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Reversal of Atopic Dermatitis with Narrow-Band UVB Phototherapy and Biomarkers for Therapeutic Response

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Abstract

Background—Atopic dermatitis (AD) is a common inflammatory skin disease exhibiting a predominantly Th2/“T22” immune activation and a defective epidermal barrier. Narrow-band UVB (NB-UVB) is considered an efficient treatment for moderate-to-severe AD. In psoriasis, NB-UVB has been found to suppress the Th1/Th17-polarization with subsequent reversal of epidermal hyperplasia. The immunomodulatory effects of this treatment are largely unknown in AD.

Objective—To evaluate the effects of NB-UVB on immune and barrier abnormalities in AD, aiming to establish reversibility of disease and biomarkers of therapeutic response.

Methods—12 moderate-to-severe chronic AD patients received NB-UVB phototherapy 3 times weekly for up to 12 weeks. Lesional and non-lesional skin biopsies were obtained before and after treatment and evaluated by gene-expression and immunohistochemistry studies.

Results—All patients had at least a 50% reduction in SCORing of AD (SCORAD) index with NB-UVB phototherapy. The Th2, “T22,” and Th1 immune pathways were suppressed and measures of epidermal hyperplasia and differentiation normalized. The reversal of disease activity was associated with elimination of inflammatory leukocytes, Th2/“T22”- associated cytokines and chemokines, and normalized expression of barrier proteins.

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Clinical Implications

Resolution of Th2- and “T22”-associated inflammation is strongly correlated with therapeutic response to narrow-band UVB in atopic dermatitis and targeting these pathways might be beneficial in disease treatment.

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Conclusions—Our study shows that resolution of clinical disease in patients with chronic AD is accompanied by reversal of both the epidermal defects and the underlying immune activation. We have defined a set of biomarkers of disease response that associate resolved Th2 and “T22” inflammation in chronic AD patients with reversal of barrier pathology. By showing reversal of the AD epidermal phenotype with a broad immune-targeted therapy, our data argues against a fixed genetic phenotype.

Keywords

Atopic dermatitis; phototherapy; narrow-band UVB; Th2; T22; biomarker; skin

INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease¹ characterized by: 1) immune activation; 2) marked epidermal hyperplasia; and 3) defective barrier function, reflecting underlying alterations in keratinocyte differentiation.²⁻⁴ The pathogenesis of AD is still debated. A primary barrier dysfunction that leads to immune polarization (the “