU incorporation in keratinocytes in the back skin was detected by immunohistochemistry. Bars on the right represent the mean number of BrdU positive cells +/- SD. **E.** X-gal staining (blue) of heterozygous GATA3LacZ skin samples from mice treated with imiquimod with or without NB-UVB. X-Gal staining that in these mice correlates with GATA3 expression is lower in the inflamed epidermis compared to control and is induced during NB-UVB treatment. See page 158 for a full-color representation of this figure.

GATA3 expression is reduced in the regenerating epidermis

The tissue response in psoriasis is often compared to that during epidermal regeneration (23). During epidermal regeneration (e.g. upon wounding) keratinocyte proliferation is stimulated and an inflammatory response is initiated to prevent infection through the injured epidermal barrier. We thus investigated whether decreased GATA3 expression was specific to psoriatic inflammation or was also seen under conditions of epidermal regeneration. First, the epidermal barrier was disrupted in non-lesional skin of patients with psoriasis by removal of the stratum corneum via repeated tape stripping. Before tape stripping and 5 h later skin biopsies were taken from the tape-stripped area, the epidermis was separated from the dermis, and mRNA expression of epidermal GATA3 was studied. GATA3 expression in the epidermis was reduced to 60% on average of the baseline expression in six of the seven patients (Figure 3A).

We also investigated GATA3 expression during wound healing by performing X-Gal staining on sections taken from the wounded back skin of a GATA3 LacZ knock in mouse. Whereas X-Gal staining was observed in all epidermal layers of the epidermis adjacent to the wounded area, only a few X-Gal positive cell nuclei were visible in the stratum granulosum

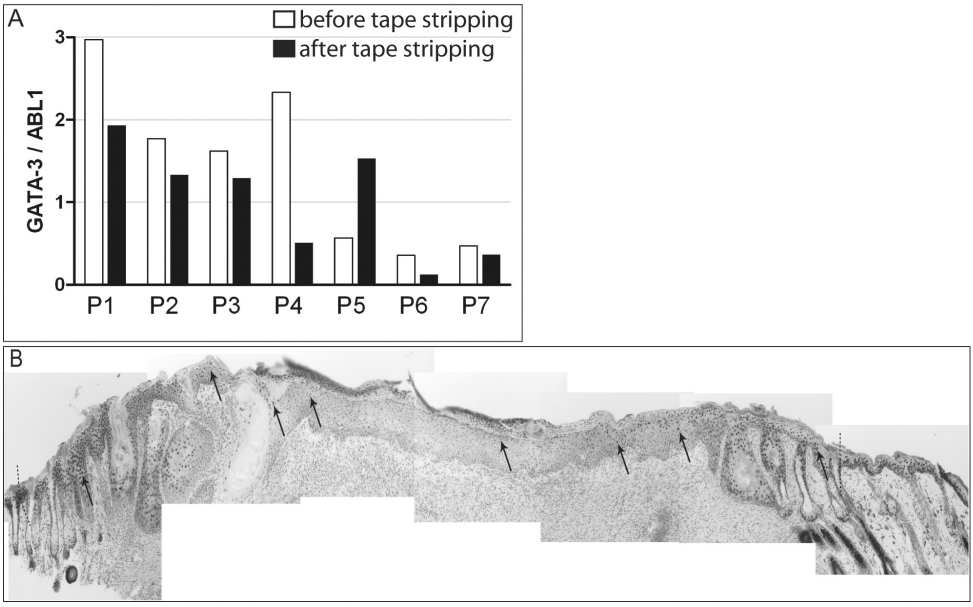

Figure 3. GATA3 expression is reduced in the regenerating epidermis.
A. The stratum corneum of non-lesional skin of seven psoriatic patients was removed by repeated tape stripping to induce regeneration. Before and 5 h after tape stripping biopsies were taken and epidermal GATA3 expression was determined using RT-PCR, relative to ABL1 as a housekeeping control gene. Relative expression values of individual patients are shown. **B.** GATA3 expression is downregulated in the healing wound of murine skin. X-gal staining (blue) of wounded skin of a heterozygous GATA3LacZ mouse shows downregulation of the LacZ transgene under the control of GATA3 in the highly proliferative zone of the healing wound. Arrows indicate X-Gal positive cells; the border between regenerating and adjacent normal skin is marked. See page 159 for a full-color representation of this figure.

and straum corneum in the healing wound. The number of X-Gal positive cells increased gradually towards the adjacent healthy skin (Figure 3B).

In conclusion, GATA3 expression is reduced in conditions in which the epidermis is forced to regenerate.

Comparison of expression profiles in psoriasis and in epidermal GATA3-deficient mice

We then investigated which cellular processes are affected by low GATA3 expression in the psoriatic epidermis. For this we compared gene expression profiles obtained from hair follicles of epidermis-specific GATA3-/- mice (7) with those from lesional epidermis of patients with psoriasis. A total of 5922 genes were differentially expressed in the hair follicle of epidermis-specific GATA3-/- mice when compared to wild type mice ($p<0.05$, ≥1.2-fold up- or downregulated (7)). In human lesional psoriatic epidermis, which is characterized by low GATA3 expression, 2412 genes were differentially expressed when compared to non-lesional epidermis ($p<0.05$, ≥1.2-fold up- or downregulated) (Racz *et al.,* submitted). Genes were identified that were upregulated in both GATA3 -/- mouse skin and in lesional

Table 1. Genes differentially expressed in both psoriasis and GATA3 -/- mice.

Symbol	Cell differentiation	Fold change human	Fold change mouse	Up/ down
SOD2	SUPEROXIDE DISMUTASE 2, MITOCHONDRIAL	6.0	1.2	U
ALDH1A3	ALDEHYDE DEHYDROGENASE 1 FAMILY, MEMBER A3	3.9	1.5	U
TXNDC5	THIOREDOXIN DOMAIN CONTAINING 5	2.5	1.3	U
EHF	ETS HOMOLOGOUS FACTOR	2.3	1.3	U
CTSB	CATHEPSIN B	2.1	1.3	U
S100A6	S100 CALCIUM BINDING PROTEIN A6 (CALCYCLIN)	1.8	1.4	U
SGK1	SERUM/GLUCOCORTICOID REGULATED KINASE	1.8	2.4	U
EIF2B2	EUKARYOTIC TRANSLATION INITIATION FACTOR 2B, SUBUNIT 2 BETA	1.7	1.7	U
DICER1	DICER1, RIBONUCLEASE TYPE III	1.6	1.6	U
POLB	POLYMERASE (DNA DIRECTED), BETA	1.6	1.4	U
PHB	PROHIBITIN	1.6	1.4	U
BOK	BCL2-RELATED OVARIAN KILLER	1.4	1.5	U
TXNL1	THIOREDOXIN-LIKE 1	1.3	1.3	U
CHL1	CELL ADHESION MOLECULE WITH HOMOLOGY TO L1CAM	4.5	2.0	D
TNFAIP3	TUMOR NECROSIS FACTOR, ALPHA-INDUCED PROTEIN 3	2.6	1.3	D
TNFRSF19	TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 19	2.3	1.4	D
FAS	FAS (TNF RECEPTOR SUPERFAMILY, MEMBER 6)	2.0	1.5	D
DLG5	DISCS, LARGE HOMOLOG 5	1.9	1.6	D
FOXO1	FORKHEAD BOX O1A	1.9	1.6	D

Table 1. Genes differentially expressed in both psoriasis and GATA3 -/- mice (continued).

Symbol	Cell differentiation	Fold change human	Fold change mouse	Up/ down
MYST3	MYST HISTONE ACETYLTRANSFERASE 3	1.9	1.5	D
TNFRSF25	TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 25	1.9	1.2	D
RYBP	RING1 AND YY1 BINDING PROTEIN	1.8	1.5	D
SPRY1	SPROUTY HOMOLOG 1, ANTAGONIST OF FGF SIGNALING	1.8	1.5	D
MIB1	MINDBOMB HOMOLOG 1	1.7	1.4	D
PDCD6IP	PROGRAMMED CELL DEATH 6 INTERACTING PROTEIN	1.6	1.4	D
UBE2B	UBIQUITIN-CONJUGATING ENZYME E2B	1.6	1.5	D
JAG2	JAGGED 2	1.5	1.5	D
DHCR24	24-DEHYDROCHOLESTEROL REDUCTASE	1.2	2.1	D
	Cell cycle			
KLK10	KALLIKREIN 10	2.3	2.0	U
S100A6	S100 CALCIUM BINDING PROTEIN A6 (CALCYCLIN)	1.8	1.4	U
GSPT1	G1 TO S PHASE TRANSITION 1	1.7	1.4	U
PHB	PROHIBITIN	1.6	1.4	U
AURKAIP1	AURORA KINASE A INTERACTING PROTEIN 1	1.5	1.4	U
PA2G4	PROLIFERATION-ASSOCIATED 2G4	1.5	1.3	U
MCC	MUTATED IN COLORECTAL CANCERS	3.0	1.5	D
WEE1	WEE1 HOMOLOG	2.5	1.5	D
KAT2B	K(LYSINE) ACETYLTRANSFERASE 2B	2.2	1.7	D
FOXN3	FORKHEAD BOX N3	2.1	1.5	D
MAP2	MICROTUBULE/ASSOCIATED PROTEIN 2	2.1	1.6	D
DLG5	DISCS, LARGE HOMOLOG 5	1.9	1.6	D
BUB3	BUB3 BUDDING UNINHIBITED BY BENZIMIDAZOLES 3 HOMOLOG	1.6	1.6	D
JAG2	JAGGED 2	1.5	1.5	D
WNK1	WNK LYSINE DEFICIENT PROTEIN KINASE 1	1.5	2.3	D
DHCR24	24-DEHYDROCHOLESTEROL REDUCTASE	1.2	2.2	D
CCNI	CYCLIN I	1.2	1.4	D
	Apoptosis			
SOD2	SUPEROXIDE DISMUTASE 2, MITOCHONDRIAL	6.0	1.2	U
ALDH1A3	ALDEHYDE DEHYDROGENASE 1 FAMILY, MEMBER A3	3.9	1.5	U
TXNDC5	THIOREDOXIN DOMAIN CONTAINING 5	2.5	1.3	U
CTSB	CATHEPSIN B	2.1	1.3	U

Table 1. Genes differentially expressed in both psoriasis and GATA3 -/- mice (continued).

Symbol	Cell differentiation	Fold change human	Fold change mouse	Up/ down
SGK1	SERUM/GLUCOCORTICOID REGULATED KINASE	1.8	2.4	U
POLB	POLYMERASE (DNA DIRECTED), BETA	1.6	1.4	U
PHB	PROHIBITIN	1.6	1.4	U
BOK	BCL2-RELATED OVARIAN KILLER	1.4	1.5	U
TXNL1	THIOREDOXIN-LIKE 1	1.3	1.3	U
TNFAIP3	TUMOR NECROSIS FACTOR, ALPHA-INDUCED PROTEIN 3	2.6	1.3	D
TNFRSF19	TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 19	2.3	1.4	D
FAS	FAS (TNF RECEPTOR SUPERFAMILY, MEMBER 6)	2.0	1.5	D
DLG5	DISCS, LARGE HOMOLOG 5 (DROSOPHILA)	1.9	1.6	D
FOXO1	FORKHEAD BOX O1A	1.9	1.6	D
TNFRSF25	TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 25	1.9	1.2	D
RYBP	RING1 AND YY1 BINDING PROTEIN	1.8	1.5	D
PDCD6IP	PROGRAMMED CELL DEATH 6 INTERACTING PROTEIN	1.6	1.4	D
DHCR24	24-DEHYDROCHOLESTEROL REDUCTASE	1.2	2.2	D
	Transcription regulation			
EHF	ETS HOMOLOGOUS FACTOR	2.3	1.3	U
SUB1	SUB1 HOMOLOG	1.8	1.4	U
ASCC3	ACTIVATING SIGNAL COINTEGRATOR 1 COMPLEX 3	1.5	1.4	U
PA2G4	PROLIFERATION-ASSOCIATED 2G4	1.5	1.3	U
FOXP1	FORKHEAD BOX P1	1.4	1.4	U
PHB2	PROHIBITIN 2	1.3	1.4	U
TSC22D1	TSC22 DOMAIN FAMILY, MEMBER 1	3.0	1.7	D
HLF	HEPATIC LEUKEMIA FACTOR	2.3	1.5	D
NFIB	NUCLEAR FACTOR I/B	2.3	1.4	D
KAT2B	K(LYSINE) ACETYLTRANSFERASE 2B	2.2	1.7	D
FOXN3	FORKHEAD BOX N3	2.1	1.5	D
FOXO1	FORKHEAD BOX O1A	1.9	1.6	D
MYST3	MYST HISTONE ACETYLTRANSFERASE 3	1.9	1.5	D
SMARCA2	SWI/SNF RELATED, MATRIX ASSOCIATED, ACTIN DEPENDENT REGULATOR OF CHROMATIN, SUBFAMILY A, MEMBER 2	1.9	1.6	D
ZNF329	ZINC FINGER PROTEIN 329	1.9	1.4	D
RYBP	RING1 AND YY1 BINDING PROTEIN	1.8	1.5	D
TCF12	TRANSCRIPTION FACTOR 12	1.8	1.3	D

Table 1. Genes differentially expressed in both psoriasis and GATA3 -/- mice (continued).

Symbol	Cell differentiation	Fold change human	Fold change mouse	Up/ down
CTNND1	CATENIN DELTA 1	1.7	1.4	D
PBX3	PRE-B-CELL LEUKEMIA TRANSCRIPTION FACTOR 3	1.7	1.5	D
TFAP2A	TRANSCRIPTION FACTOR AP-2 ALPHA	1.7	1.7	D
SUV420H1	SUPPRESSOR OF VARIEGATION 4-20 HOMOLOG 1	1.6	1.4	D
ZNF148	ZINC FINGER PROTEIN 148	1.6	1.3	D
KLF13	KRUPPEL-LIKE FACTOR 13	1.5	1.5	D
SSBP3	SINGLE STRANDED DNA BINDING PROTEIN 3	1.5	1.2	D
SNAI2	SNAIL HOMOLOG 2	1.5	1.2	D
SERTAD2	SERTA DOMAIN CONTAINING 2	1.4	1.4	D
Other				
DSC2	DESMOCOLLIN 2	4.0	1.9	U
TF	TRANSFERRIN	2.2	2.0	U
LNX1	LIGAND OF NUMB-PROTEIN X 1	4.3	1.6	D
INSIG2	INSULIN INDUCED GENE 2	2.9	1.4	D
IGFBP5	INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 5	2.7	1.3	D

psoriatic skin (77 genes), or were downregulated in both (97 genes) (Table 1). This list of genes was subjected to gene annotation using the DAVID Gene Annotation Tool. Low GATA3 expression in both human psoriatic and GATA3 -/- murine epidermis coincided with the altered expression of genes involved in cell differentiation, cell proliferation and apoptosis (Table 1). In addition, the expression of several transcription factors was regulated in coordination with GATA3 (Table 1), e.g. FOXP1, FOXO1, FOXN3, TFAP2A, KLF13 and SNAI2, which are transcription factors that are involved in organ development and differentiation.

GATA3 expression in primary human keratinocytes during normal proliferation, induced differentiation and cell cycle arrest

To better understand the behavior of GATA3 during epidermal/keratinocyte differentiation, immunofluorescent staining of GATA3 was performed in undifferentiated and differentiated epidermal keratinocytes. Primary human keratinocytes were cultured under high Ca^{2+} conditions in order to stimulate differentiation, and then harvested after 24 h, fixed and stained with anti-GATA3 antibody. In differentiating keratinocytes GATA3 expression was localized to the nucleus, whereas in normal proliferating control keratinocytes GATA3 was randomly expressed in the nuclei and in the cytoplasm (Figure 4A, B, D). In addition, cell cycle arrest was induced in keratinocytes by TGF-β1 at both low and high Ca^{2+} concentrations. Primary keratinocytes were cultured with TGF-β1, harvested after 24 h and stained for GATA3. Interestingly, GATA3 expression in TGF-β1-treated cells was mostly localized to the cytoplasm, indicating transcriptional inactivity (Figure 4C, D).

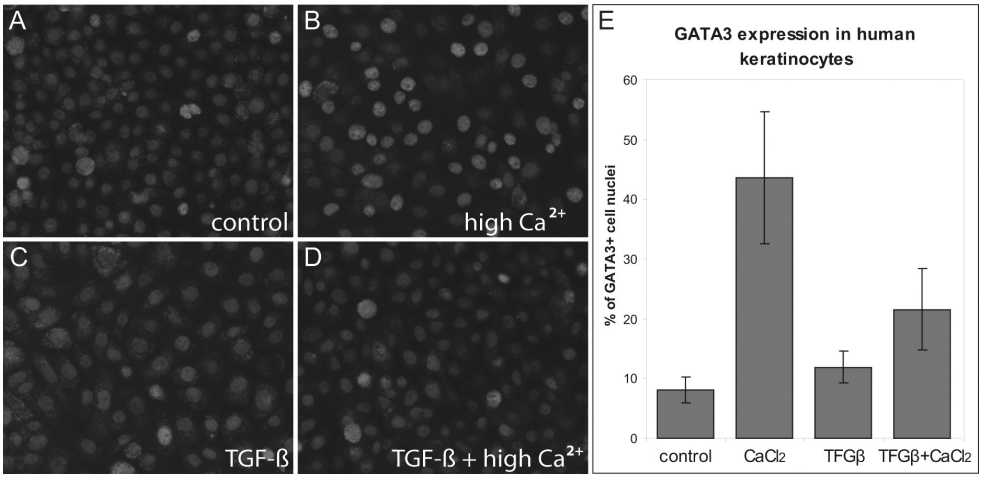

Figure 4. GATA3 translocates to the nucleus in differentiating keratinocytes, whereas it stays in the cytoplasm during cell cycle arrest.
Primary human epidermal keratinocytes were cultured on chamber slides. When cells reached ~75% confluency, $CaCl_2$ (**B, D**) and/or TGF-β1 (**C, D**) were added to the culture medium. After 24 h cells were fixed and immunofluorescent staining for GATA3 protein (pink) was performed. **E.** GATA3 positive cell nuclei were counted and are shown as a percentage of the total number of cell nuclei. See page 160 for a full-color representation of this figure.

In conclusion, GATA3 is transcriptionally active in differentiated keratinocytes, whereas no nuclear GATA3 was observed during cell cycle arrest, indicating a role for GATA3 during cell differentiation and proliferation.

DISCUSSION

Here we show that the epidermal expression of the zinc finger transcription factor GATA3 is consistently downregulated in psoriasis, in the imiquimod-induced psoriasis-like mouse model and under conditions of epidermal regeneration. Our finding that GATA3 expression is reduced in both psoriasis and epidermal regeneration confirms previously described parallels between these conditions.

The involvement of GATA3 in the pathophysiology of psoriasis is a novel finding. Although GATA3 has occasionally been reported in microarray studies of psoriasis (24-26), its role and the modulation of its expression in skin has never been pursued in much detail. Polymorphisms in the GATA3 gene or genetic associations have not been reported to be associated with psoriasis, but, interestingly, are associated with atopic dermatitis, a chronic inflammatory skin disease with epidermal hyperplasia (27). Haploinsufficiency of the GATA3 gene causes the very rare HDR (hypoparathyroidism, sensorineural deafness and renal dysplasia) syndrome (28). Generalized psoriasis has been reported in a patient with the HDR syndrome (29). This finding and our current studies indicate that GATA3 might be an essential component of the pathophysiology of psoriasis as well.

Most of our current insight into the function of GATA3 originates from T cell biology, whereas much less is known about GATA3 molecular function in keratinocytes. In T cells GATA3 determines Th2 cell differentiation and selectively activates the promoters of IL-4, IL-5, and IL-13 through chromatin remodelling. For the latter, GATA3 must translocate from the cytoplasm into the nucleus to access its target genes.

Our initial mechanistic explanation was that downregulation of GATA3 could be caused by cytokines in the highly pro-inflammatory milieu in psoriasis lesions. However, GATA3 expression was not suppressed by proinflammatory cytokines such as IFN-γ, IFN-α and IL-22, which are critical in the pathogenesis of psoriasis. The possibility that suppression of GATA3 is inducible by other specific mediators or cytokines or combinations that we did not test, cannot be ruled out.

To identify the effects of decreased GATA3 expression in psoriasis, based on our microarray results, we first listed all genes known to be causally related to GATA3 i.e. differentially regulated in epidermis-specific GATA3 -/- mice. Genes from this list that were regulated in the same way (i.e. up or down) in lesional psoriatic skin versus healthy, non-lesional skin were selected. The resulting group of genes included negative regulators of inflammation, such as the psoriasis susceptibility gene TNFAIP3 (19, 30) and the Notch ligand jagged 2 (31), genes regulating epidermal differentiation, such as the transcription factor AP2-α (TFAP2A) (32), and the apoptosis-inducing FAS molecule. In addition, the transcription factors FOXN3, FOXO1 and FOXP1 were also regulated together with GATA3. These molecules play regulatory roles in organ development (33), metabolism (34) and cell proliferation (35), respectively. Parallel to the induction of GATA3, AP2-α (TFAP2A) and the anti-inflammatory TNFAIP3 and jagged 2 were also induced by NB-UVB therapy of psoriasis. Induction of these molecules might contribute to the anti-psoriatic effects of NB-UVB.

In conclusion, this study shows that the epidermal expression of the transcription factor GATA3 is consistently downregulated under conditions of keratinocyte hyperproliferation and altered differentiation such as in psoriasis, in murine psoriasis-like dermatitis and in wound healing. Our results indicate that further studies on the epidermal function of GATA3 in psoriasis could provide novel insights into the pathophysiology of the disease.

METHODS

Patients, NB-UVB treatment and biopsy samples

Seventeen patients (11 men, 6 women, age range 20-73) with psoriasis were recruited after informed consent (METC registration number 234.237/2003/210). All patients had a Psoriasis Area and Severity Index (PASI) score of at least 10, and had not received systemic therapy for at least one month or topical therapy for at least two weeks prior to the start of the study. Ten patients were treated with standard NB-UVB phototherapy until total clearance of psoriasis was achieved, or for a maximum of three months. From these 10 patients 3-mm biopsies were taken from lesional and non-lesional skin before the start of NB-UVB therapy and after the last treatment session. When PASI scores reached a reduction of 50% of the baseline score, additional biopsies from lesional and non-lesional skin were taken. NB-UVB treatment was applied three times a week using a Waldmann 7001 UVB cabinet equipped with Philips TL-01

bulbs. The initial UVB dose was 0.1-0.3 J/cm^2 (depending on the skin type of the patient); the mean cumulative UVB dose was 42.0 J/cm^2 (range 30-60 J/cm^2). During the course of UVB treatment PASI scores were evaluated every two weeks.

Biopsy samples were taken from the seven additional patients from non-lesional skin, from which the stratum corneum was removed by successive application of adhesive tape (tape stripping) of approximately 2 cm^2. Five hours after tape stripping biopsies were taken from the treated area.

Skin organ culture

Skin biopsies (3 mm diameter) were obtained, after informed consent, from healthy volunteers undergoing breast reduction in the Department of Plastic Surgery of the Sint Franciscus Gasthuis, Rotterdam, The Netherlands. Biopsies were cultured in a transwell system as described previously, with the dermis immersed in medium, and the epidermis exposed to the air interface (36). Recombinant human IFN-α (500 U/ml), IFN-γ (500 U/ml) or IL-22 (50 ng/ml) (R&D Systems, Abingdon, UK) was added to the culture medium. These concentrations were previously demonstrated to yield optimal biological responses in this organ culture system. Biopsies were collected 24 h later.

Keratinocyte culture

Primary human epidermal keratinocytes were obtained from healthy donors as described previously (37) and cultured in Dermalife medium (LifieLine Cell Technology, Walkersville, MD) on Lab-Tek Chamber slides. Keratinocytes from passages 3 to 4 were used. Cells were cultured with or without $CaCl_2$ (final Ca^{2+} concentration was 1.2 mM). In addition, TGF-β1 was added to the cells at a concentration of 3 ng/ml.

RNA isolation

The epidermis was separated from the dermis after incubation in 1 mg/ml protease X (Sigma Aldrich, Zwijndrecht, The Netherlands) for 90 min at 37°C, and stored in RNA lysis buffer at -80°C until further processing. Total messenger RNA was isolated from the epidermis only, using a GenElute Mammalian Total RNA Miniprep kit (Sigma Aldrich). RNA purity and integrity was verified by scanning with an Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip.

Array hybridization and analysis

For hybridization on gene expression arrays, RNA samples of four individual patients per array (250 ng mRNA per patient) were pooled. Patients were divided into two groups in order to have duplicate arrays for each time point and condition. Biotinylated target RNA was prepared from the pooled (1 μg) total RNA, and hybridized on GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA). Array hybridization and scanning was performed as described previously (38). The data were read and robust multichip analysis (RMA) (39) was used to remove the background and normalize the data across arrays (40). These values were $\log_2$-transformed for further analysis, yielding numbers between 0 and 16. A two-way ANOVA with factors "probe" and "condition" was used for each probeset to calculate both average expression levels per condition (41) and a *p*-value for the difference between conditions. The resulting *p*-values were adjusted for multiple testing using Šidák step-up adjustment (42). Genes were considered differentially expressed when *p*-values were < 0.05.

Gene expression analysis of isolated hair follicles of GATA3-/- mice was described previously (7). Functional annotation was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (43).

Quantitative RT-PCR

RNA of individual patients was transcribed into cDNA, and RT-PCR was performed as described previously (44). ABL1 was used as a housekeeping control gene. The sequences of newly designed primers and probe numbers of the Exiqon probe library system (Exiqon, Vedbaek, Denmark) are listed in Table 2.

Mice and treatments

Induction of skin inflammation by daily imiquimod application in BALB/c and LacZ knock in GATA3 mice (22) was performed as described previously (20). 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 UVB dose was 70% of the minimal erythema dose (MED) on the first day and it was increased by 10% in each treatment. Scoring of the severity of skin inflammation was performed as described previously (20). On the 6th day, mice were sacrificed and 3 mm biopsy samples were taken from the back skin. Total RNA was isolated, transcribed into cDNA, and the expression of GATA3 was determined by RT-PCR, using ABL1 as a housekeeping control gene.

To determine the MED in BALB/c mice, animals were irradiated with increasing doses of NB-UVB. Ear thickness was measured with a micrometer (Mitutoyo, Veenendaal, The Netherlands) before UVB irradiation and 48 h later. The lowest NB-UVB dose at which ear thickness was significantly increased was 1680 mJ/cm^2. Seventy percent of this dose (thus 70% of the MED) was used as the starting dose in further experiments.

Table 2. Primers and probes for RT-PCR.

Gene	Forward primer	Reverse primer	Probe
hGATA3	GCTTCGGATGCAAG TCCA	GCCCCACAGTTCAC ACACT	Nr. 8 [1]
mGATA3	CATTACCACCTATC CGCCCTATG	CACACACTCCCTGCC TTCTGT	CGAGGCCCAAGG CACGATCCAG
hGAPDH	TCCACTGGCGTCTT CAC	GGCAGAGATGATGAC CCTTTT	Nr. 45 [1]
h/mABL1	TGGAGATAACACTCT AAGCA TAACTAAAGGT	GATGTAGTTGCTTG GGACCCA	CCATTTTTGGTTTGGG CTTCACACCATT

[1] Probe from the Exiqon probe library system (Exiqon, Vedbaek, Denmark).

Prior to the irradiation experiment one of the LacZ knock in GATA3 mice was critically bitten and had to be sacrificed. We took advantage of this unintentional skin wounding to assess whether this insult and concomitant skin regeneration affected GATA3 expression. The wounded back skin of this mouse as well as unaffected adjacent tissue was embedded in Tissue-Tec embedding medium (Sakura, Zoeterwoude, The Netherlands) and snap-frozen.

Immunohistochemistry, X-Gal staining and BrdU labelling

For immunofluorescent staining, cryosections or cells were fixed for 10 min in 4% paraformaldehyde (PFA) in PBS. Primary antibodies included anti-β-defensin 2 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), anti-GATA-3 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), anti-phosphorylated STAT3 (1:50; Cell Signaling Technology Inc, Danvers, MA) and K10 (mouse 1:50; Sigma, clone k8.60). Relevant FITC-, TxR- or HRP-conjugated antibodies (1:100, Abcam) were used to detect primary antibodies. All fluorescent images were taken with an Axio Imager (Zeiss) fluorescence microscope. Immunohistochemical staining procedures were applied as described previously (45). Primary antibodies were hamster anti-mouse CD11c (undiluted) to detect myeloid dendritic cells, rat anti-mouse GR-1 (1:80) to identify neutrophil granulocytes, and rat anti-mouse Meca20 (undiluted) to visualize blood vessel endothelium. For standard histology PFA-fixed cryosections were stained with hematoxylin and eosin.

Mice were injected with 50 mg/kg bodyweight 5-bromo-2'-deoxyuridine (BrdU) and sacrificed 2 h later. Cryosections were fixed for 10 min in 4% PFA in PBS and washed three times for 5 min in PBS. A block/diluent was used: 1% BSA, 0.05% Tween in PBS. For BrdU immunohistochemistry with BrdU (mouse 1:100; DAKO, clone Bu20a), tissue samples were fixed in 4% PFA in PBS at 4°C overnight. Skin samples were subsequently embedded in paraffin and sectioned at 5 μm. After deparaffination sections were boiled in 0.01 M citrate buffer (pH 6.0) for 15 min prior to incubation with primary antibody. Sections were analyzed and photographed with an Olympus BX40 light microscope.

For X-gal staining sections were fixed for 1 min in 0.5% glutaraldehyde, 1% PFA, washed in PBS and incubated in X-gal staining solution (5 mM $K_3Fe(CN)_6$, 5mM $K_4Fe(CN)_{6*}3H_20$, 2 mM $MgCl_2$, 0.01% Nadeoxycholate, 0.02% NP40, 1 mg/ml bromo-chloro-indolyl-galactopyranoside) for 5 h at room temperature. Sections were then fixed in 4% PFA for 10 min and counterstained with neutral red.

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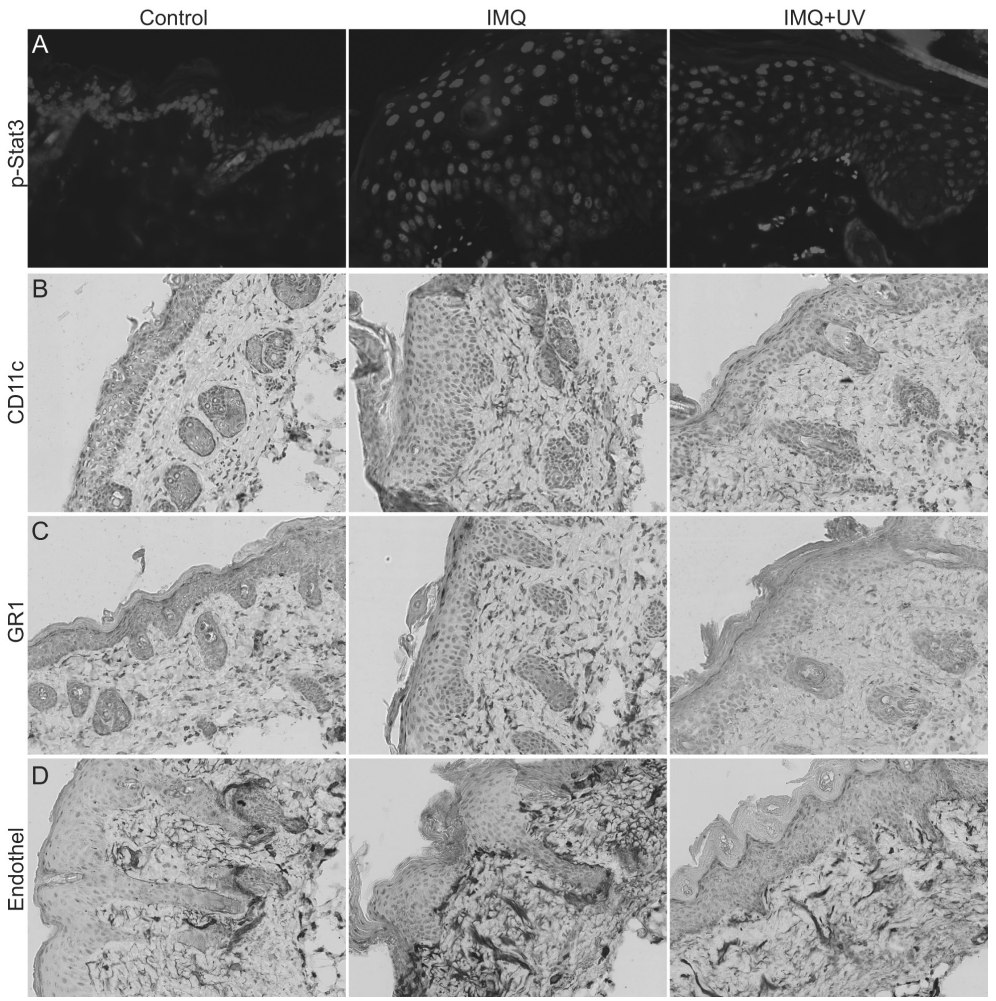
REFERENCES

1. Tschachler, E. (2007). Psoriasis: the epidermal component. *Clin Dermatol* **25**(6): 589-95.
2. Ghadially, R., Reed, J. T. and Elias, P. M. (1996). Stratum corneum structure and function correlates with phenotype in psoriasis. *J Invest Dermatol* **107**(4): 558-64.

3. Haider, A. S., *et al.* (2006). Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. *J Invest Dermatol* **126**(4): 869-81.
4. Yamamoto, M., *et al.* (1990). Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. *Genes Dev* **4**(10): 1650-62.
5. Chikh, A., *et al.* (2007). Expression of GATA-3 in epidermis and hair follicle: relationship to p63. *Biochem Biophys Res Commun* **361**(1): 1-6.
6. Kaufman, C. K., *et al.* (2003). GATA-3: an unexpected regulator of cell lineage determination in skin. *Genes Dev* **17**(17): 2108-22.
7. Kurek, D., *et al.* (2007). Transcriptome and phenotypic analysis reveals Gata3-dependent signalling pathways in murine hair follicles. *Development* **134**(2): 261-72.
8. de Guzman Strong, C., *et al.* (2006). Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3. *J Cell Biol* **175**(4): 661-70.
9. Son do, N., *et al.* (2009). Abundant expression of Kallikrein 1 gene in human keratinocytes was mediated by GATA3. *Gene* **436**(1-2): 121-7.
10. George, K. M., *et al.* (1994). Embryonic expression and cloning of the murine GATA-3 gene. *Development* **120**(9): 2673-86.
11. Pandolfi, P. P., *et al.* (1995). Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis. *Nat Genet* **11**(1): 40-4.
12. van der Wees, J., *et al.* (2004). Hearing loss following Gata3 haploinsufficiency is caused by cochlear disorder. *Neurobiol Dis* **16**(1): 169-78.
13. Oosterwegel, M., *et al.* (1992). Expression of GATA-3 during lymphocyte differentiation and mouse embryogenesis. *Dev Immunol* **3**(1): 1-11.
14. Schwarz, B. A. and Bhandoola, A. (2006). Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. *Immunol Rev* **209**: 47-57.
15. Zheng, W. and Flavell, R. A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**(4): 587-96.
16. Zhang, D. H., *et al.* (1997). Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem* **272**(34): 21597-603.
17. Ouyang, W., *et al.* (2000). Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* **12**(1): 27-37.
18. Ouyang, W., *et al.* (1998). Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* **9**(5): 745-55.
19. Nair, R. P., *et al.* (2009). Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* **41**(2): 199-204.
20. van der Fits, L., *et al.* (2009). Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* **182**(9): 5836-45.
21. Nestle, F. O., *et al.* (2005). Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* **202**(1): 135-43.
22. Hendriks, R., *et al.* (1999). Expression of the transcription factor GATA-3 is required for the development of the earliest T cell progenitors and correlates with stages of cellular proliferation in the thymus. *Eur J Immunol* **29**(6): 1912-8.
23. Nickoloff, B. J., *et al.* (2006). Lessons learned from psoriatic plaques concerning mechanisms of tissue repair, remodeling, and inflammation. *J Investig Dermatol Symp Proc* **11**(1): 16-29.
24. Oestreicher, J. L., *et al.* (2001). Molecular classification of psoriasis disease-associated genes through pharmacogenomic expression profiling. *Pharmacogenomics J* **1**(4): 272-87.
25. Zhou, X., *et al.* (2003). Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* **13**(1): 69-78.
26. Reischl, J., *et al.* (2007). Increased expression of Wnt5a in psoriatic plaques. *J Invest Dermatol* **127**(1): 163-9.
27. Arshad, S. H., *et al.* (2008). Polymorphisms in the interleukin 13 and GATA binding protein 3 genes and the development of eczema during childhood. *Br J Dermatol* **158**(6): 1315-22.
28. Van Esch, H., *et al.* (2000). GATA3 haplo-insufficiency causes human HDR syndrome. *Nature* **406**(6794): 419-22.

29. Aksoylar, S., *et al.* (2004). HDR (hypoparathyroidism, sensorineural deafness, renal dysplasia) syndrome presenting with hypocalcemia-induced generalized psoriasis. *J Pediatr Endocrinol Metab* **17**(7): 1031-4.
30. Coornaert, B., Carpentier, I. and Beyaert, R. (2009). A20: central gatekeeper in inflammation and immunity. *J Biol Chem* **284**(13): 8217-21.
31. Choi, K., *et al.* (2009). Distinct biological roles for the notch ligands jagged-1 and jagged-2. *J Biol Chem* **284**(26): 17766-74.
32. Fuchs, E. and Horsley, V. (2008). More than one way to skin. *Genes and Development* **22**(8): 976-85.
33. Schuff, M., *et al.* (2007). FoxN3 is required for craniofacial and eye development of Xenopus laevis. *Dev Dyn.* **236**(1): 226-39.
34. Gross, D., van den Heuvel, A. and Birnbaum, M. (2008). The role of FoxO in the regulation of metabolism. *Oncogene* **27**(16): 2320-36.
35. Koon, H., *et al.* (2007). FOXP1: a potential therapeutic target in cancer. *Expert Opin Ther Targets.* **11**(7): 955-65.
36. Companjen, A. R., *et al.* (2001). A modified *ex vivo* skin organ culture system for functional studies. *Arch Dermatol Res* **293**(4): 184-90.
37. Prens, E. P., *et al.* (2008). IFN-alpha enhances poly-IC responses in human keratinocytes by inducing expression of cytosolic innate RNA receptors: relevance for psoriasis. *J Invest Dermatol* **128**(4): 932-8.
38. Staal, F. J., *et al.* (2004). Wnt target genes identified by DNA microarrays in immature CD34+ thymocytes regulate proliferation and cell adhesion. *J Immunol* **172**(2): 1099-108.
39. Irizarry, R. A., *et al.* (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**(2): 249-64.
40. Bolstad, B. M., *et al.* (2003). A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**(2): 185-93.
41. Dik, W. A., *et al.* (2005). New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. *J Exp Med* **201**(11): 1715-23.
42. Ge, U., Dudoit, S. and Speed, T. (2003). Resampling-based multiple testing for microarray data analysis. *TEST* **12**(1): 1-44.
43. Dennis, G., Jr., *et al.* (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* **4**(5): P3.
44. van der Fits, L., *et al.* (2003). Psoriatic lesional skin exhibits an aberrant expression pattern of interferon regulatory factor-2 (IRF-2). *J Pathol* **199**(1): 107-14.
45. van der Fits, L., *et al.* (2004). In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *J Invest Dermatol* **122**(1): 51-60.



Supplemental Figure 1. NB-UVB treatment affects epidermal differentiation, inflammatory infiltrate and vascular alterations in imiquimod-induced skin inflammation in mice.
BALB/c mice were treated daily with imiquimod 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. Mice were sacrificed on day 6. Immunofluorescent staining for phosphorylated STAT3 (**A**), is shown, as well as immunohistochemical analysis of myeloid dendritic cells (CD11c, **B**), granulocytes (GR1, **C**), and endothelial cells (MECA-20, **D**). See page 161 for a full-color representation of this figure.

5

Cellular and molecular effects of pulsed dye laser and local narrow-band UVB therapy in psoriasis

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ABSTRACT

Background and Objective: Pulsed dye laser (PDL) therapy is effective in clearing psoriasis plaques, but the mechanism of action is incompletely understood. Narrow-band UVB (NB-UVB) is an effective standard treatment for psoriasis, with a better defined mode of action.

Our aim was to evaluate the cellular and molecular effects of PDL and to compare them with those of local NB-UVB, in order to gain further insight into their mechanisms of action in psoriasis.

Study design/Patients and Methods: Nineteen patients with stable plaque-type psoriasis were treated either with PDL or NB-UVB. Lesional punch biopsies were obtained from all patients before treatment. Additional biopsies were obtained at 3h and 24h after PDL treatment in 5 of these patients. In 14 patients additional biopsies were taken after 7 and 13 weeks of treatment. Samples were analyzed for expression of molecules that play critical roles in the pathogenesis of psoriasis, including epidermal (β-defensin-2), immune cell-derived (tumor necrosis factor (TNF)-α) and vascular components (VEGFR).

Results: Clinical high responders to PDL treatment showed decreased expression of VEGFR2, VEGFR3 and E-selectin within 24 h after PDL treatment. After 2 PDL treatments IL-23, TNF-α mRNA and E-selectin protein expression were significantly reduced, whereas after 4 treatments all epidermal markers and dermal T cell infiltrates normalized. In NB-UVB-treated plaques, epidermal activation markers and E-selectin expression were significantly reduced after 13 weeks.

Conclusions: At the end of the treatment period both PDL and NB-UVB resulted in reduced expression of epidermal activation markers and reduced dermal T cell infiltrates. We show early effects of PDL on vascular endothelial growth factor receptors, followed by downregulation of TNF-α and IL-23p19, which might contribute to PDL efficacy in psoriasis.

INTRODUCTION

The dilation and proliferation of the dermal papillary microvasculature is one of the early changes seen in a new psoriatic plaque (1). The increased dermal microvasculature facilitates the traffic of leukocytes from the circulation into the skin and therefore plays an important role in maintaining inflammation in psoriasis (2). Interference with leukocyte trafficking via selective destruction of the dilated capillaries may be an effective therapeutic intervention in psoriasis. Selective targeting of bloodvessels may be achieved with the flash-lamp pumped pulsed dye laser (PDL) (3). The PDL technique is based on the selective absorption of short pulses of 585 nm light by oxyhemoglobine inducing photothermolysis of capillaries, leaving the other nearby structures in the skin undamaged (4).

Several studies reported that psoriatic plaques were partly or completely cleared by PDL treatment (5-8). In a comparative study, PDL showed a significantly higher efficacy than a class II topically applied corticosteroid (8). PDL was also effective in clearing recalcitrant psoriatic plaques (9), whereas palmoplantar psoriasis responded well to treatment with PDL alone or in combination with topical calcipotriol or salicylic acid (10).

Narrow-band UVB (NB-UVB) therapy is a standard treatment modality for psoriasis. The mechanism of action of UVB has been investigated more thoroughly than that of PDL. It is known that UVB targets the epidermal compartment, inhibiting the proliferation of keratinocytes, abrogating antigen presentation, migration of Langerhans cells (11) and inducing apoptosis of activated skin-homing T cells (12, 13). Prolonged exposure to UVB light and a high cumulative dose may result in premature ageing of the skin and lead to a higher risk of skin cancer. However, recent follow-up studies in patients receiving NB-UVB for psoriasis did not report any increase in the incidence of skin cancer as compared to controls (14, 15). Long-term side effects of PDL treatment have not yet been reported.

No significant differences in clinical efficacy were demonstrated between PDL and NB-UVB in a single-blind, prospective, paired randomized controlled study (16), indicating that PDL is a valid treatment modality for psoriasis. However, to date we lack insight into the cellular and molecular mechanisms induced by PDL explaining its efficacy in psoriasis. The aim of the present study was to investigate the effects of PDL treatment on the cellular and molecular markers of disease activity in psoriasis using immunohistochemical and quantitative RT-PCR techniques and lesional biopsies taken before, during and at the end of the treatment and compare them with those of NB-UVB treatment.

MATERIALS AND METHODS

Patients and biopsies

After providing written informed consent (Medical Ethical Committee, Erasmus University Medical Center, Rotterdam, approval nr: MEC-2004-154) 19 subjects with stable plaque type psoriasis were enrolled in the study. Patients intolerant to light (toxic or allergic), using drugs with phototoxic or photo-allergic potency, younger than 18 years, were pregnant, or with pre-existing or manifest skin malignancy were excluded. A wash-out period of 2 weeks was indicated for topical drugs and of 4 weeks for photo(chemo)therapy and systemic drugs. Emollients were allowed.

In each patient the most representative psoriasis plaque was selected for a baseline biopsy.

In 5 patients (3 men and 2 women aged 45 to 59 years) additional 3-mm biopsies were taken at 3 h and 24 h after the first PDL treatment for the analysis of the short term effects. In 14 patients (7 men and 7 women aged 20 to 65 years) psoriasis plaques were randomly selected and treated either with PDL or with NB-UVB or left untreated. In these patients 3 mm biopsies were taken (in each patient one from a UVB-treated site and one from a PDL-treated site) at week 7 (after 2 treatments of PDL and 15 treatments with NB-UVB) and at week 13 (after 4 treatments of PDL and 30 to 33 treatments of NB-UVB). The tissue samples were snap-frozen in Tissue-Tek OCT (Miles, Elkhart, IN, USA) and stored at -80°C until further processing.

Treatments

PDL treatment was performed using the V Star PDL (Cynosure Inc., Chelmsford, MA), emitting yellow light at a wavelength of 585 nm. During treatment, the skin was air-cooled using a Zimmer 6 air cooler (Zimmer Elktromedizin, Neu-Ulm, Germany). PDL treatment parameters were: pulse duration 0.50 ms, spot diameter 7 mm, spots overlapping ± 20%, and fluences between 5.5 and 6.5 J/cm^2. Patients were treated with PDL every 3 weeks for 10 weeks, a total of four treatments.

Narrow-band ultraviolet B treatment was performed using the UVB-TL01 S (wavelength 311 nm) handheld device (Cosmedico Medizintechnik GmbH, Villingen-Schwenningen, Germany). Mean UV-output was 19.4 mWatt/cm^2. Calibration of the devices was performed before the treatment with the Optometer P 9710 (Gigahertz Optik, Puchheim, Germany). During treatment, the aperture of the appliance (5.0 × 10.0 cm) was held against the skin assuring that the distance between the device and the skin was constant in each patient. Minimal erythema dose (MED) was determined in each patient prior to the study. Mean MED NB-UVB was 0.86 J/cm^2 (range 0.34–1.2 J/cm^2). UVB treatment (3 times per week) parameters were: a start dose of 70% of the MED, dose increments of 20% of the previous treatment until persistent erythema appeared or clearing of the lesion was achieved. During 11 weeks in total 30–33 treatments were given. Mean initial dose was 0.62 J/cm^2 (range 0.24–0.86 J/cm^2), mean cumulative dose 84 J/cm^2 (range 40–120 J/cm^2).

In each patient plaques were randomly chosen, and treated with UVB, PDL or only with emollient. Concomitant topical treatment with salicylic acid 5% in petrolatum was permitted for all plaques in between the treatment sessions, to reduce reflectance of the PDL and the NB-UVB beam by scales, but was discontinued 2 days before PDL treatment and directly before UVB treatment. Clinical efficacy was assessed as described previously (16).

Immunohistochemistry

Cryosections (6 µm) were cut from each punch biopsy, mounted on glass slides, fixed in acetone and stained for markers of psoriasis using antibodies shown in Table 1. Endogenous peroxidase activity was neutralized using 4-chloro-1-naphtol. After washing in phosphate buffered saline (PBS) containing 0.05% Tween 20 and 0.5% bovine serum albumin (BSA), slides were incubated with the primary antibodies (Table 1) for 1 h at room temperature. They were subsequently incubated with biotinylated secondary antibodies (rabbit-anti-mouse

Table 1. Antibodies used in the study.

Antibody	Target molecule	Supplier	Species	Titer
anti-TGK	Transglutaminase K	Biomedical Technologies Inc.	Mouse	1:200
anti-CD3	CD3, a cell surface molecule on T lymphocytes	Dako	Rabbit	1:100
anti-vWF	Von Willebrand factor, a coagulation factor synthesized by endothelial cells	Abcam	Rabbit	1:25000
anti-E-selectin	E-selectin (CD62E), an adhesion molecule on endothelial cells	R&D systems Inc.	Mouse	1:20

IgG (Dako, 1:400) or donkey-anti-rabbit IgG (Amersham Biosciences, 1:800)) for 30 min and with horseradish peroxidase (HRP)-linked streptavidin (Dako, High Wycombe, UK) for 1 h at room temperature. Any non-specific staining was prevented by adding unlabeled (normal) secondary antibody and normal human AB serum. HRP activity was visualized with amino-9-ethylcarbazole (Sigma-Aldrich, St. Louis, MO, USA) as a chromogen, resulting in a bright red staining. Sections were counterstained with haematoxylin and mounted in glycerin-gelatin (Dako).

From each section a representative region was selected and photographed at 100x magnification using an AxioCam MRc5 camera (Zeiss, Goettingen, Germany). The acquired images were analyzed by computer-assisted image analysis using the WCIF VisionJ (http://rsb.info.nih.gov/ij) software. The area occupied by stained tissue was expressed as a fraction of the total area of interest (i.e. epidermis or dermis). For statistical analysis of the quantitative data, the Wilcoxon Signed Ranks test and the Kruskal-Wallis test were used for unpaired and paired sets of data, respectively.

RNA extraction and RT-PCR

For total RNA extraction, twenty 10 μm cryosections were cut under RNAase-free conditions from each biopsy sample, directly placed in RNA lysis buffer (Sigma) followed by mRNA extraction. RNA was transcribed into cDNA and RT-PCR was performed using newly designed primers and probes. ABL1 was used as a housekeeping control gene. Primer and probe sequences are listed in Table 2.

RESULTS

Clinical assessment

The Physician's Global Assessment (PGA) score was used to establish clinical improvement. The PGA score is a 5 point scoring system, where score 0 denotes symptom-free state, whereas scores 1-5 represent increasing severity. Definition of each score, as well as the clinical outcomes of the study have been reported in more detail elsewhere (16). The results of present study showed that the mean clinical improvement (mean reduction in the PGA score) at week 13 was 46% for the PDL-treated plaques and 52% for the UVB-

Table 2. Primers and probes for quantitative real-time PCR.

	Forward primer	Reverse primer	Probe [1]
KRT17	TTGAGGAGCTGCAGAACAAG	AGTCATCAGCAGCCAGACG	76
HBD2	TCAGCCATGAGGGTCTTGTA	GGATCGCCTATACCACCAAA	35
TNF-α	CAGCCTCTTCTCCTTCCTGAT	GCCAGAGGGCTGATTAGAGA	29
IL-23p19	GTTCCCCATATCCAGTGTGG	TCCTTTGCAAGCAGAACTGA	76
E-selectin	ACCAGCCCAGGTTGAATG	GGTTGGACAAGGCTGTGC	86
VEGF-A	TGCCCGCTGCTGTCTAAT	TCTCCGCTCTGAGCAAGG	1
VEGFR2	GCTCAAGACAGGAAGACCAAG	GGTGCCACACGCTCTAGG	27
VEGFR3	CAAGAAAGCGGCTTCAGGTA	GCAGAGAAGAAAATGCTGACG	8
Bcl2	CAACACGCAGAGAATGTAAAGC	GGTAGGAGCTGTGGCGACT	45
ABL1	TGGAGATAACACTCTAAGCA TAACTAAAGGT	GATGTAGTTGCTTGGGACCCA	CCATTTTTGGTT TGGGCTTCACAC CATT

[1] Probe numbers of the Exiqon probe library system (Exiqon, Vedbaek, Denmark).

treated plaque indicating that the clinical efficacy of both treatments were comparable. High responders (defined as a PGA reduction > 50%) were selected in order to evaluate the effects of both treatments. Clinical improvement during the treatments as assessed by the PGA score in the total group and in the 6 high responders is shown in Figure 1.

Early effects of the pulsed dye laser

In order to elucidate the mechanism of action of PDL in psoriasis, early changes (within 24 h) in psoriasis plaques upon PDL treatment were investigated. In biopsies taken 3 h and 24 h after PDL-treatment no changes were observed in mRNA expression levels of markers of the activated epidermal psoriasis phenotype, such as β-defensin 2 (HBD2) and keratin 17 (KRT17), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF)-A (data not shown). Expression of the anti-apoptotic, heat-regulated molecule Bcl2 showed no alteration within this time period of time (data not shown). Expression of the endothelial molecules E-selectin and vascular endothelial growth factor receptor (VEGFR)-2 was significantly reduced in the biopsies taken 3 h after PDL treatment (Figure 2 a, b). In the biopsies taken at 24 h, the expression of E-selectin was still reduced, whereas the expression of VEGFR2 returned to baseline, indicating that the reduction of VEGFR2 was transient (Figure 2 a, b). Interestingly, mRNA expression of the lymphatic marker VEGFR3 showed an alteration similar to VEGFR2 (Figure 2c). The expression of interleukin (IL)-23p19 mRNA was significantly reduced at 3h, and still reduced although not significantly at 24 h (Figure 2d).

In summary, PDL affected the mRNA expression of VEGFR2 and VEGFR3 as early as 3 h after treatment, whereas E-selectin expression was significantly reduced 24 h after treatment. The mRNA expression of IL-23p19 was also affected by PDL treatment, although the expression change did not reach significance at 24 h.

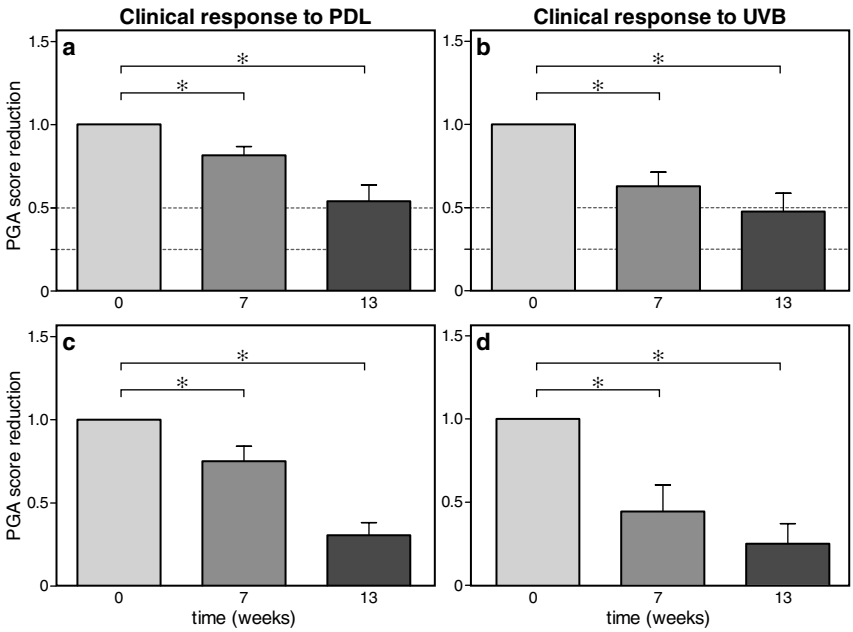

Figure 1. Clinical improvement of psoriasis lesions during PDL and local NB-UVB treatment from baseline to week 13.

In each patient, stable psoriatic plaques were randomly selected and treated with PDL or local NB-UVB. Clinical improvement was evaluated using the Physician's Global Assessment (PGA) score. (a)-(b) The average clinical improvement in the entire study group (14 patients). (c)-(d) The average clinical improvement in 6 patients with high clinical response. Error bars represent the standard error of the mean (SEM).

Long-term alterations at the mRNA level

In 14 patients, selected psoriasis plaques were treated either with PDL or with local NB-UVB. PDL treatment was carried out every 3 weeks for 10 weeks, in total four treatments, whereas NB-UVB was applied three times weekly with increasing doses. The mRNA expression of keratinocyte-, immune cell- and endothelial cell-associated activation markers was assessed by quantitative RT-PCR in biopsies taken before the start, at week 7 and week 13 of therapy in the group of clinical responders, in order to determine the course of the molecular changes during the treatment. The expression of keratin 17 and β-defensin 2 decreased significantly in the PDL-treated samples, corresponding with the clinical improvement. In the UVB-treated lesions no significant alterations in keratin 17 and β-defensin 2 expression were observed in responders (Figure 3 a-d).

The mRNA expression of TNF-α decreased significantly in the samples treated with PDL but not in those treated with NB-UVB (Figure 3 e, f). Expression of IL-23p19 mRNA was suppressed in the PDL-treated samples at week 7 of treatment, but returned to baseline levels after 13 weeks, whereas in the UVB-treated samples no significant alterations in the expression of IL-23p19 mRNA were detected in the clinical responders (Figure 3 g, h).

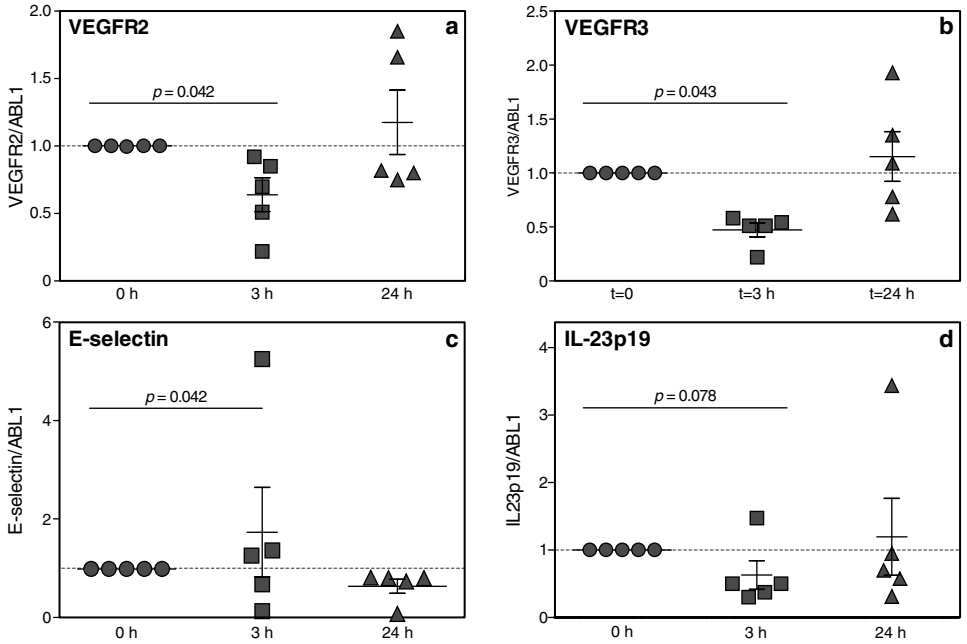

Figure 2. The effects of the PDL on the expression of vascular endothelial growth factor receptors, E-selectin and IL-23p19 in psoriatic skin.

The expression of (a) VEGFR2, (b) VEGFR3, (c) E-selectin and (d) IL-23p19 was determined by RT-PCR in biopsies taken from psoriatic lesions before, 3 h and 24 h after PDL treatment in 5 patients with psoriasis. The figures depict expression values in individual patients relative to the baseline. At each time point, the mean +/- SEM are marked. *p*-values are shown as calculated with the Wilcoxon signed ranks test.

Expression of VEGFR2 mRNA was upregulated during both treatments, indicating an activated angiogenesis (Figure 3 i, j). In addition, a reduced expression of the antiapoptotic molecule Bcl2 was observed in the biopsies after PDL treatment (data not shown).

In summary, in 6 clinical responders PDL-treatment resulted in decreased mRNA expression of TNF-α and IL-23p19 at 7 weeks, and reduced β-defensin 2, keratin 17 and Bcl2 at 13 weeks, whereas expression of the angiogenic molecule VEGFR2 was induced. In the NB-UVB-treated samples mRNA expressions of these markers showed great variation between the patients.

Immunohistochemical alterations

Skin biopsy samples of all 14 patients were analyzed by immunohistochemistry. To follow epidermal alterations, expression of transglutaminase K (TGK), an early differentiation marker with strong expression in psoriasis, was assessed. To assess the inflammatory components of disease activity, T lymphocyte infiltrates (CD3) was visualized in the dermis. Changes in blood vessel content were followed by Von Willebrand factor (VWf) staining, whereas endothelial activation was assessed by E-selectin expression.

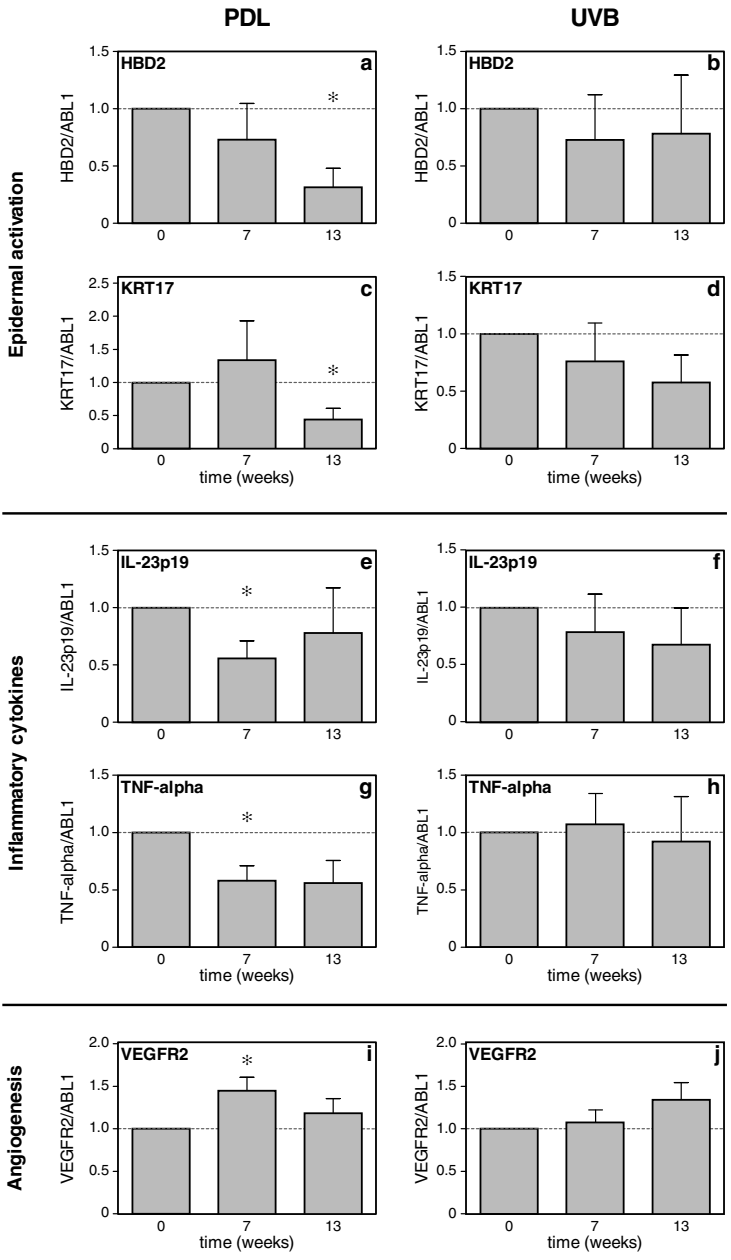

Figure 3. The effects of PDL and UVB on the expression of psoriasis markers in lesional psoriatic skin. mRNA expression of (a)-(b) β-defensin 2, (c)-(d) keratin 17, (e)-(f) IL-23p19, (g)-(h) TNF-α and (i)-(j) VEGFR2 was measured by quantitative RT-PCR in punch biopsies from 6 patients. ABL1 was used as a housekeeping control gene. Bars show average expression levels relative to the baseline, error bars represent SEM. *p*-values are shown as calculated with the Wilcoxon signed ranks test.

Global improvement of the psoriatic pathology, assessed using H&E staining, was observed in about 50% of patients, corresponding with the clinical improvement in psoriasis. No significant changes were found in the PDL-treated plaques, whereas in the NB-UVB-treated plaques TGK and E-selectin expression was significantly decreased after 13 weeks of treatment in the group of 14 patients (responders and non-responders) (Figure 4).

We analyzed biopsies from 6 clinical high responders (defined as a PGA reduction > 50%) for epidermal and dermal markers of psoriasis, in order to evaluate the effects of both treatments. After 14 weeks, epidermal TGK expression and dermal T cell infiltrates were markedly reduced by both treatments in these 6 patients (Figure 5a-d). Von Willebrand factor expression was significantly decreased in the UVB-treated plaques; a marked, but not significant decrease was also noted after PDL treatment (Figure 5 e, f). Expression of the endothelial activation marker E-selectin showed a significant decrease in the PDL-treated samples after 7 weeks, but this change did not reach significance at week 13. E-selectin expression showed a gradual persistent decrease in the NB-UVB-treated lesions (Figure 5 g, h).

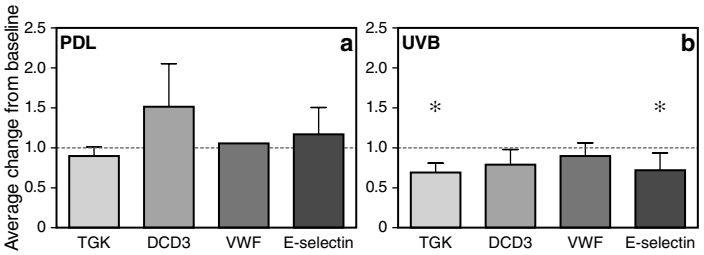

Figure 4. Immunohistochemical staining results in the entire study group.
Biopsies from 14 patients with psoriasis were taken before and 13 weeks after the start of the study. Immunohistochemical staining for epidermal transglutaminase (TGk), CD3, von Willebrand factor (VWF) and E-selectin (E-sel) was performed. Staining positivity was quantified using digital image analysis. CD3 positivity was quantified separately in the dermis (DCD3). Bars show staining positivity at 13 weeks relative to the baseline. Error bars represent the SEM.

DISCUSSION

This study shows that clinical improvement of psoriasis due to PDL treatment is accompanied by alterations in certain classic markers of psoriasis disease activity. The observed effects at the mRNA level were comparable in responders to the PDL- and the NB-UVB treatment. The relatively low clinical efficacy of NB-UVB in this study (52% improvement versus minimally 60% reported for total body irradiation) may be explained by the fact that local NB-UVB treatment was used instead of total body irradiation. It is assumed that total body UVB irradiation also exerts a systemic effect (17).

A hallmark of psoriatic skin is the remarkable transformation in the local microvascular system characterized by dilation and tortuosity of capillaries, increased permeability, and high endothelial venule formation which is usually observed in lymph nodes (18).

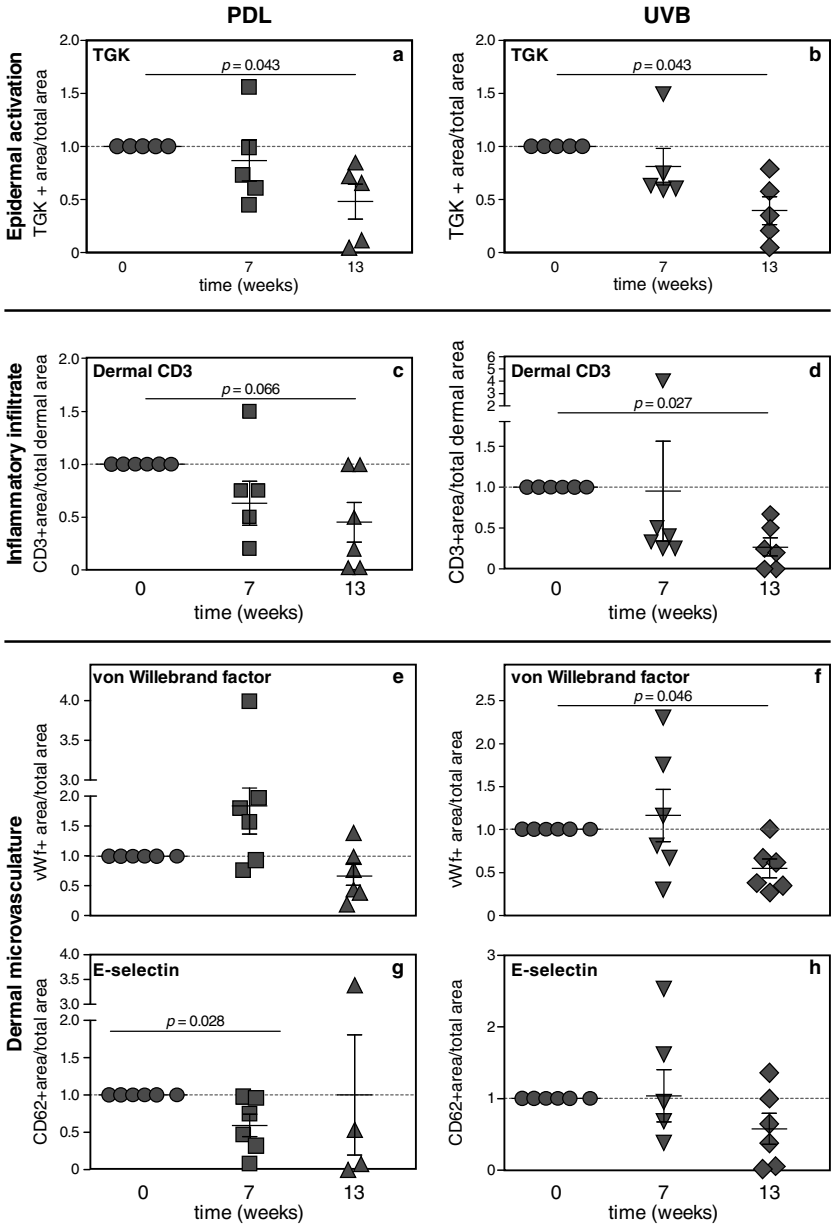

Figure 5. Immunohistochemical staining results in clinical responders.
Biopsy samples from 6 clinical responders, taken before the start of treatment and 7 and 13 weeks later were stained using antibodies against (a)-(b) transglutaminase K (TGK), (c)-(d) CD3, (e)-(f) von Willebrand factor and (g)-(h) E-selectin. Staining positivity was quantified using digital image analysis. Figures depict expression values of individual patients relative to the baseline. At each time point, the mean +/- SEM are marked. *p*-values are shown as calculated with the Wilcoxon signed ranks test.

Active angiogenesis in psoriatic lesions evidenced by upregulation of VEGF and VEGFR2, is accompanied by endothelial cell activation as observed by the upregulation of ICAM-1, vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. During this process, vascular endothelial growth factor (VEGF) signaling on endothelial cells represents the major rate-limiting step (18). VEGF acts by engaging with its tyrosine kinase receptors VEGFR1 and VEGFR2 in endothelial cells. Although VEGF binds to both receptors, it appears that most of its biological functions are mediated via VEGFR2 (18, 19).

Our observation that the early effects of the PDL involve the decreased expression of the endothelial molecules VEGFR2 and E-selectin corresponds with its proposed primary target. However, an additional early effect was also noted on VEGFR3 (FLT4), a lymph-endothelial marker (20). Lymphatics are expanded in psoriasis and VEGFR3 expression is increased in both involved and uninvolved psoriatic skin (19). Reduction of VEGFR3 expression early after PDL treatment may contribute to its efficacy in psoriasis.

Interestingly, the expression of VEGFR2 was upregulated at later time points indicating re-activation of angiogenesis. This active angiogenesis may counteract the antipsoriatic effect of the PDL treatment and may explain the insufficient clinical response in some patients. Another possibility might be that the main source of VEGF in psoriatic lesions, the activated keratinocyte, is not targeted by PDL treatment. Therefore, activated lesional keratinocytes are less inhibited and may continue to stimulate the lesional microvasculature. The upregulation of VEGFR2 may explain the enhanced efficacy of PDL treatment when combined with calcipotriol ointment, which targets activated keratinocytes (10).

TNF-α and IL-23 are critical cytokines in the pathogenesis of psoriasis, mainly produced by inflammatory dendritic cells (DC) in the dermis (21), (22). Their importance is underlined by the fact that biologics targeting TNF-α and IL12/IL23p40 are effective in treating psoriasis (23). Furthermore, both cytokines are downregulated during treatment with other effective therapies (24). Our study shows that after 7 weeks of PDL treatment, TNF-α and IL-23 mRNA was downregulated in psoriatic skin, and IL-23 even within 3 h after treatment. In combination with the observed reduction in the number of dermal CD3+ T cells, it may indicate that the vascular damage induced by PDL treatment may also affect perivascular immune cells.

Markers of keratinocyte activation and of the psoriasis phenotype were only significantly reduced at the end of the series of PDL and NB-UVB treatment sessions. Keratin 17 expression is high in the psoriatic epidermis, correlating with the clinical severity (25). Keratin 17 is considered to be a candidate autoantigen in psoriasis (26). Human β-defensin 2 is a molecule with antimicrobial activity, expressed by epidermal keratinocytes under inflammatory conditions such as psoriasis (27). Patients with psoriasis have higher genomic copy numbers of the gene encoding β-defensin 2 (28) and have higher serum β-defensin 2 levels than healthy controls. Serum β-defensin 2 levels were shown to correlate positively with the disease severity (29). Since the PDL treatment does not target the epidermis primarily, it is conceivable that the PDL-associated decrease in the expression of epidermal keratin 17 and β-defensin 2 is secondary to the dermal blood vessel damage and to the subsequent reduction in the inflammatory infiltrate and their mediators.

Our finding that PDL treatment is effective in approximately 50% (a subgroup) of patients has also been reported by others (30). In our study, the group of PDL-responders could not be distinguished from the non-responders in terms of specific baseline expression patterns (data

not shown) or immunohistochemical alterations induced by the treatment. Further studies are essential for identifying the prognostic markers of PDL-responsiveness in psoriasis.

In conclusion, PDL and local NB-UVB treatment are clinically equally effective in stable plaque-type psoriasis. At the end of the treatment period both treatments resulted in reduced expression of epidermal markers of keratinocyte activation as well as reduction of dermal T cell infiltrates. This indicates that alterations in the expression of markers of psoriasis activity do not clearly disclose the clinical treatment modality used and may just reflect clinical improvement of the disease. Furthermore we observed early effects of the PDL treatment on vascular endothelial growth factor receptors, followed by downregulation of TNF-α and IL-23p19. These are previously unrecognized factors for the efficacy of PDL treatment in psoriasis.

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REFERENCES

1. Pinkus, H. and Mehregan, A. H. (1966). The primary histologic lesion of seborrheic dermatitis and psoriasis. *J Invest Dermatol* **46**(1): 109-16.
2. Heng MCY, A. S., Haberfelde GH, Song MK. (1991). Electronmicroscopic and immunohistochemical studies of the sequence of events in psoriatic plaque formation following tape-stripping. *Br J Dermatol* **125**: 548-556.
3. Hacker, S. M. and Rasmussen, J. E. (1992). The effect of flash lamp-pulsed dye laser on psoriasis. *Arch Dermatol* **128**(6): 853-5.
4. Herd, R. M., Dover, J. S. and Arndt, K. A. (1997). Basic laser principles. Dermatol Clin **15**(3): 355-72.
5. Katugampola, G. A., Rees, A. M. and Lanigan, S. W. (1995). Laser treatment of psoriasis. *Br J Dermatol* **133**(6): 909-13.
6. Ros, A. M., *et al.* (1996). Psoriasis response to the pulsed dye laser. *Lasers Surg Med* **19**(3): 331-5.
7. Hern, S., *et al.* (2001). Immunohistochemical evaluation of psoriatic plaques following selective photothermolysis of the superficial capillaries. *Br J Dermatol* **145**(1): 45-53.
8. Zelickson, B. D., *et al.* (1996). Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. *J Am Acad Dermatol* **35**(1): 64-8.
9. Erceg, A., *et al.* (2006). Efficacy of the pulsed dye laser in the treatment of localized recalcitrant plaque psoriasis: a comparative study. *Br J Dermatol* **155**(1): 110-4.
10. de Leeuw, J., *et al.* (2006). Concomitant treatment of psoriasis of the hands and feet with pulsed dye laser and topical calcipotriol, salicylic acid, or both: a prospective open study in 41 patients. *J Am Acad Dermatol* **54**(2): 266-71.
11. Hamakawa, M., *et al.* (2006). Ultraviolet B radiation suppresses Langerhans cell migration in the dermis by down-regulation of alpha4 integrin. *Photodermatol Photoimmuno Photomed* **22**: 116-123.
12. Krueger, J. G., *et al.* (1995). Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. *J Exp Med* **182**(6): 2057-68.
13. Ozawa, M., *et al.* (1999). 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. *J Exp Med* **189**(4): 711-8.
14. Weischer, M., *et al.* (2004). No evidence for increased skin cancer risk in psoriasis patients treated with broadband or narrowband UVB phototherapy: a first retrospective study. *Acta Derm Venereol* **84**(5): 370-4.

15. Hearn, R., *et al.* (2008). Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. *Br J Dermatol* **159**: 931-935.
16. De Leeuw, J., *et al.* (2009). A comparative study on the efficacy of treatment with 585 nm pulsed dye laser and ultraviolet B-TL01 in plaque type psoriasis. *Dermatol Surg* **35**(1): 80-91.
17. Schwarz, T. (2005). Mechanisms of UV-induced immunosuppression. *Keio J Med* **54**(4): 165-71.
18. Costa, C., Incio, J. and Soares, R. (2007). Angiogenesis and chronic inflammation: cause or consequence? *Angiogenesis* **10**(3): 149-66.
19. Henno, A., *et al.* (2009). Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis. *Br J Dermatol* **160**(3): 581-90.
20. Iljin, K., *et al.* (2001). VEGFR3 gene structure, regulatory region, and sequence polymorphisms. *Faseb J* **15**(6): 1028-36.
21. Zaba, L. C., *et al.* (2007). Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* **204**(13): 3183-94.
22. Lowes, M. A., *et al.* (2005). Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* **102**(52): 19057-62.
23. Krueger, G. G., *et al.* (2007). A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* **356**(6): 580-92.
24. Zaba, L. C., Krueger, J. G. and Lowes, M. A. (2009). Resident and "inflammatory" dendritic cells in human skin. *J Invest Dermatol* **129**(2): 302-8.
25. de Jong, E. M., *et al.* (1991). Keratin 17: a useful marker in anti-psoriatic therapies. *Arch Dermatol Res* **283**(7): 480-2.
26. Bonnekoh, B. and Bockelmann, R. (2007). Keratin 17/interferon-gamma autoimmune loop as a vicious circle driving psoriasis pathogenesis. *J Am Acad Dermatol* 56(1): **162**; author reply 162-4.
27. Harder, J. and Schroder, J. M. (2005). Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins. *J Leukoc Biol* **77**(4): 476-86.
28. Hollox, E. J., *et al.* (2008). Psoriasis is associated with increased beta-defensin genomic copy number. *Nat Genet* **40**(1): 23-5.
29. Jansen, P. A., *et al.* (2009). Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. *PLoS ONE* **4**(3): e4725.
30. Taibjee, S. M., *et al.* (2005). Controlled study of excimer and pulsed dye lasers in the treatment of psoriasis. *Br J Dermatol* **153**(5): 960-6.

6

General Discussion

GENERAL DISCUSSION

Narrow-band UVB (NB-UVB) therapy is a standard treatment for moderate to severe psoriasis. It is safe in terms of limited carcinogenicity, and achieves high efficacy scores in a large proportion of patients (1-5). The mode of action of NB-UVB in psoriasis is incompletely understood and much of current knowledge is based on *in vitro* studies and studies in mice. In general, the efficacy of NB-UVB is thought to be mediated via: 1) inhibition of keratinocyte proliferation, 2) induction of apoptosis in intraepidermal bone marrow-derived cells (dendritic cells, (natural killer) T cells, pDCs), 3) skewing of T cell differentiation from Th1 and Th17 towards the Th2 phenotype. However, it has become clear that the skin immune system of the mouse and the mouse genome differ more from man than previously anticipated (6), underscoring the need for studies in patients with active disease. Molecular analysis of the impact of NB-UVB on (innate) inflammatory pathways may generate more insight into the mode of action of NB-UVB in modifying the pathophysiology of psoriasis and to define potential therapeutic target molecules.

In this thesis global transcriptomic effects of NB-UVB therapy in psoriasis are analyzed. In our studies we focused on the epidermal compartment, as most UVB radiation is absorbed there. In general, the following conclusions were drawn:

1) epidermal Th17 and interferon signalling pathways are suppressed by NB-UVB;
2) NB-UVB downregulates innate cytosolic RNA receptors in psoriasis; and
3) epidermal GATA3 expression is induced by NB-UVB, and might be the basis of the antiproliferative effects of UVB (see also Box 1).

In addition, we demonstrated that UVB-effects in non-lesional skin cannot simply be extrapolated to the mode of action in active lesions, which emphasizes the need for *in vivo* studies in lesional psoriatic skin. In order to clarify the involvement of Th17 pathway components such as the double-stranded RNA receptors melanoma differentiation-associated gene (MDA) 5, retinoic acid-inducible gene (RIG)-I, the transcription factor GATA3 in the therapeutic effectiveness of NB-UVB*, in vitro, ex vivo* and murine experiments were performed. All these molecules are known to be expressed by epidermal keratinocytes (7-9), and our epidermal samples, both lesional and non-lesional, are composed of 95% keratinocytes. Therefore, while investigating the roles of these molecules in psoriasis, we decided to study only epidermal cells *in vivo* and keratinocytes *in vitro*. However, since other cell types are also present in the epidermis, changes in expression within these cells (e.g. Langerhans cells, melanocytes, Merkel cells), or the UVB-induced change in the cellular composition of the epidermis (e.g. depletion of T lymphocytes, myeloid dendritic cells (DC) and neutrophilic granulocytes) probably also contributed to the gene expression changes we observed. However, the DC/Langerhans cell markers CD1a, CD1c and CD207/Langerin and the T cell markers CD3, CD4 and CD8 were not differentially expressed between samples taken before and after UV-therapy. We did detect an induction of genes involved in melanin synthesis, such as tyrosinase and melan-A, indicating melanocyte activation. We concluded that our expression data mainly represented gene expression profiles of cells other than DC, Langerhans cells or T lymphocytes. However, when evaluating the function of STAT3, MDA5 or GATA3 in psoriasis, roles of these molecules in inflammatory cells and melanocytes should also be considered. Below, the results on the molecular targets of NB-UVB therapy in psoriasis are summarized and discussed in view of the future prospects of this field of research.

Box 1. Main conclusions

- Clearance of psoriasis by NB-UVB therapy is associated with suppression of type I and type II interferon signalling and downregulation of the Th17 pathway in the lesional epidermis. NB-UVB inhibits the phosphorylation of STAT3, resulting in reduced expression of its transcriptional targets, for example the antimicrobial peptide β-defensin 2.
- NB-UVB treatment downregulates the expression of the activating dsRNA receptors RIG-I and MDA-5 in lesional epidermis of patients with psoriasis. Activation of these innate antigen receptors by virus- or host cell-derived dsRNA induces the production of type I interferons, which in turn activate adaptive immune responses. Thus, inhibition of these molecules by NB-UVB contribute to the anti-inflammatory effects of the treatment.
- NB-UVB therapy induces the expression of the transcription factor GATA3 in psoriasis. GATA3 in the epidermis induces the expression of anti-inflammatory and proapoptotic molecules, and regulators of epidermal differentiation. These GATA3 targets might be involved in the improvement of psoriatic inflammation by NB-UVB. We also provide evidence that proinflammatory mediators that are critical in the pathogenesis of psoriasis do not suppress or regulate epidermal GATA3 expression.
- The pulsed dye laser (PDL) is an effective therapeutic option for recalcitrant psoriasis. We show early effects of PDL on the expression of E-selectin and vascular endothelial growth factor receptors, followed by downregulation of TNF-α and IL-23p19. NB-UVB and PDL treatments were found to induce quite similar expression changes after 14 weeks of treatment.

The Th17 pathway as potential therapeutic target in psoriasis

Th17 cells are induced primarily by DC-derived IL-23, via the IL-23 receptor on their surface, made up of two subunits, IL-23R and IL-12Rβ1 (10). In the presence of IL-23, Th17 cells produce Th17 cytokines, including IL-17A, IL-17F, IL-6, TNF-α, IL-21 and IL-22. Th17 pro-inflammatory cytokines stimulate keratinocyte proliferation as well as drive inflammatory cell activation, migration and action, especially that of neutrophil granulocytes, in the skin. Thus they contribute to the formation of psoriatic lesions and lead to the persistent, low-grade inflammation characteristic of psoriasis. Many actions of the Th17 cytokines IL-21, IL-22 and IL-6 are mediated by the transcription factor STAT3.

We have found that NB-UVB therapy downregulated several components of the Th17 pathway, such as STAT3 and its transcriptional targets (e.g. β-defensin 2, keratin 16, IL-20R, S100A7, S100A9) in the psoriatic epidermis (Figure 1). Direct inhibition of STAT3 phosphorylation was shown in an *ex vivo* skin organ culture system where the number of IL-22-induced phosphorylated STAT3-positive cell nuclei was reduced by subsequent NB-UVB treatment, as early as 6 h after irradiation. In addition, IL-22-induced expression of β-defensin 2 mRNA was inhibited by NB-UVB.

Other anti-psoriatic therapies also affect the Th17 pathway, which emphasizes the importance of this pathway in the resolution of psoriatic inflammation. The Th17 pathway

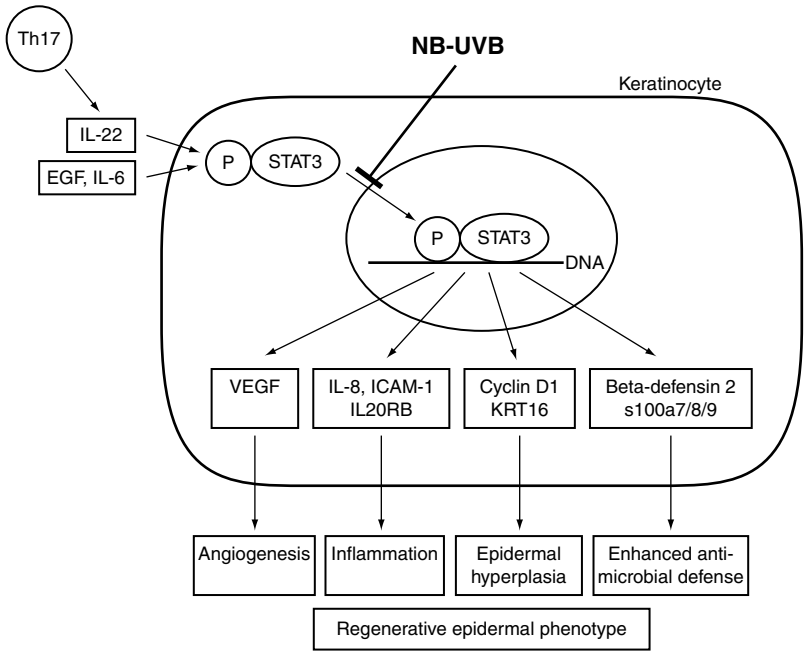

Figure 1. NB-UVB inhibits the phosphorylation of STAT3.
IL-22, a cytokine secreted by Th17 cells, induces the phosphorylation of STAT3. Phosphorylated STAT3 translocates to the nucleus and induces the transcription of genes involved in the depicted functions. NB-UVB inhibits the phosphorylation of STAT3, which leads to decreased expression of the STAT3 target genes.

is modulated in the psoriatic skin during treatment with Cyclosporine A, a standard effective therapy for psoriasis (11). This modulation was observed already 2 weeks after the start of treatment and correlated well with the clinical improvement of the disease (11). Etanercept (TNF receptor – immunoglobulin fusion protein), another effective treatment modality in psoriasis, also had early inhibitory effects on Th17 cells (12).

Direct inhibition of this pathway is achieved by the novel anti IL-12/IL-23 p40 therapies, such as the recently approved ustekinumab and ABT-874. These drugs seem to clear psoriasis very effectively and their clinical effect lasts much longer than the half-life of the drugs (13-15).

Even more specific inhibition of this pathway could be achieved by inhibition of IL-22 or IL-17. IL-17 targeted approaches are being developed for the treatment of psoriasis and other autoinflammatory diseases, and have now progressed towards Phase II clinical trial testing in patients with psoriasis (16). IL-22 has been successfully targeted using an anti-IL-22 antibody and IL-22-small interfering RNA in animal and *in vitro* models of lung cancer where its expression is also increased (17). These approaches might find their way to into the clinic for the treatment of psoriasis.

In addition, selective inhibition of STAT3 phosphorylation is another way of targeting the Th17 pathway. Constitutive STAT3 activation has been linked to cancer initiation, proliferation, promotion of angiogenesis and inhibition of apoptosis. STAT3 is involved in

the induction and survival of cancer cells. Several strategies have been pursued to inhibit the STAT3 pathway as a therapeutic approach in treating cancers. Small molecules such as LLL-3 (18), STA-21 (19), Stattic (20) and DNA decoys (21) bind to STAT3, abrogating its ability to bind to its response elements on DNA. STAT3 DNA decoys were successfully applied in experimental models of squamous cell carcinoma in combination with EGFR and BCL2 antagonists (22). In summary, several strategies have been developed for the inhibition of STAT3 phosphorylation and activation, although it has yet to be demonstrated that inhibition of STAT3 phosphorylation is sufficient to clinically improve psoriasis.

All above mentioned agents require extensive testing in *in vitro* and in murine models of psoriatic inflammation, before their efficacy and safety can be evaluated in patients with psoriasis.

Involvement of MDA5 and RIG-I in the anti-inflammatory effect of NB-UVB

Retinoic acid inducible gene (RIG)-I and melanoma differentiation-associated gene (MDA) 5 bind virus- or host cell-derived double-stranded (ds)RNA with or without a 5' triphosphate end. Upon activation these cytosolic receptors stimulate the production of type I interferons, via activation of the transcription factor IRF3 (8). Type I interferons play essential roles in the initiation of psoriatic inflammation (23). Furthermore, type I IFN signalling is activated in psoriatic plaques (24). We postulated previously that dsRNA receptors play a role in the maintenance of chronic inflammation in psoriasis. We have shown that NB-UVB treatment downregulates the expression of RIG-I and MDA-5 in lesional epidermis of patients with psoriasis (Figure 2). A rapid effect of NB-UVB on dsRNA receptor expression was demonstrated in cultured primary keratinocytes. NB-UVB irradiation suppressed the type I and type II IFN-induced expression of all activating dsRNA-receptors as early as 2 hours after irradiation, whereas expression of the negative regulator LGP2 remained unaffected. The proinflammatory response induced by dsRNA in keratinocytes was reduced by NB-UVB-irradiation. NB-UVB irradiation, in such a manner, interrupts the positive feedback loop of epidermal inflammation in psoriasis (Figure 2).

The exact mechanism of this suppressive effect requires further investigation. Broadband UVB inhibits IFN-γ-induced STAT1 phosphorylation in mouse keratinocytes (25). It would be worthwhile investigating whether inhibition of STAT1 phosphorylation also occurs in primary human keratinocytes upon NB-UVB radiation, and whether the expression of activating dsRNA receptors depends on STAT1 phosphorylation in these cells. Alternatively, NB-UVB might directly affect these receptors in the cytoplasm, or again through formation of ROS. Effect of NB-UVB on cytosolic innate RNA receptors was not demonstrated previously. The inhibitory effect we describe might contribute to the worsening or reactivation of herpes simplex virus (HSV) infection upon UVB exposure, as RIG-I-like helicases are involved in the immune response to HSV (26). In addition, many questions on the role of these receptors in the pathophysiology of psoriasis and their usefulness as therapeutic targets remain open. Further studies need to clarify whether specific inhibition or blockade of MDA5 or RIG-I affects psoriatic inflammation. Our imiquimod-induced skin inflammation model could be used to answer this question. In addition, overexpression of MDA5 and RIG-I in keratinocytes prior to UVB irradiation could demonstrate whether these molecules are indeed involved in the suppression of the inflammatory response to dsRNA by NB-UVB.

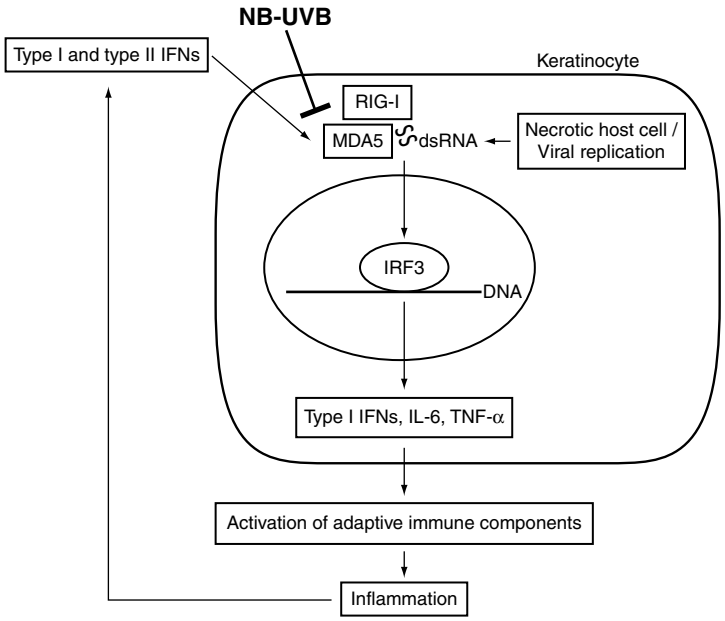

Figure 2. NB-UVB downregulates the expression of MDA-5 and RIG-I.
Psoriatic keratinocytes are characterized by high expression of the activating cytoplasmic dsRNA receptors MDA-5 and RIG-I, and thereby display enhanced sensitivity to virus- or host cell-derived dsRNA. Binding of these receptors to dsRNA results in the production of type I interferons. NB-UVB downregulates the expression of MDA-5 and RIG-I in the psoriatic epidermis, and limits thereby the sensitivity of these cells to dsRNA. IRF3: interferon regulatory factor 3

Role of GATA3 induction in the improvement of psoriatic inflammation

The transcription factor GATA3 has regulatory roles during the formation of the epidermal barrier and during desquamation (27-29). In addition, GATA3 also regulates the differentiation of T lymphocytes, stimulating the development of Th2 cells and inhibiting the skewing towards the Th1 phenotype (30, 31). In addition, in breast cancer low GATA3 expression is associated with early metastasis and poor prognosis, whereas high GATA3 expression with less metastasis and better prognosis (32).

NB-UVB phototherapy upregulated the expression of GATA3 in lesional psoriatic epidermis (Figure 3), where GATA3 expression was lower than in non-lesional skin. Interestingly, GATA3 was also upregulated by Cyclosporin A treatment, correlating with the clinical improvement (33). We demonstrated that low epidermal expression of the transcription factor GATA3 is associated with altered keratinocyte proliferation and differentiation in psoriasis and a murine psoriasis-like skin inflammation model. We also showed that proinflammatory mediators that are critical in the pathogenesis of psoriasis did not suppress epidermal GATA3 expression. This finding suggests that either low GATA3 expression is an inherent abnormality in the psoriatic epidermis, resulting in hyperproliferation and barrier abnormalities comparable to GATA3-/- mice, or it may be that other mediators are responsible for its suppression.

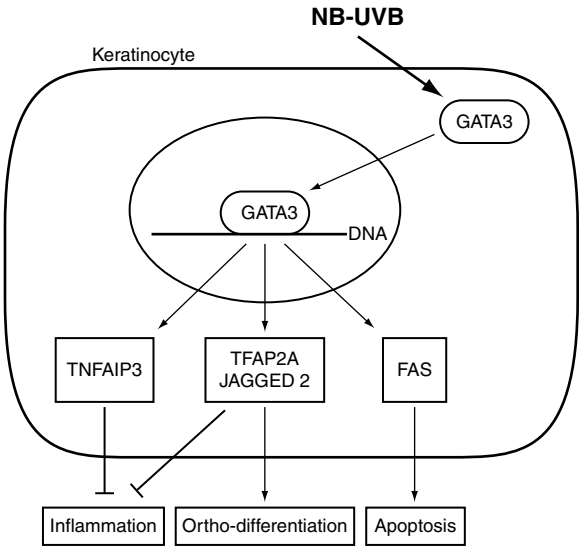

Figure 3. NB-UVB induces the expression of the transcription factor GATA3.
Expression of the transcription factor GATA3 is low in the psoriatic epidermis, potentially leading to low expression of anti-inflammatory and pro-apoptotic GATA3 targets. NB-UVB induces the expression of GATA3 which might contribute to the antipsoriatic effect of this treatment. TNFAIP3: Tumor necrosis factor, alpha-induced protein 3
TFAP2A: Transcription factor AP-2 alpha

Interestingly, GATA3 overexpression resulted in reduced STAT3 expression in mice (34), suggesting that upregulation of GATA3 by NB-UVB might be upstream of the suppression of STAT3-dependent events as discussed above.

To identify the molecular effects of low GATA3 expression in psoriasis, we first constructed a list of genes known to be related to GATA3, i.e. differentially regulated in epidermis-specific GATA3-/- mice as compared to wild type mice. We then selected those genes from this list that were regulated in the same way (i.e. up or down) in lesional psoriatic skin (characterized by low GATA3 expression) versus non-lesional skin. The resulting group of genes included negative regulators of inflammation, such as the psoriasis susceptibility gene TNFAIP3 (35, 36) and the Notch ligand jagged 2 (37), genes regulating epidermal differentiation, such as the transcription factor AP2-α (TFAP2A) (38), or the apoptosis-inducing FAS molecule. NB-UVB therapy induced the expression of these genes in the psoriatic epidermis, together with that of GATA3, which might lead to the anti-psoriatic effects of NB-UVB (Figure 3). In addition, the transcription factors FOXN3, FOXO1 and FOXP1 were also regulated together with GATA3. These molecules play regulatory roles in organ development (39), metabolism (40) and cell proliferation (41), respectively.

An upstream regulator of GATA3, the p53 homolog molecule p63 (42) is induced by DNA damage as caused by UV radiation, Actinomycin D and other genotoxic agents (43, 44). Induction of p63 leads to differentiation of cancer cells (in contrast to the induction of p53 by the same agents, which resulted in apoptosis) (43). Although we did not detect p63 induction in lesional psoriatic skin by NB-UVB at the time point studied, induction of p63 expression

might be an upstream event to the observed upregulation of GATA3 in our experimental systems.

The dermal vasculature as therapeutic target in psoriasis

This study shows that clinical improvement of psoriasis due to PDL treatment is accompanied by alterations in some classic markers of psoriasis disease activity. In responders to PDL and NB-UVB treatment, the treatment effects observed at the mRNA level were comparable. Our observation that the early effects of PDL involve reduction of the endothelial molecules VEGFR2 and E-selectin, corresponds with its proposed primary target (Figure 4). However, an additional early effect was detected on VEGFR3 (FLT4), a lymph-endothelial marker (45). Lymphatics are expanded in psoriasis, and VEGFR3 expression is high in both involved and uninvolved psoriatic skin (46). Reduction of VEGFR3 expression early after PDL treatment may contribute to its efficacy in psoriasis. After 2 treatment sessions with PDL, TNF-α and IL-23p19 mRNA expression was suppressed in the psoriatic plaques, which is probably a consequence of the primary PDL-effects on the dermal vasculature (Figure 4).

Interestingly, expression of VEGFR2 was upregulated at later time points, which is suggestive of re-activation or recovery of angiogenesis. This active angiogenesis might counteract the antipsoriatic effect of PDL treatment and might account for insufficient clinical response in some patients. Another possibility might be that the main source of VEGF in psoriatic lesions, the activated keratinocyte, is not targeted by PDL treatment. Activated lesional keratinocytes therefore, are less inhibited and may keep stimulating the lesional microvasculature. The enhanced efficacy of PDL treatment when combined with calcipotriol ointment, which targets activated keratinocytes, may be explained by the inhibitory effects of calcipotriol ointment on keratinocyte-derived VEGF (47).

In conclusion, PDL and local NB-UVB treatment are clinically equally effective in chronic plaque psoriasis. At the end of the treatment period both treatments resulted in reduced expression of epidermal markers of keratinocyte activation as well as reduction of dermal T cell infiltrates. This indicates that alterations in the expression of markers of disease activity in psoriasis do not clearly disclose the clinical treatment modality used and seem to reflect clinical improvement of the disease. Furthermore we show early effects of the pulsed dye laser on vascular endothelial growth factor receptors, followed by downregulation of TNF-α and IL-23p19, previously unrecognized factors of PDL efficacy in psoriasis.

Limitations

As all research, the studies described in this thesis were also limited by several factors. In the microarray study we were in the first place restricted in the number of skin biopsies per patient because of ethical reasons. Our initial experimental design was based on collecting 10 biopsies per patient. This relatively high number of biopsies hampered the inclusion of patients, which therefore took a longer period than foreseen. It is difficult, in terms of kinetics, to determine the optimal time point to look for targets directly affected by UVB. During NB-UVB therapy incremental UVB doses are applied in order to maintain the safety of the treatment, and the first irradiation (70% MED) is too low to have therapeutically relevant effects. The low starting dose is probably the explanation of the limited gene expression effects we observed on the short term.

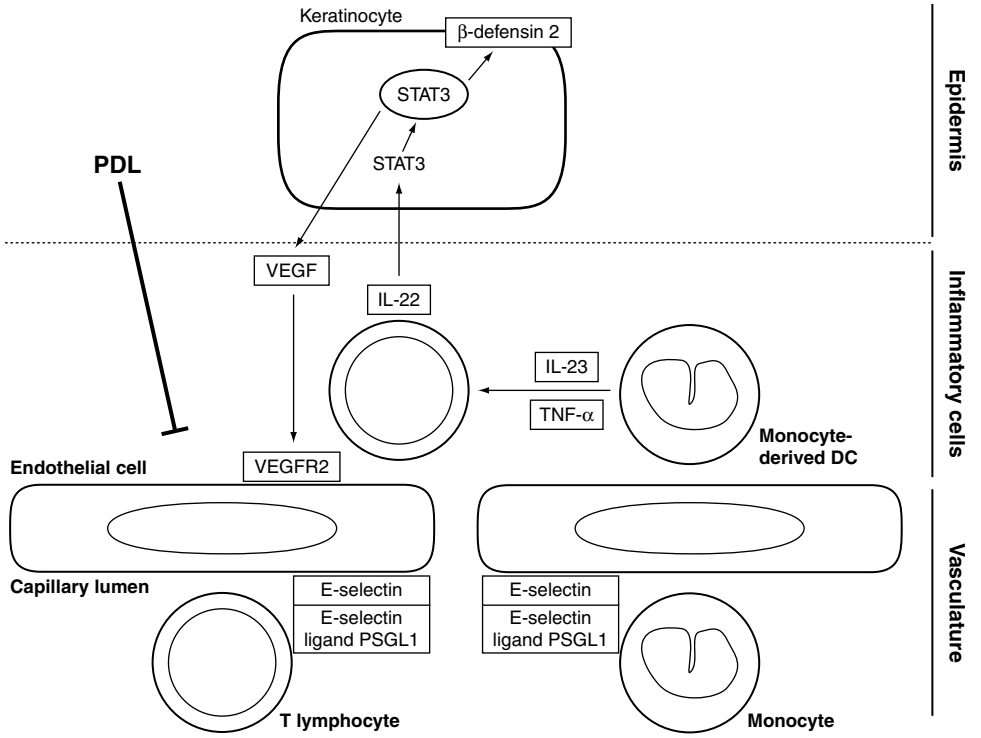

Figure 4. The 585 nm pulsed dye laser disrupts the vicious circle of psoriatic inflammation.
The dermal microvasculature contributes to the maintenance of chronic inflammation by high expression of E-selectin, a homing receptor of leukocytes, and the angiogenic VEGFR2. PDL destroys dermal blood vessels, resulting eventually in decreased expression of inflammatory components of psoriasis, such as TNF-α and IL-23p19. PSGL1: P-selectin glycoprotein ligand-1

We have chosen to focus on the epidermis in our studies, as most of NB-UVB radiation is absorbed there. However, some of the radiation might reach the dermis, and as immune cells are more sensitive for UVB effects (48, 49), dermal effects might be therapeutically relevant. Furthermore, the epidermal samples we used are still composed of different types of cells. An elegant approach would be to study cell-specific gene expression profiles, however, as in this study 3 mm biopsy samples were taken, further reduction of the studied sample size by separation of specific cell types would have lead to insufficient RNA quantities for the gene expression arrays.

For the validation of the induction of GATA3 expression by NB-UVB, the imiquimod-induced psoriasis-like mouse model was used. In spite of the striking similarities of the imiquimod-induced skin inflammation in mice with human psoriasis there are also important differences, one being that inflammation induced by imiquimod treatment was not maintained chronically in this model at that time. Accordingly, NB-UVB treatment, which requires application for several weeks before being efficacious in humans, could only be applied three times in the mouse model.

In the comparative study between the pulsed dye laser and the topical NB-UVB, limited cellular and molecular effects of NB-UVB were found, especially at mRNA level. However, clinical efficacy of NB-UVB in this study was also somewhat lower than would be expected based on published results. The lower clinical efficacy of NB-UVB in our study (52% improvement versus minimally 65-70% reported for total body irradiation) may be explained by the fact that local NB-UVB treatment was used instead of total body irradiation. It is assumed that total body UVB irradiation also exerts a systemic effect (50).

Areas for future research

Gene expression profiling gives a global picture of actual mRNA levels in tissue samples at a certain time point. Such an approach is a great instrument to generate hypotheses on cellular and molecular mechanisms. Based on gene expression profiling during NB-UVB therapy of psoriasis and on additional experiments *in vitro*, *ex vivo* and in mice we discovered and subsequently hypothesized that STAT3 phosphorylation, cytosolic innate dsRNA receptors and GATA3 are involved in the resolution of psoriatic inflammation. Future studies are necessary to further unravel the exact mechanism by which UVB affects their expression and function, and whether these molecules are suitable therapeutic targets in psoriasis, and to establish whether specific targeting of any of those molecules is sufficient to improve psoriasis.

The involvement of GATA3 in both epidermal and Th2 differentiation suggests a possible regulatory role for GATA3 in psoriasis, a disease characterized by disturbed epidermal differentiation and enhanced Th1/Th17 responses. Interestingly, polymorphisms in the GATA3 gene are described in atopic dermatitis, another chronic inflammatory skin disease with epidermal hyperplasia (51). Haploinsufficiency of the GATA3 gene causes the very rare HDR (hypoparathyroidism, sensorineural deafness and renal dysplasia) syndrome (52). Generalized psoriasis has been described in a patient with the HDR syndrome (53). Taken together, these data point out that it might be worthwhile to look for genetic variations in the GATA3 gene in patients with psoriasis.

As in our study complete epidermis was used as a basis for gene expression profiling, further studies may investigate whether cells other than keratinocytes contribute to the described expression changes. Because of the low starting dose of standard NB-UVB therapy (70% MED), we could not detect direct targets of NB-UVB in our *in vivo* study. Other experimental approaches are required in order to identify these targets. Involvement of DNA damage in anti-inflammatory NB-UVB-effects can be studied by cell-specific overexpression of DNA repair photolyases (54). With this method identification of the cell type where DNA damage has to occur for the anti-inflammatory effect of NB-UVB would be possible, e.g. in the IMQ-induced skin inflammation model in mice, which we showed to be improved by NB-UVB. Involvement of reactive oxygen species could in turn be studied by application of blockers such as N-acetylcystein.

This thesis did not address the quantitative and functional changes in epidermal sub-populations such as Langerhans cells and regulatory T cells upon NB-UVB treatment. As Langerhans cells and regulatory T cells display clear abnormalities in psoriasis, and UVB is known to affect these cells in normal skin, it is tempting to speculate that the efficacy of NB-UVB therapy in psoriasis is partly due to its effect on these cells.

As of the targeting of dermal microvasculature by the pulsed dye laser, we found that VEGFR2 and VEGFR3 were downregulated at very early time points. Later on during therapy VEGFR2 expression was induced by PDL. We speculated that this upregulation of VEGFR2 would probably counteract the therapeutic effect of PDL. However, this hypothesis should be tested in larger group of patients and also during other therapies, and correlation of the clinical response with VEGFR2 expression should be examined. Our finding that PDL treatment is effective in approximately 50% (a subgroup) of patients has also been reported by others (55). In our study, the group of PDL-responders could not be distinguished from the non-responders in terms of specific baseline expression patterns or immunohistochemical alterations induced by treatment. This may be addressed in future studies.

Besides psoriasis, NB-UVB phototherapy effectively clears a broad array of skin diseases such as atopic dermatitis, vitiligo, early-stage T cell lymphoma, chronic urticaria, lichen planus, polymorphic light eruption, seborrhoic dermatitis, pruritus and acquired perforating dermatosis (reviewed by Gambichler (56) and by Berneburg (57)). An interesting question is to which extent the mechanisms of NB-UVB action we identified in psoriasis are comparable with its effect in other skin diseases. Examination of expression changes in different target genes in these diseases during treatment would also extend the understanding of their pathophysiology.

Box 2. Outstanding research questions

- What is the primary target of UVB in the psoriatic epidermis? If it is DNA, in which cells does the UVB-induced mutated DNA result in the resolution of inflammation?
- Are reactive oxygen species and cytoprotective enzymes important for the improvement of inflammation by NB-UVB? Does the higher expression of cytoprotective enzymes in lesional psoriatic skin play a role in the efficacy of NB-UVB?
- Are STAT3, MDA5, RIG-I and GATA3 valid therapeutic targets in psoriasis? Does inhibition (or, in the case of GATA3, induction) of only one of these molecules sufficient to limit psoriatic inflammation?
- Do the migratory properties of Langerhans cells and the suppressive function of regulatory T cells change during NB-UVB therapy of psoriasis?
- Are the interferon signalling and the Th17 pathways, cytosolic RNA receptors and GATA3 involved in the pathophysiology of other skin diseases that can be treated with NB-UVB?

Concluding remarks

In conclusion, gene expression profiling of psoriatic epidermal samples before, during and after NB-UVB therapy revealed interferon signalling and Th17 (STAT3-regulated) pathways, as well as the cytosolic innate RNA receptors MDA5 and RIG-I and the transcription factor GATA3 as target molecules of the therapy that might account for the efficacy of NB-UVB in

psoriasis. These results at the same time indicate critical involvement of these pathways and molecules in the pathophysiology of psoriasis. They might be essential for the development and maintenance of the activated, regenerative epidermal phenotype which is characteristic for psoriasis.

REFERENCES

1. Hearn, R. M., *et al.* (2008). Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. *Br J Dermatol* **159**(4): 931-5.
2. Coven, T. R., *et al.* (1997). Narrowband UV-B produces superior clinical and histopathological resolution of moderate-to-severe psoriasis in patients compared with broadband UV-B. *Arch Dermatol* **133**(12): 1514-22.
3. Walters, I. B., *et al.* (1999). Suberythemogenic narrow-band UVB is markedly more effective than conventional UVB in treatment of psoriasis vulgaris. *J Am Acad Dermatol* **40**(6 Pt 1): 893-900.
4. Ibbotson, S. H., *et al.* (2004). An update and guidance on narrowband ultraviolet B phototherapy: a British Photodermatology Group Workshop Report. *Br J Dermatol* **151**(2): 283-97.
5. Carrascosa, J. M., *et al.* (2005). [Consensus document on phototherapy: PUVA therapy and narrow-band UVB therapy]. *Actas Dermosifiliogr* **96**(10): 635-58.
6. Church, D., *et al.* (2009). Lineage-specific biology revealed by a finished genome assembly of the mouse. *PLoS Biol* **7**(5): e1000112.
7. Sano, S., *et al.* (2005). Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* **11**(1): 43-9.
8. Kalali, B. N., *et al.* (2008). Double-stranded RNA induces an antiviral defense status in epidermal keratinocytes through TLR3-, PKR-, and MDA5/RIG-I-mediated differential signaling. *J Immunol* **181**(4): 2694-704.
9. Kaufman, C. K., *et al.* (2003). GATA-3: an unexpected regulator of cell lineage determination in skin. *Genes Dev* **17**(17): 2108-22.
10. McKenzie, B. S., Kastelein, R. A. and Cua, D. J. (2006). Understanding the IL-23-IL-17 immune pathway. *Trends Immunol* **27**(1): 17-23.
11. Haider, A. S., *et al.* (2008). Identification of cellular pathways of "type 1," Th17 T cells, and TNF- and inducible nitric oxide synthase-producing dendritic cells in autoimmune inflammation through pharmacogenomic study of cyclosporine A in psoriasis. *J Immunol* **180**(3): 1913-20.
12. Zaba, L. C., *et al.* (2007). Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* **204**(13): 3183-94.
13. Papp, K. A., *et al.* (2008). Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* **371**(9625): 1675-84.
14. Leonardi, C. L., *et al.* (2008). Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* **371**(9625): 1665-74.
15. Kimball, A. B., *et al.* (2008). Safety and efficacy of ABT-874, a fully human interleukin 12/23 monoclonal antibody, in the treatment of moderate to severe chronic plaque psoriasis: results of a randomized, placebo-controlled, phase 2 trial. *Arch Dermatol* **144**(2): 200-7.
16. De Sanctis, J. B., *et al.* (2009). Pharmacological modulation of Th17. *Recent Pat Inflamm Allergy Drug Discov* **3**(2): 149-56.
17. Zhang, W., *et al.* (2008). Antiapoptotic activity of autocrine interleukin-22 and therapeutic effects of interleukin-22-small interfering RNA on human lung cancer xenografts. *Clin Cancer Res* **14**(20): 6432-9.
18. Fuh, B., *et al.* (2009). LLL-3 inhibits STAT3 activity, suppresses glioblastoma cell growth and prolongs survival in a mouse glioblastoma model. *Br J Cancer* **100**(1): 106-12.
19. Song, H., *et al.* (2005). A low-molecular-weight compound discovered through virtual screening inhibits Stat3 function in breast cancer cells. *Proc Natl Acad Sci U S A* **102**: 4700–4705.

20. Schust, J., *et al.* (2006). Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem Biol* **13**: 1235–1242.
21. Lui, V., *et al.* (2007). Antiproliferative mechanisms of a transcription factor decoy targeting signal transducer and activator of transcription (STAT) 3: the role of STAT1. *Mol Pharmcol* **71**: 1435–1443
22. Boehm, A. L., *et al.* (2008). Combined targeting of epidermal growth factor receptor, signal transducer and activator of transcription-3, and Bcl-X(L) enhances antitumor effects in squamous cell carcinoma of the head and neck. *Mol Pharmacol* **73**(6): 1632-42.
23. Nestle, F. O., *et al.* (2005). Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* **202**(1): 135-43.
24. van der Fits, L., *et al.* (2004). In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *J Invest Dermatol* **122**(1): 51-60.
25. Aragane, Y., *et al.* (1997). Down-regulation of interferon gamma-activated STAT1 by UV light. *Proc Natl Acad Sci U S A* **94**(21): 11490-5.
26. Rasmussen, S., *et al.* (2009). Herpes simplex virus infection is sensed by both Toll-like receptors and retinoic acid-inducible gene- like receptors, which synergize to induce type I interferon production. *J Gen Virol* **90** (1): 74-8.
27. Kurek, D., *et al.* (2007). Transcriptome and phenotypic analysis reveals Gata3-dependent signalling pathways in murine hair follicles. *Development* **134**(2): 261-72.
28. de Guzman Strong, C., *et al.* (2006). Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3. *J Cell Biol* **175**(4): 661-70.
29. Son do, N., *et al.* (2009). Abundant expression of Kallikrein 1 gene in human keratinocytes was mediated by GATA3. *Gene* **436**(1-2): 121-7.
30. Zheng, W. and Flavell, R. A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**(4): 587-96.
31. Ouyang, W., *et al.* (1998). Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* **9**(5): 745-55.
32. Dydensborg, A., *et al.* (2009). GATA3 inhibits breast cancer growth and pulmonary breast cancer metastasis. *Oncogene* Epub ahead of print.
33. Oestreicher, J. L., *et al.* (2001). Molecular classification of psoriasis disease-associated genes through pharmacogenomic expression profiling. *Pharmacogenomics J* **1**(4): 272-87.
34. van Hamburg, J. P., *et al.* (2008). Enforced expression of GATA3 allows differentiation of IL-17-producing cells, but constrains Th17-mediated pathology. *Eur J Immunol* **38**(9): 2573-86.
35. Nair, R. P., *et al.* (2009). Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* **41**(2): 199-204.
36. Coornaert, B., Carpentier, I. and Beyaert, R. (2009). A20: central gatekeeper in inflammation and immunity. *J Biol Chem* **284**(13): 8217-21.
37. Choi, K., *et al.* (2009). Distinct biological roles for the notch ligands jagged-1 and jagged-2. *J Biol Chem* **284**(26): 17766-74.
38. Fuchs, E. and Horsley, V. (2008). More than one way to skin. *Genes and Development* **22**(8): 976-85.
39. Schuff, M., *et al.* (2007). FoxN3 is required for craniofacial and eye development of Xenopus laevis. *Dev Dyn.* **236**(1): 226-39.
40. Gross, D., van den Heuvel, A. and Birnbaum, M. (2008). The role of FoxO in the regulation of metabolism. *Oncogene* **27**(16): 2320-36.
41. Koon, H., *et al.* (2007). FOXP1: a potential therapeutic target in cancer. *Expert Opin Ther Targets.* **11**(7): 955-65.
42. Candi, E., *et al.* (2006). p63 is upstream of IKK alpha in epidermal development. *J Cell Sci* **119**(Pt 22): 4617-22.
43. Katoh, I., *et al.* (2000). p51A (TAp63gamma), a p53 homolog, accumulates in response to DNA damage for cell regulation. *Oncogene* **19**(27): 3126-30.
44. Vilgelm, A., El-Rifai, W. and Zaika, A. (2008). Therapeutic prospects for p73 and p63: rising from the shadow of p53. *Drug Resist Updat* **11**(4-5): 152-63. .
45. Iljin, K., *et al.* (2001). VEGFR3 gene structure, regulatory region, and sequence polymorphisms. *Faseb J* **15**(6): 1028-36.

46. Henno, A., *et al.* (2009). Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis. *Br J Dermatol* **160**(3): 581-90.
47. de Leeuw, J., *et al.* (2006). Concomitant treatment of psoriasis of the hands and feet with pulsed dye laser and topical calcipotriol, salicylic acid, or both: a prospective open study in 41 patients. *J Am Acad Dermatol* **54**(2): 266-71.
48. Aufiero, B. M., *et al.* (2006). Narrow-band UVB induces apoptosis in human keratinocytes. *J Photochem Photobiol B* **82**(2): 132-9.
49. Ozawa, M., *et al.* (1999). 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. *J Exp Med* **189**(4): 711-8.
50. Schwarz, T. (2005). Mechanisms of UV-induced immunosuppression. *Keio J Med* **54**(4): 165-71.
51. Arshad, S. H., *et al.* (2008). Polymorphisms in the interleukin 13 and GATA binding protein 3 genes and the development of eczema during childhood. *Br J Dermatol* **158**(6): 1315-22.
52. Van Esch, H., *et al.* (2000). GATA3 haplo-insufficiency causes human HDR syndrome. *Nature* **406**(6794): 419-22.
53. Aksoylar, S., *et al.* (2004). HDR (hypoparathyroidism, sensorineural deafness, renal dysplasia) syndrome presenting with hypocalcemia-induced generalized psoriasis. *J Pediatr Endocrinol Metab* **17**(7): 1031-4.
54. Jans, J., *et al.* (2006). Differential role of basal keratinocytes in UV-induced immunosuppression and skin cancer. *Mol Cell Biol* **26**(22): 8515-26.
55. Taibjee, S. M., *et al.* (2005). Controlled study of excimer and pulsed dye lasers in the treatment of psoriasis. *Br J Dermatol* **153**(5): 960-6.
56. Gambichler, T., *et al.* (2005). Narrowband UVB phototherapy in skin conditions beyond psoriasis. *J Am Acad Dermatol* **52**(4): 660-70.
57. Berneburg, M., Rocken, M. and Benedix, F. (2005). Phototherapy with narrowband vs broadband UVB. *Acta Derm Venereol* **85**(2): 98-108.

SUMMARY

Psoriasis is a chronic inflammatory skin disease, characterized by elevated, red, scaly plaques at specific body sites. Psoriasis affects 2-3% of the Western population. Treatment of patients with psoriasis is often challenging as long-term use of most available therapies is limited by their side-effects. The aim of this thesis was to illuminate the molecular effects of narrow-band (NB-) UVB phototherapy, a highly effective systemic treatment modality in psoriasis, in order to clarify the mechanism of the resolution of psoriatic skin inflammation and the immune pathogenesis of the disease. In addition, by identification of pathways that are responsible for the effectiveness of NB-UVB we aimed for detecting novel potential therapeutic target molecules for the treatment of psoriasis. For this, *in vivo* and in vitro approaches were applied.

First, patients with psoriasis undergoing standard NB-UVB phototherapy were recruited, and skin biopsy samples were collected before, during and after the therapy both from lesional and non-lesional skin. To analyze immediate effects of NB-UVB, samples were collected six hours after the first treatment session. To be able to correlate the results with the clinical severity, additional samples were collected when PASI scores reached 50% of the baseline. The epidermis was separated from the dermis, RNA was extracted, and gene expression profiling was performed. **Chapter 2** describes the global results of this *in vivo* study. NB-UVB phototherapy significantly downregulated Th17 and interferon signalling pathways in psoriatic epidermis. IL-22, a T-cell-derived Th17 cytokine that signals via phosphorylation of signal transducer and activator of transcription (STAT) 3, induces the expression of β-defensin 2, S100A7 and S100A9, lipocalin 2 and the IL-20 receptor B. All these molecules were suppressed by NB-UVB therapy. We referred to this pathway as Th17 pathway because of this IL-22-dependent regulation. In addition, pathways that are known therapeutic targets in psoriasis, such as the glucocorticoid, vitamin D, peroxisome proliferator-activated receptor and IL-4 signalling were affected by phototherapy. The response to NB-UVB six hours after the first treatment session involved different, and much fewer genes in lesional skin than in non-lesional skin. Direct inhibition of the Th17 pathway by UVB was confirmed in an *ex vivo* organ culture system by reduced STAT3 phosphorylation and β-defensin-2 production.

Since our group previously demonstrated high expression of innate cytosolic receptors for double-stranded RNA (dsRNA) in psoriatic lesions, we used the microarray data to specifically search for this interferon-inducible pathway. As described in **Chapter 3**, expression of the dsRNA receptors melanoma differentiation associated gene (MDA) 5 and retinoic acid-inducible gene (RIG)-I were higher in lesional epidermis than in non-lesional samples, and NB-UVB therapy downregulated the expression of these two molecules in lesional skin, leaving the expression of the other three dsRNA receptors (TLR3, PKR and LGP2) unaffected. The ligand of RIG-I and MDA5 is dsRNA with or without a 5' triphosphate end, which can be a byproduct of viral replication or derived from necrotic host cells. Activation of these receptors leads to type I interferon production. Type I interferons play essential roles in the initiation of psoriatic plaque formation. Furthermore, type I IFN signalling is activated in psoriatic plaques. In psoriasis, high epidermal expression of MDA5 and RIG-I is found in keratinocytes. To investigate whether downregulation of MDA5 and RIG-I by NB-UVB is only an epiphenomenon of resolving psoriasis or is a direct effect of UV, primary keratinocytes

were cultured with IFN-α or IFN-γ to upregulate dsRNA receptors (to mimic the psoriatic situation). Subsequent NB-UVB treatment suppressed the expression of all activating dsRNA receptors already within 2 hours after irradiation, and not affected the negative regulator LGP2. Thus, NB-UVB irradiation crippled the local innate inflammatory response induced by dsRNA. Since high expression of MDA5 and RIG-I possibly contributes to the maintenance of chronic inflammation in psoriasis, suppression of dsRNA receptor expression might play a role in the clinical efficacy of NB-UVB phototherapy in psoriasis.

We detected low expression of the transcription factor GATA3 in lesional samples before treatment as compared to non-lesional samples, by microarray analysis. GATA3 has regulatory roles during the differentiation of both epidermal keratinocytes and T helper lymphocytes. In the skin GATA3 regulates epidermal barrier formation and desquamation. In T lymphocytes it stimulates the development of the T helper 2 phenotype and at the same time inhibits Th1 differentiation. GATA3 expression was induced by NB-UVB, inversely correlating with the clinical improvement of psoriasis. The correlation of GATA3 expression and skin inflammation is further analysed in **Chapter 4**. The imiquimod (IMQ)-induced murine skin inflammation model was used. After demonstrating the beneficial effect of NB-UVB in IMQ-induced skin inflammation in mice, similarly to its effectiveness in human psoriasis, GATA3 expression was determined in the mouse skin at mRNA and protein levels. GATA3 expression was lower in IMQ-treated skin than in skin treated with control cream. Additional NB-UVB treatment induced GATA3 expression. In addition, low GATA3 expression was detected in wounded mouse skin, demonstrating the association of low GATA3 expression with the regenerative epidermal phenotype. Epidermal GATA3 expression was not suppressed by IFN-α, IFN-γ or IL-22 in a human ex vivo skin culture system, suggesting that low GATA3 expression in psoriasis is not secondary to the presence of immune cell-derived inflammatory mediators.

Most of NB-UVB radiation is absorbed by the epidermis, which prompted us to focus on the epidermis in all the studies discussed above. However, psoriatic inflammation cannot be maintained without cells and molecules that are located in the dermis. Not only immunocytes, such as plasmacytoid and myeloid dendritic cells and T lymphocytes are critical for the formation of psoriasis plaques, but also vascular factors play a role in the inflammatory process. Although vascular remodelling is a hallmark of psoriasis, anti-vascular strategies for treating these conditions receive little attention. The pulsed dye laser (PDL) selectively destroys the dermal microvasculature which can result in partial or complete disappearance of psoriasis plaques. In **Chapter 5** cellular and molecular effects of the PDL are compared with those of the epidermis-targeted NB-UVB. At the end of the 14-week-long treatment period no significant differences were detected between the cellular and molecular effects of the two therapies, and the clinical efficacy was also comparable. However, PDL suppressed the expression of the receptors for vascular endothelial growth factor 2 and 3 already three hours after treatment, and that of E-selectin after 24 hours. After 2 PDL treatments IL-23 and TNF-α mRNA and E-selectin protein expression were significantly reduced. These early effect of PDL might contribute to its efficacy in psoriasis.

In conclusion, in this thesis novel mechanisms of NB-UVB action in psoriasis are revealed. We have shown that NB-UVB therapy affects 1) STAT3 and the Th17 pathway which in the epidermis is represented by antimicrobial peptides; 2) the interferon-induced innate dsRNA receptors MDA5 and RIG-I, and 3) the transcription factor GATA3 with involvement in

the proliferation and differentiation of epidermal keratinocytes. Our findings indicate that these molecules and pathways are critical in the pathophysiology of psoriasis and might represent novel therapeutic targets. Further studies need to investigate the possibilities of specifically modulate these molecules and to determine their usefulness as therapeutic targets in psoriasis.

SAMENVATTING

Psoriasis, een chronische ontsteking van de huid wordt gekenmerkt door erythemateuze schilferende plaques op specifieke lichaamsdelen. Twee tot drie procent van de westerse populatie lijdt aan psoriasis. De behandeling van psoriasispatiënten is uitdagend want langetermijngebruik van de meeste effectieve therapieën wordt beperkt door bijwerkingen.

Het doel van dit proefschrift was om de moleculaire effecten van smalband UVB (SB-UVB) therapie, een zeer effectieve systemische antipsoriatische therapie, te identificeren om inzicht te krijgen in de mechanismen van de opklaring van psoriatische huidontsteking en in de immunopathogenese van psoriasis. Een verder doel was om moleculaire pathways te detecteren die verantwoordelijk zijn voor de verbetering van de psoriatische ontsteking en daardoor targets van toekomstige therapieën kunnen zijn. *In vivo* en *in vitro* methoden zijn toegepast.

Ten eerste zijn er huidbiopten genomen van aangedane en niet aangedane huid van psoriasispatiënten voor, tijdens en na SB-UVB therapie. Om de directe targets van SB-UVB te kunnen identificeren zijn er biopten genomen 6 uur na de eerste UVB belichting. Om een verband te kunnen leggen tussen de moleculaire resultaten en het klinisch effect van de behandeling zijn er verdere biopten verzameld enkele weken na de start van de behandeling, toen de PASI scores 50 % van de baseline hadden bereikt. Epidermis werd gescheiden van de dermis, epidermale RNA werd geïsoleerd, en de globale genexpressie werd geanalyseerd met behulp van microarrays. De resultaten hiervan zijn beschreven in **Hoofdstuk 2**. Th17 en interferon signalering pathways werden geremd in de psoriatische epidermis door SB-UVB. IL-22 is een van de zogenaamde Th17 cytokinen. IL-22 stimuleert de fosforylatie van het molecuul signal transducer and activator of transcription (STAT) 3, en dit leidt tot verhoogde expressie van o.a. β-defensine 2, S100A7, S100A9, lipocalin 2 en de IL-20 receptor B. Al deze moleculen werden geremd door NB-UVB fototherapie. Dit pathway werd in dit proefschrift Th17 pathway genoemd, vanwege de inductie hiervan door IL-22. Ook pathways die bekende therapeutische targets zijn in psoriasis werden beïnvloed door SB-UVB therapie, onder andere glucocorticoid, vitamine D, peroxisome proliferator-activated receptor en IL-4 signalering pathways. In aangedane en niet aangedane huid van de patiënten werden volkomen verschillende genen beïnvloed door SB-UVB, wat het belang benadrukt van *in vivo* studies in ontstoken huid. Expressie van weinig genen veranderde 6 uur na de eerste belichting in aangedane huid. Een direct effect van SB-UVB op het Th17 pathway werd aangetoond in *ex vivo* gekweekte normale huidbiopten, waar SB-UVB de fosforylatie van STAT3 en de mRNA expressie van β-defensine 2 heeft geremd.

Twee type I interferongeïnduceerde dubbelstrengs RNA (dsRNA) receptoren, melanoma differentiation-associated gene (MDA) 5 en retinoic acid-inducible gene (RIG)-I werden ook geremd door SB-UVB in de psoriatische epidermis. In **Hoofdstuk 3** hebben wij verder onderzocht welke rol deze moleculen in het antipsoriatische effect van SB-UVB zouden kunnen spelen. MDA5 en RIG-I binden aan dsRNA die afkomstig kan zijn uit virussen of uit necrotische cellen van de host. Activatie van MDA5 en RIG-I leidt tot de synthese van type I interferonen, die belangrijk zijn in de beginfase van de ontwikkeling van psoriatische plaques. Type I interferon signalering is geactiveerd in aangedane psoriatische epidermis. Wij hebben eerder al aangetoond dat deze receptoren hoog tot expressie kwamen in psoriasis, en dat ze

vooral in epidermale keratinocyten werden gelokaliseerd. Om verder te onderzoeken of de lage dsRNA receptor expressie na SB-UVB therapie een direct effect is van UVB of alleen een gevolg van de verbetering van de ontsteking, hebben we kweek van primaire humane keratinocyten gebruikt. Expressie van dsRNA receptoren werd geïnduceerd (om de situatie in psoriatische huid na te bootsen) door toevoeging van IFN-α of IFN-γ. In cellen die hiernaast ook zijn belicht met SB-UVB ging de expressie van de activerende dsRNA receptoren TLR3, MDA5, RIG-I en PKR niet omhoog, maar die van de negatieve regulator LGP2 wel. SB-UVB heeft dus de lokale response op dsRNA verlamd. De hoge expressie van MDA5 en RIG-I draagt mogelijk bij aan het in stand houden van de chronische ontsteking in psoriasis, en de remming van dit pathway kan een rol spelen in de klinische effectiviteit van SB-UVB in psoriasis.

Door middel van microarray onderzoek lage expressie van de transcriptiefactor GATA3 werd gedetecteerd in aangedane huid van de patiënten, en expressie van deze transcriptiefactor werd gestimuleerd door SB-UVB therapie. GATA3 is betrokken in de differentiatie van zowel keratinocyten als T lymfocyten. In de huid reguleert GATA3 de vorming van de epidermale barriere. In T lymfocyten stimuleert GATA3 de vorming van Th2 cellen en remt die van Th1 cellen. In **Hoofdstuk 4** is de analyse van GATA3 expressie in verschillende ontstekingsmodellen beschreven. Hier werd gebruik gemaakt van het imiquimod (IMQ)-geïnduceerde huidontstekingsmodel in de muis. Eerst hebben wij aangetoond dat deze huidontsteking, die in veel opzichten op psoriasis lijkt, milder wordt onder invloed van SB-UVB. In de muis, net als in de mens, was GATA3 expressie lager in ontstoken huid dan in niet ontstoken huid, en SB-UVB heeft de expressie omhoog gemoduleerd in ontstoken huid. Ook in een wond die op de muizenrug is ontstaan was GATA3 expressie lager dan in gezonde huid, wat bevestigt dat het regeneratieve huidfenotype, ook kenmerkend voor psoriasis, samengaat met lage GATA3 expressie. IFN-α, IFN-γ en IL-22 hadden geen remmende effect op epidermale GATA3 expressie in gekweekte normale humane huidbiopten, wat erop wijst dat de lage GATA3 expressie in psoriasis niet het gevolg is van de aanwezigheid van deze cytokinen.

De voorgaande hoofdstukken waren vooral gericht op de epidermis, omdat SB-UVB grotendeels hier wordt geabsorbeerd. Dermale huidcomponenten zijn echter ook onmisbaar voor het in stand houden van de psoriatische huidontsteking. Cellen van het immuunsysteem spelen essentiële rollen in de formatie van psoriatische plaques, maar vasculaire factoren zijn ook betrokken in het ontstekingsproces. Hoewel vasculaire remodelling een kenmerk van psoriasis is, is er weinig aandacht voor therapieën die specifiek de bloedvaten beïnvloeden. Dermale capillairen kunnen selectief vernietigd worden met de pulsed dye laser (PDL), en dit kan leiden tot gedeeltelijke of complete genezing van psoriatische plaques. In **Hoofdstuk 5** zijn de cellulaire en moleculaire effecten van PDL vergeleken met die van SB-UVB, een "epidermale" behandeling. Na 14 weken behandeling waren er geen significante verschillen tussen de moleculaire effecten van de twee therapieën, dus het aangrijpingspunt van de behandeling was op dit tijdstip niet meer doorslaggevend voor de expressie van markers van psoriasis. Klinische effectiviteit van beide behandelingen waren ook vergelijkbaar. De bloedvatspecifieke moleculen VEGFR2 en E-selectine hadden een lage expressie binnen de eerste 24 uur na PDL behandeling. Na 2 behandelingen met PDL ging de expressie van IL-23 en TNF-α omlaag. Deze effecten dragen waarschijnlijk bij aan de effectiviteit van PDL in psoriasis.

Ter conclusie: in dit proefschrift zijn nieuwe mechanismen van SB-UVB effectiviteit in psoriasis in kaart gebracht. 1) STAT3 en de Th17 pathway, die in de epidermis vertegenwoordigd wordt door antimicrobiële eiwitten; 2) de interferongeïnduceerde cytosolische dsRNA receptoren MDA5 en RIG-I; en 3) GATA3, een transcriptiefactor met regulerende rol in de proliferatie en differentiatie van de epidermis. Onze resultaten wijzen erop dat deze moleculen essentiële rollen spelen in de pathosfysiologie van psoriasis en potentiële therapeutische targets zijn. Verdere studies zijn nodig om mogelijkheden te testen voor het beïnvloeden van deze moleculen en hun nuttigheid als therapeutische targets in psoriasis vast te stellen.

ABBREVIATIONS

ABL1	Abelson murine leukemia viral (v-abl) oncogene homolog 1
ANOVA	analysis of variance
BB-UVB	broad-band UVB
BrdU	5-bromo-2-deoxyuridine
CCND1	cyclin D1
CHS	contact hypersensitivity
COX2	cyclo-oxygenase 2
CPD	cyclobutane pyrimidine dimer
CRIP1	cysteine-rich intestinal protein 1
CYP1B1	cytochrome P450, subfamily I, polypeptide 1
DC	dendritic cell
dsRNA	double-stranded RNA
EGF	epidermal growth factor
FOX	forkhead box
GR-1	granulocyte-differentiation antigen-1 (Ly-6G)
hBD2	human beta-defensin 2
HDR	hypoparathyroidism, sensorineural deafness and renal dysplasia
IFIH1/MDA5	interferon induced with helicase C domain 1/ melanoma differentiation associated gene 5
IFI27	interferon, alpha-inducible protein 27
IFN	interferon
IGF1	insulin-like growth factor
IGFBP7	insulin-like growth factor binding protein 7
IKK1	IκB kinase 1
IL	interleukin
IMQ	imiquimod
INSIG1	insulin-induced gene 1
KLF13	Kruppel-like factor 13
KRT	keratin
LCE	late cornified envelope
LFA3	lymphocyte function-associated antigen 3
LGP2	laboratory of genetics and physiology-2
MAPK	mitogen-activated protein kinase
MDA5	melanoma differentiation associated gene 5
MED	minimal erythema dose
MHC	major histocompatibility complex
NB-UVB	narrow-band UVB
NF-κB	nuclear factor κB
NK	natural killer
OAS1	2’,5’-oligoadenylate synthase 1
PASI	Psoriasis Area and Severity Index
PBMC	peripheral blood mononuclear cells

pDC	plasmacytoid dendritic cell
PDL	pulsed dye laser
PFA	paraformaldehyde
PGA	Physicians' Global Assessment
PGE2	prostaglandin E2
PKR	double-stranded RNA-dependent protein kinase
PPAR	peroxisome proliferator-activated receptor
PRR	pattern recognition receptor
RANKL	receptor activator of NF-κB ligand
RIG-I	retinoic acid-inducible gene-I
RLH	RIG-I-like helicase
ROS	reactive oxygen species
RT-PCR	real time polymerase chain reaction
SD	standard deviation
SEM	standard error of the mean
SNAI2	SNAIL homolog 2
STAT3	signal transducer and activator of transcription 3
STEAP4	six-transmembrane epithelial antigen of prostate 4
TFAP2A	transcription factor AP2 alpha
TGF-β1	transforming growth factor β1
TGK	keratinocyte transglutaminase
Th	T helper
TLR	Toll-like receptor
TNF	tumor necrosis factor
TNFAIP3	tumor necrosis factor, alpha-induced protein 3
UCA	urocanic acid
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
vWf	von Willebrand factor

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- Ruth die mij gedurende de laatste jaren veel heeft geholpen
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- De arts-assistenten van de afdeling Dermatologie
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- Tar, Wendy en Marcia voor hun hulp bij het maken van het boekje
- Mijn familie waarvoor één promotie al voldoende was
- Eli en Geerteke voor hun steun en hulp, Jilt voor zijn geduld.

CURRICULUM VITAE

3rd November 1975	Born in Budapest, Hungary
1994 - 2001	M.D. degree aquired at the Semmelweis University Budapest, General Medical Faculty *Doctoral thesis:* • Clinical follow-up of patients with bullous pemphigoid. Supervisor: Prof.Dr. Sarolta Kárpáti *Internships abroad, clinical experience:* • ERASMUS scholarship for 6-month internship at the Albert-Ludwigs-University of Freiburg, Germany: internal medicine, surgery, dermatology (2 months each, February - August 2001) • Obstetrics and gynaecology; Heinrich-Heine-University of Düsseldorf, Germany (August 2000) • Part-time paramedic at the Hungarian Ambulance Service (2000 - 2001) • Guest volunteer in Coleg Elidyr, Wales, UK (April - December 1999)
2001 - 2004	PhD research ”Mutation analysis and gene expression studies in Darier’s disease and Hailey-Hailey disease” (Supervised by Prof. Dr. Sarolta Kárpáti), Department of Dermatology and Venereology, Semmelweis University Budapest, Hungary. Defense of the thesis: 23rd May 2006. *Courses followed:* • Basics of human molecular genetics (2001/02) • Molecular biology of hereditary skin diseases (2002) • Methodology and ethics of medical research (2002/03) *September 2003 - September 2004* Research scholar Dept. of Dermatology University of California, San Francisco, USA Supervisor: Theodora Mauro, M.D. Main projects: Differentiation and cell adhesion in Hailey-Hailey disease
2005 - 2009	PhD research ”Molecular targets of narrow-band UVB phototherapy in psoriasis” (Supervised by Prof.Dr. Errol P. Prens, Prof.Dr. Jon D. Laman, Dr. Leslie van der Fits), Departments of Dermatology and Immunology, Erasmus University Medical Center, The Netherlands
2008 -	Residency in dermatology, Department of Dermatology, Erasmus University Medical Center, Rotterdam, The Netherlands

LIST OF PUBLICATIONS

E. Rácz, D. Kurek, M. Kant, E. Florencia S. Mourits, D. De Ridder, J.D. Laman, L. van der Fits, E.P. Prens. GATA3 expression is reduced in psoriasis and under conditions of epidermal regeneration, and is induced by narrow-band UVB. *Submitted for publication.*

E. Rácz, E.P. Prens. Molecular pathophysiology of psoriasis and molecular targets of anti-psoriatic therapy. *Expert Reviews Molecular Medicine, accepted.*

E. Rácz, J. de Leeuw, E.M. Baerveldt, M. Kant, H.A.M. Neumann, L. van der Fits, E.P. Prens. Cellular and molecular effects of pulsed dye laser and local narrow-band UVB therapy in psoriasis. *Submitted for publication.*

E. Rácz, E.P. Prens, M. Kant, D. de Ridder, D. Kurek, J.D. Laman, F.J.T. Staal, L. van der Fits. Narrow-band UVB phototherapy inhibits epidermal interferon and Th17 pathways in patients with psoriasis. *Submitted for publication.*

E. Rácz, E.P. Prens, M. Kant, E. Florencia, J.D. Laman, D. de Ridder, L. van der Fits. Narrow-band UVB strongly inhibits expression of activating innate cytosolic RNA receptors: in keratinocytes *in vitro* and in psoriatic skin. *Submitted for publication.*

K. Aberg, **E. Rácz**, M. Behne, T. Mauro. Involucrin expression is decreased in Hailey-Hailey keratinocytes due to increased involucrin mRNA degradation. *J Invest Dermatol. 2007; 127: 1973-9.*

E. Rácz, Z. Kornsée, M. Csikós, M. Dobos, P. Salacz, S. Kárpáti. Darier's Disease Associated with Cutis Verticis Gyrata, Hyperprolactinaemia and Depressive Disorder. *Acta Dermato-Venereologica 2006; 86: 59-60.*

E. Rácz, M. Csikos, S. Karpati. Novel mutations in the *ATP2C1* gene in two patients with Hailey-Hailey disease. *Clin Exp Dermatol. 2005; 30: 575-7.*

E. Rácz, M. Csikós, R. Benko, Z. Kornseé, S. Kárpáti. Three novel mutations in the *ATP2A2* gene in Hungarian families with Darier's disease, including a novel splice site generating intronic nucleotide change. *J Dermatol Sci. 2005; 38: 231-4.*

E. Rácz, M. Csikos, Z. Kornsee, A. Horvath, S. Karpati. Identification of mutations in the *ATP2A2* gene in patients with Darier's disease from Hungary. *Exp Dermatol. 2004; 13: 396-9.*

M. Csikos, P. Hollo, K. Becker, **E. Rácz**, A. Horvath, S. Karpati. Novel N160I Mutation of Keratin 9 in a Large Pedigree from Hungary with Epidermolytic Palmoplantar Keratoderma. *Acta Derm Venereol. 2003; 83: 303-5.*

E. Rácz, J. de Leeuw, R. van Lingen, A. van Tuyll van Serooskerken, H. Both, E.P. Prens, L. van der Fits. De effecten van de pulsed-dye laser (PDL) in vergelijking met UVB-TL-01-behandeling in gelokaliseerde chronische plaque psoriasis. *Nederlands Tijdschrift voor Dermatologie en Venereologie 2006; 16: 11-3. (Dutch).*

M. Csikos, K. Becker, **E. Rácz,** A. Bona, R. Benko, A. Czippan, M. Katona, L. Bruckner-Truderman, S. Karpati, A. Horvath. Molecular genetic analysis of herediter epidermolysis bullosa. *Borgyogyaszati es Venereologiai Szemle 2004; 80: 195-202. (Hungarian).*

PHD PORTFOLIO SUMMARY

Summary of PhD training and teaching activities

Name PhD student: Emőke Rácz	PhD period: 1 jan 2005 – 30 jun 2009
Erasmus MC Department: Dermatology, Immunology	Promotor(s): Prof.Dr. E.P. Prens, Prof.Dr. J.D. Laman
Research School: Molecular Medicine	Supervisor: Prof.Dr. E.P. Prens, Prof.Dr. J.D. Laman, Dr. L. van der Fits

1. PhD training

	Year	Workload (Hours/ ECTS)
General academic skills		
- Biomedical English Writing and Communication	2006 - 2007	30 hours
- Research Integrity	2007	9 hours
Research skills		
- Statistics (Classical Methods of Data Analysis, NIHES)	2006	3 weeks
- Methodology: Biomedical research techiques IV.	2005	40 hours
- Research management for PhD students and postdocs (NIBI)	2007	20 hours
In-depth courses (e.g. Research school, Medical Training)		
- Molecular Immunology (Molmed)	2005	7 days
Presentations		
Poster		
- In vitro irradiation of keratinocytes with narrow-band UVB light inhibits the Toll-like receptor 3-induced production of inflammatory mediators. ESDR 2005, Tübingen, Germany.	2005	
- A comparative study on the effect of treatment with 585 nm Pulsed Dye Laser (PDL) and with UVB-TL-01 in plaque-type psoriasis. Psoriasis from Gene to Clinic, London, UK.	2005	
- A comparative study on the effects of the 585 nm Pulsed Dye Laser and narrow-band UVB in plaque-type psoriasis. ESDR 2006, Paris, France.	2006	
- Narrow-band UVB phototherapy for psoriasis: Normalization of epidermal gene expression and differential responses in lesional versus non-lesional skin. ESDR 2007, Zürich, Switzerland.	2007	

Oral

- Effecten van smalband UVB (TL-01) belichting op het inflammatoire fenotype van keratinocyten. Jaarvergadering Nederlandse Vereniging voor Experimentele Dermatologie. Lunteren. 26-27 januari 2006. 2006
- De effecten van de pulsed-dye laser (PDL) in vergelijking met UVB-TL-01-behandeling in gelokaliseerde chronische plaque psoriasis. 309de Wetenschappelijke Vergadering van de Nederlandse Vereniging voor Dermatologie en Venereologie, 10 februari 2006, Rotterdam. 2006
- Effecten van TL-01 fototherapie op epidermale genexpressie in psoriasis. Jaarvergadering NVED, Lunteren 2007. 2007
- Receptoren voor virale dubbelstrengs RNA: target moleculen van smalband UVB fototherapie in psoriasis?Jaarvergadering NVED, Lunteren 2008. 2008

International conferences

- 35th Annual ESDR Meeting, Tübingen, Germany. 2005
- 4th International Congress, Psoriasis from Gene to Clinic, London, UK. 2005
- 36th Annual ESDR Meeting, Paris, France.
- 37th Annual ESDR Meeting, Zürich, Zwitserland. 2006
  2007

Seminars and workshops

- Voorjaarsvergadering Nederlandse Vereniging voor Immunologie.	2005	2 days
- "What is the immunological risk associated with UV exposure"	2005	8 hours
- Najaarsvergadering Nederlandse Vereniging voor Immunologie.	2006	2 days
- 10th Molecular Medicine Day, Rotterdam.	2006	8 hours
- Voorjaarsvergadering Nederlandse Vereniging voor Immunologie.	2006	2 days
- Symposium 'Health and Evolution'. Rotterdam.	2006	2 days
- NWO Talentendag: Onderhandelen, Subsidie aanvragen.	2006	8 hours
- Laser cursus (Molecular Medicine).	2007	8 hours
- Najaarsvergadering Nederlandse Vereniging voor Immunologie	2006 2006	8 hours 2 days
- 11th Molecular Medicine Day, Rotterdam.	2007	8 hours

Didactic skills

- Teach the teacher, preclinical	2007	16 hours

Other

2. Teaching activities

	Year	**Workload (Hours/ ECTS)**
Lecturing		
Supervising practicals and excursions		
Supervising Master's theses		
- Ewout Baerveldt, 7 aug 2006 - 20 april 2007. Histological examination of the effects of PDL in psoriasis.	2006-07	
Other		
- Immunological case discussions for 2nd year mediacal students	2005/06 2006/07 2007/08	24 hours 24 hours 24 hours

APPENDIX

Chapter 1: Figure 1

Figure 1. Clinical appearance of psoriatic plaques.

Chapter 2: Figure 1

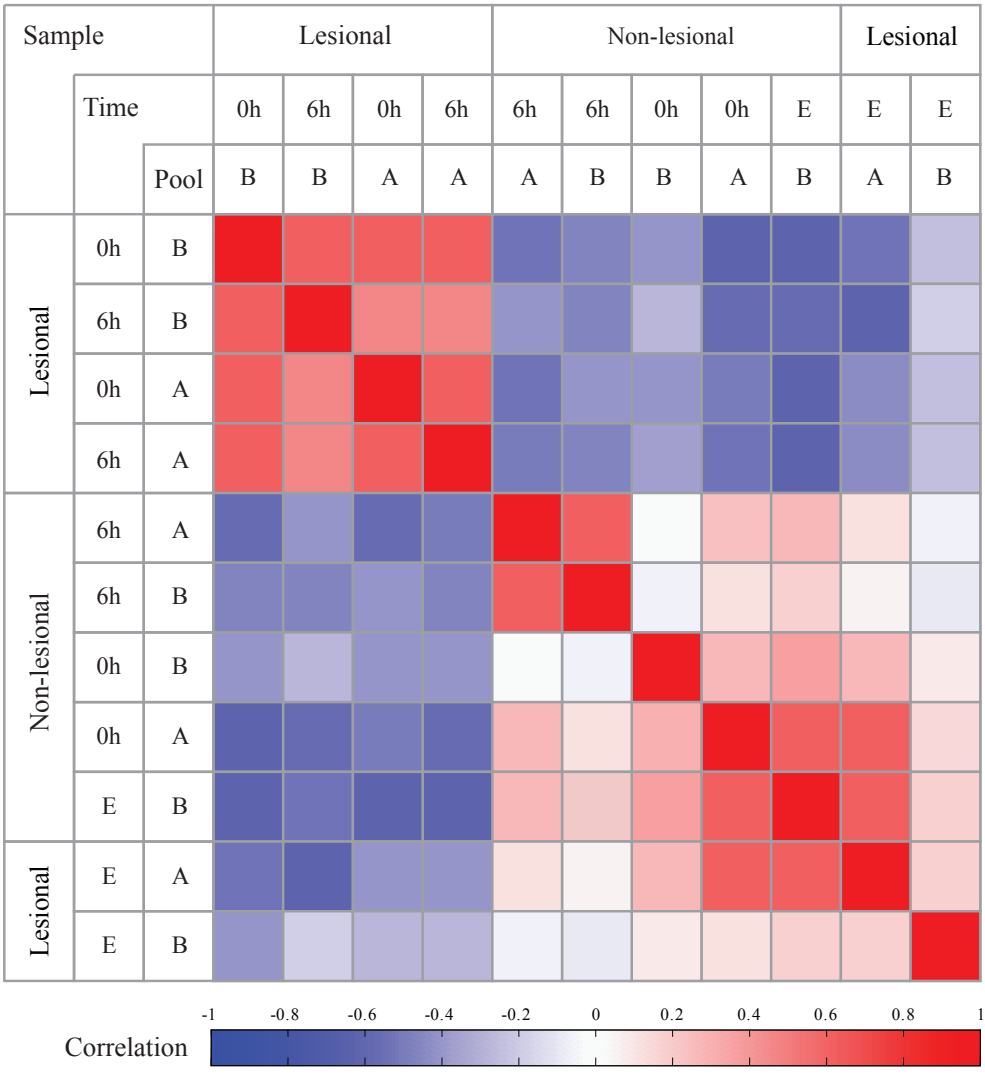


Figure 1. Correlation view of expression profiles of the different RNA pools.
Red squares indicate positive pairwise correlations and blue squares indicate negative pairwise correlations. L: lesional samples, N: non-lesional samples; 0 h: sample taken before the first irradiation, 6 h: sample taken 6 h after the first irradiation, E: sample taken after completion of NB-UVB therapy; A, B: patient pools.

Chapter 2: Figure 2

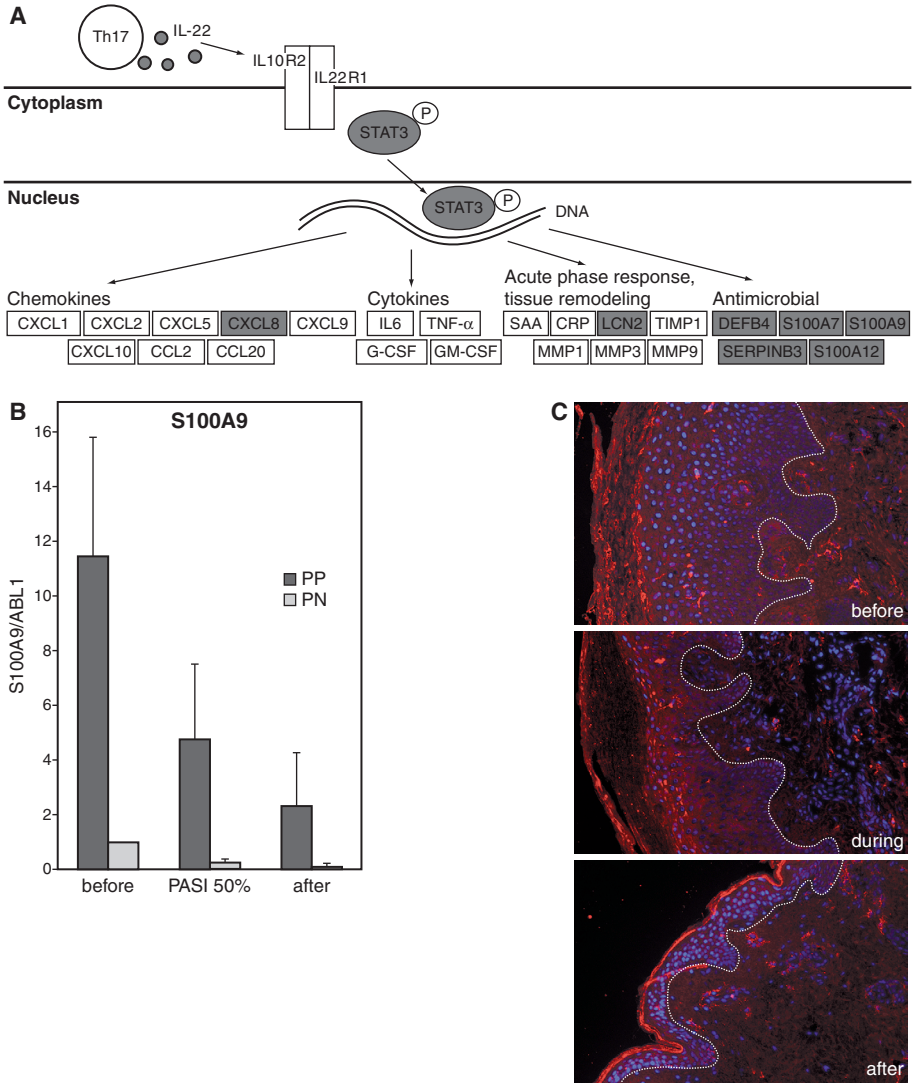


Figure 2. NB-UVB therapy suppresses the Th17 pathway.
A. Th17 pathway. Grey indicates gene downregulation. **B.** S100A9 expression measured by RT-PCR. PP: lesional, PN: non-lesional samples; 50%: sample taken at 50% PASI score reduction. Error bars indicate SEM, n=4 representative patients. **C.** β-defensin-2 in psoriatic skin samples during NB-UVB therapy. G-CSF: granulocyte colony-stimulating factor, GM-CSF: granulocyte macrophage colony-stimulating factor, SAA: serum amyloid A, CRP: C-reactive protein, LCN2: lipocalin 2, TIMP1: TIMP metallopeptidase inhibitor 1, MMP1: matrix metalloprotease 1, DEFB4: β-defensin 2, SERPINB3: serine proteinase inhibitor, clade B, member 3.

Chapter 2: Figure 3

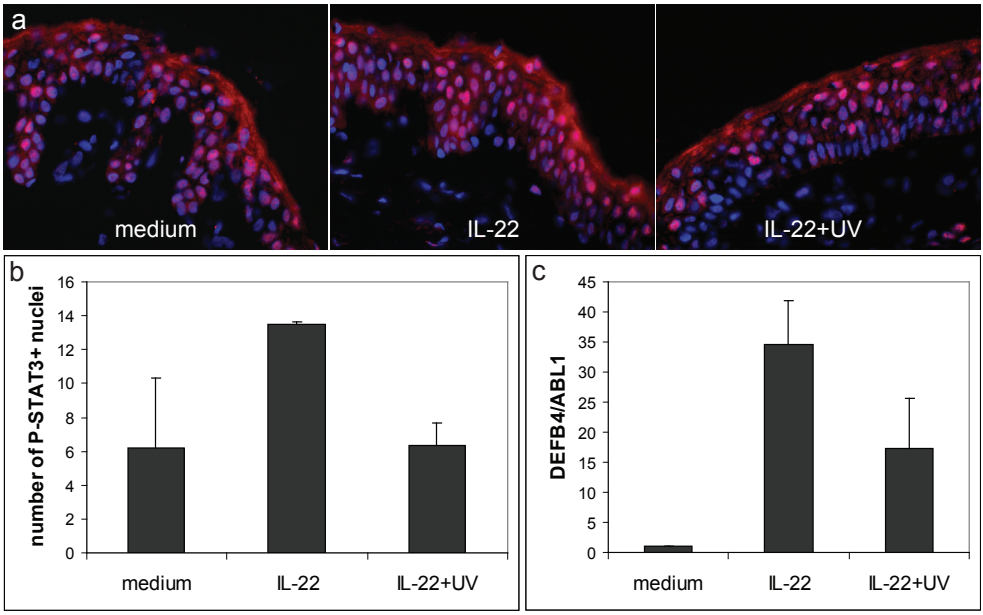


Figure 3. NB-UVB inhibits epidermal STAT3 activation and β-defensin 2 production.
A. Phosphorylated STAT3 in skin biopsies of healthy controls, treated *in vitro* with IL-22 +/- NB-UVB. **B.** Counts of phospho-STAT3+ nuclei in the epidermis. Error bars indicate SEM, n=4 subjects. **C.** β-defensin 2 mRNA expression in the epidermis of healthy controls. Error bars indicate SEM, n=4 subjects.

Chapter 2: Figure 4

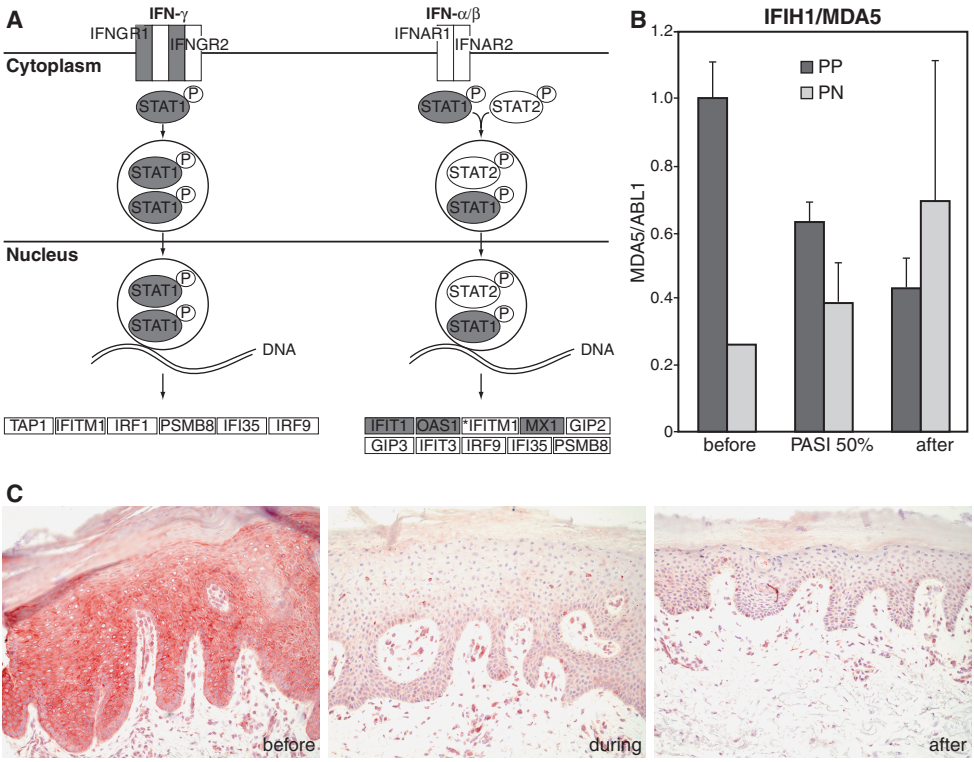


Figure 4. NB-UVB therapy suppresses IFN signalling pathways.
A. IFN signalling pathways. Grey colour indicates gene downregulation. **B.** IFIH1/MDA5 expression measured by RT-PCR. PP: lesional, PN: non-lesional samples; 50%: sample taken at 50% PASI score reduction. Error bars indicate SEM, n=5 patients. **C.** MxA expression during NB-UVB therapy. IFNGR: IFN-γ receptor, IFNAR: IFN-α receptor, TAP1: transporter 1, ATP-binding cassette, B, IFITM1: interferon induced transmembrane protein 1, IRF1: interferon regulatory factor 1, PSMB8: proteasome beta 8, IFI35: interferon-induced protein 35, IRF9:interferon regulatory factor 9.

Chapter 2: Figure 5

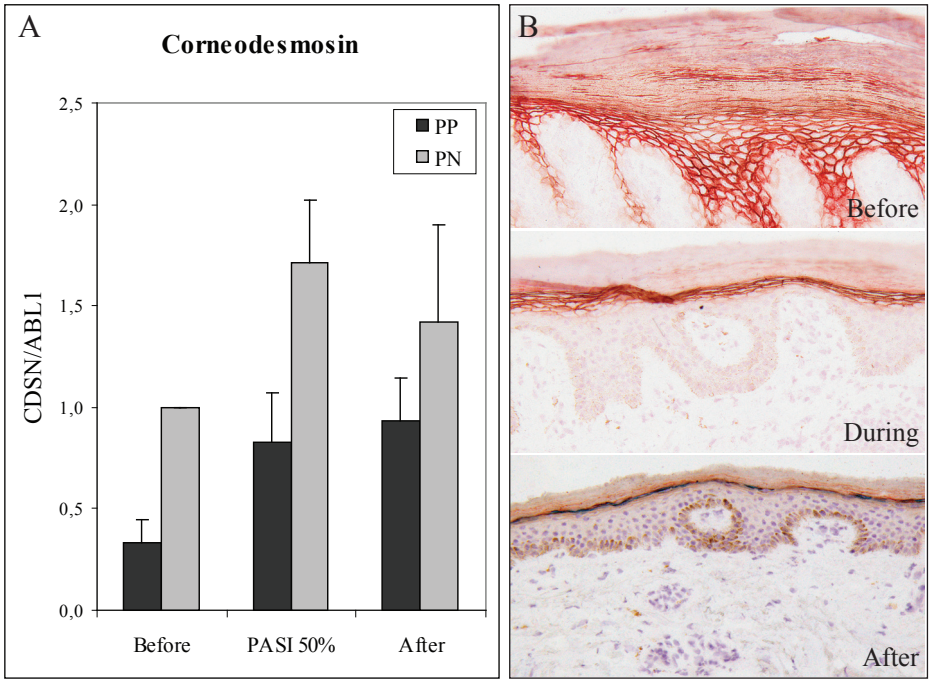


Figure 5. NB-UVB therapy suppresses epidermal differentiation.
A. Corneodesmosin (CDSN) expression measured by RT-PCR. PP: lesional, PN: non-lesional samples; 50%: sample taken at 50% PASI score reduction. Error bars indicate SEM, n=5 patients. **B.** Transglutaminase K expression during NB-UVB therapy.

Chapter 2: Figure 6

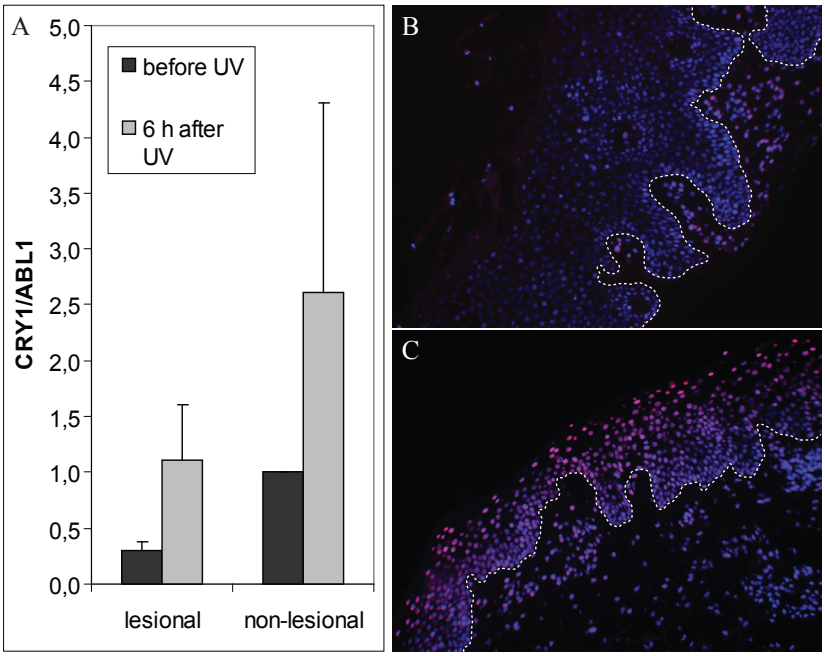
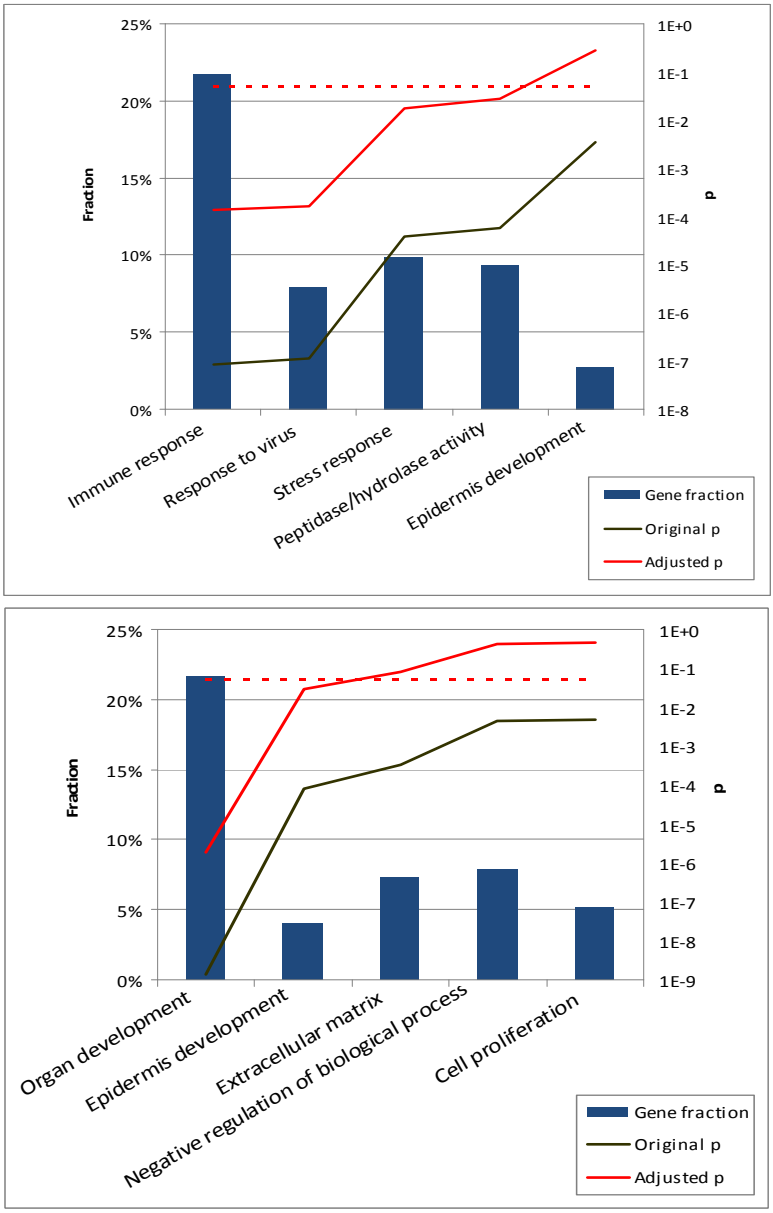


Figure 6. Immediate epidermal effects of NB-UVB.
A. mRNA expression of cryptochrome 1 (CRY1) immediately before and 6 h after the first irradiation, relative to ABL1. Error bars indicate SEM, n=6 patients. **B-C.** Immunohistochemical staining for CPDs in lesional skin before (**B**) and 15 min after (**C**) irradiation with 70% MED.

Chapter 2: Supplemental Figure 1



Supplemental Figure 1. Top 5 GO annotation clusters as found by DAVID (Database for Annotation, Visualization, and Integrated Discovery) in the list of genes downregulated (a) or upregulated (b) in the lesional epidermis before vs. after NB-UVB therapy.
Each cluster contains multiple GO terms. Shown are the average fraction of genes assigned to the terms in the cluster and the geometric means of the original and Benjamini-Hochberg adjusted *p*-values of the terms in the cluster, respectively. The dashed line indicates the significance threshold (adjusted *p*-value = 0.05).

Chapter 4: Figure 1

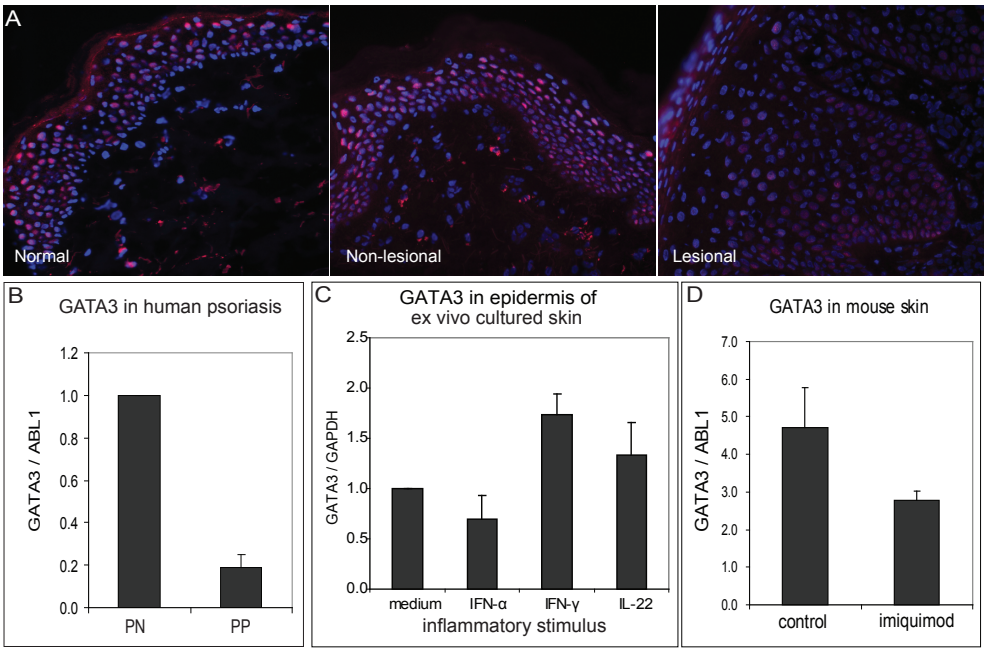


Figure 1. Epidermal GATA3 expression is reduced in psoriatic lesions.
A. GATA3 protein was present in the nuclei of differentiating layers of the epidermis in normal skin. In non-lesional skin expression of GATA3 was also present in the basal layer of the epidermis whereas in lesional skin GATA3 expression was downregulated. **B.** Expression of GATA3 was lower in lesional skin (PP) compared to non-lesional skin (PN). Epidermal GATA3 mRNA expression was determined by RT-PCR using ABL1 as a housekeeping control gene. Bars represent mean +/- SEM (n=5 patients). **C.** 3 mm biopsy samples from normal human skin were cultured in the presence of proinflammatory cytokines for 24 h. The epidermis was separated from the dermis and GATA3 expression was determined by RT-PCR in epidermal RNA, using ABL1 as a housekeeping control gene. Bars represent mean +/- SEM (n=4 healthy donors). **D.** GATA3 mRNA expression in the imiquimod-induced psoriasis-like dermatitis mouse model. GATA3 mRNA expression was determined in imiquimod- or control cream-treated back skin of Balb/C mice. ABL1 was used as a housekeeping control gene. Bars indicate the mean +/- SEM (n=3 mice per group).

Chapter 4: Figure 2

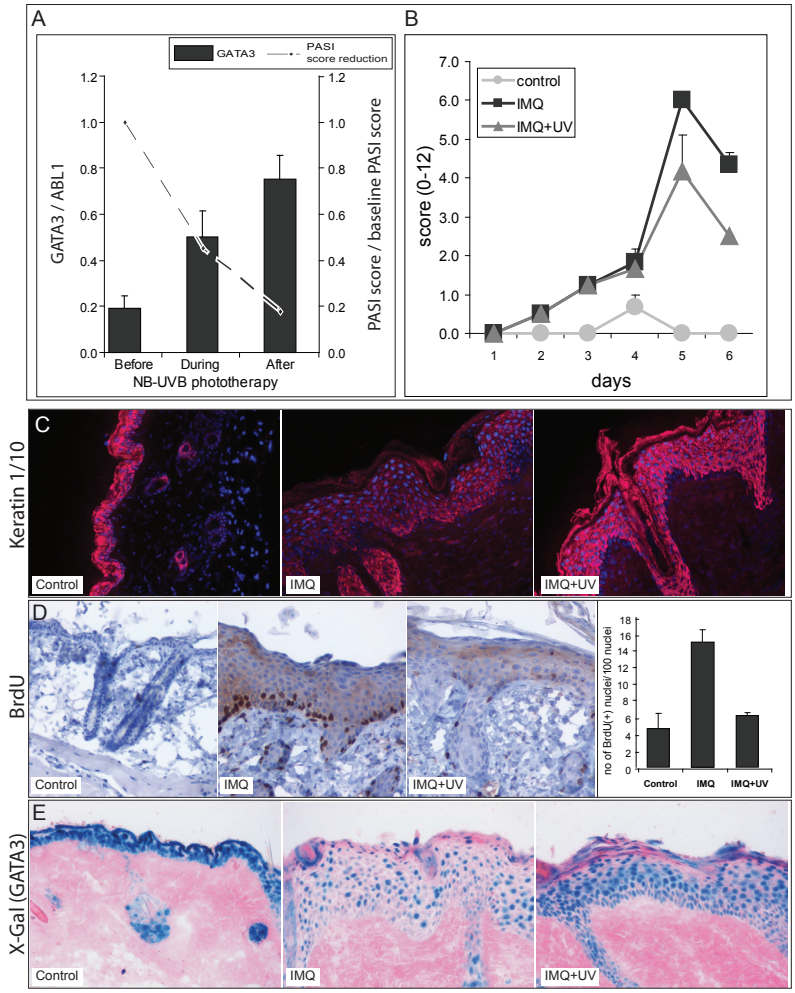


Figure 2. Upregulation of GATA3 expression during NB-UVB phototherapy.
A. GATA3 expression is upregulated during the course of NB-UVB phototherapy in patients with psoriasis. Three- mm biopsy samples were collected from lesional (PP) and non-lesional skin of patients with psoriasis before, during and after NB-UVB phototherapy. Epidermal GATA3 mRNA expression was determined with RT-PCR using ABL1 as a housekeeping control gene. Bars represent mean +/- SEM (n=5 patients). The broken line shows the change in the PASI score, relative to the baseline score. **B**. BALB/c mice were treated daily with imiquimod 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. Erythema, scaling, and the 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. **C.** Mice were sacrificed on day 6. Imiquimod-induced inflammation was studied on sections made from the back skin of the mice. Keratin 1/10 immunofluorescent staining of the back of the mice is shown. **D.** BrdU incorporation in keratinocytes in the back skin was detected by immunohistochemistry. Bars on the right represent the mean number of BrdU positive cells +/- SD. **E.** X-gal staining (blue) of heterozygous GATA3LacZ skin samples from mice treated with imiquimod with or without NB-UVB. X-Gal staining that in these mice correlates with GATA3 expression is lower in the inflamed epidermis compared to control and is induced during NB-UVB treatment.

Chapter 4: Figure 3

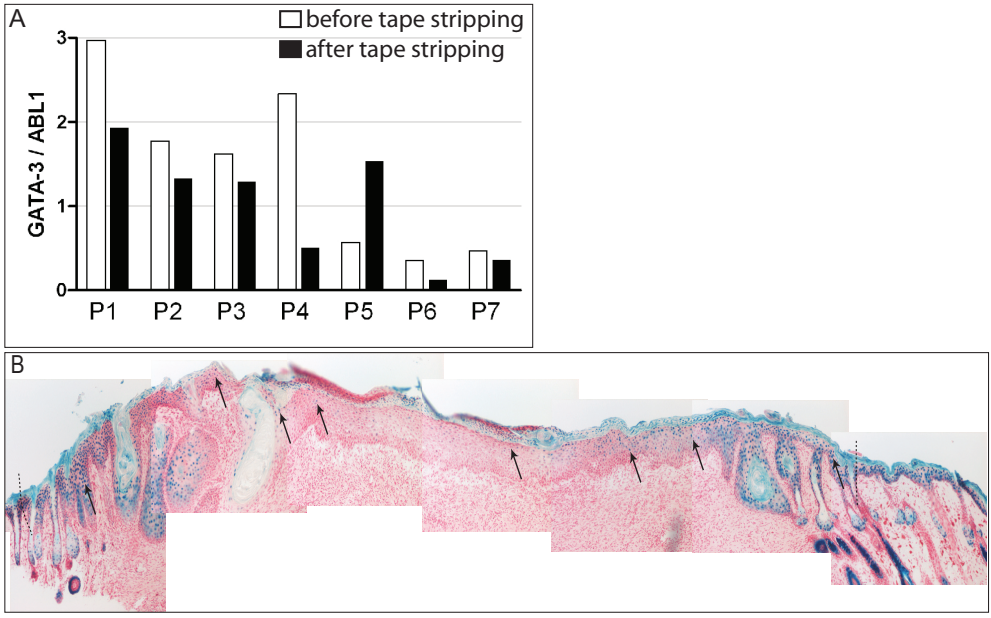


Figure 3. GATA3 expression is reduced in the regenerating epidermis.
A. The stratum corneum of non-lesional skin of seven psoriatic patients was removed by repeated tape stripping to induce regeneration. Before and 5 h after tape stripping biopsies were taken and epidermal GATA3 expression was determined using RT-PCR, relative to ABL1 as a housekeeping control gene. Relative expression values of individual patients are shown. **B.** GATA3 expression is downregulated in the healing wound of murine skin. X-gal staining (blue) of wounded skin of a heterozygous GATA3LacZ mouse shows downregulation of the LacZ transgene under the control of GATA3 in the highly proliferative zone of the healing wound. Arrows indicate X-Gal positive cells; the border between regenerating and adjacent normal skin is marked.

Chapter 4: Figure 4

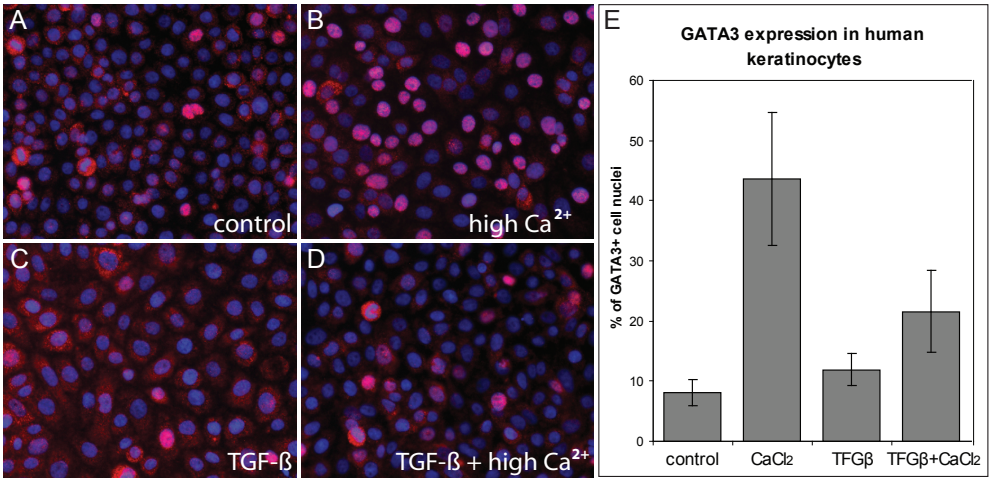
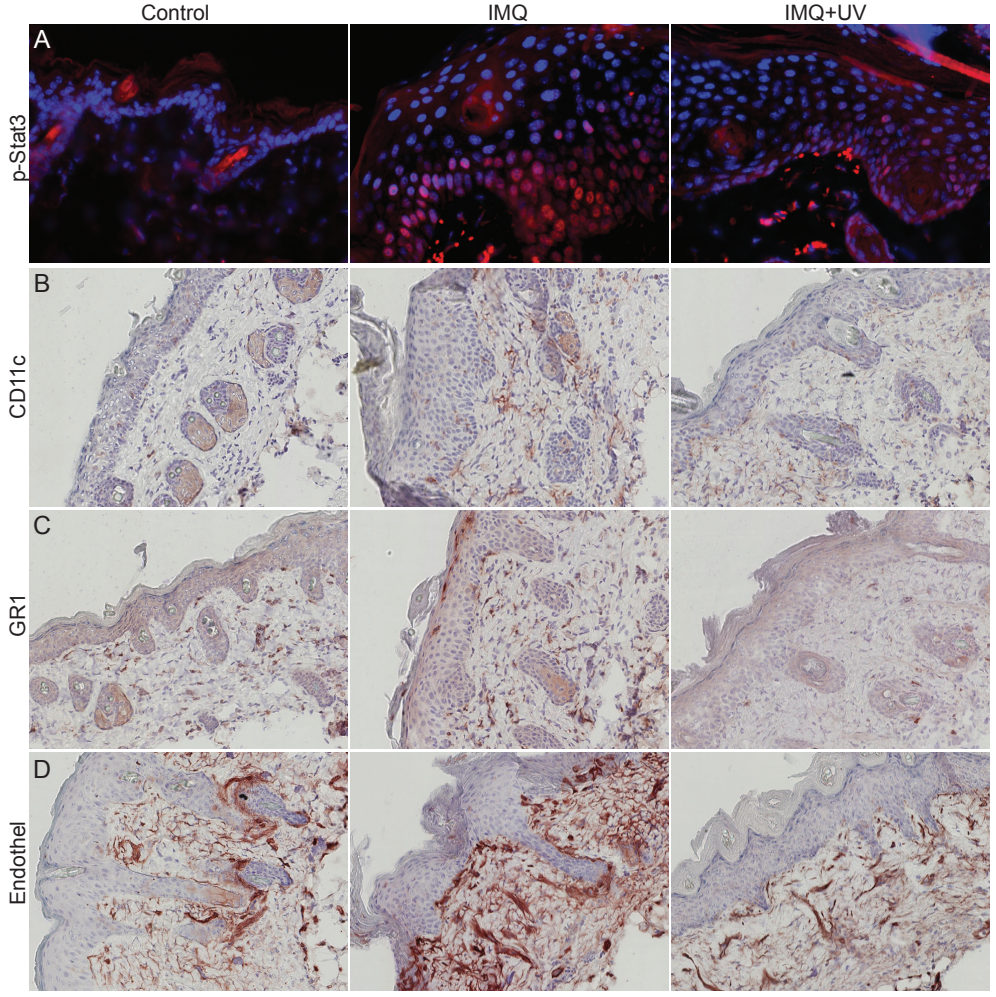


Figure 4. GATA3 translocates to the nucleus in differentiating keratinocytes, whereas it stays in the cytoplasm during cell cycle arrest.
Primary human epidermal keratinocytes were cultured on chamber slides. When cells reached ~75% confluency, $CaCl_2$ (**B, D**) and/or TGF-β1 (**C, D**) were added to the culture medium. After 24 h cells were fixed and immunofluorescent staining for GATA3 protein (pink) was performed. **E.** GATA3 positive cell nuclei were counted and are shown as a percentage of the total number of cell nuclei.

Chapter 4: Supplemental Figure 1



Supplemental Figure 1. NB-UVB treatment affects epidermal differentiation, inflammatory infiltrate and vascular alterations in imiquimod-induced skin inflammation in mice.
BALB/c mice were treated daily with imiquimod 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. Mice were sacrificed on day 6. Immunofluorescent staining for phosphorylated STAT3 (**A**), is shown, as well as immunohistochemical analysis of myeloid dendritic cells (CD11c, **B**), granulocytes (GR1, **C**), and endothelial cells (MECA-20, **D**).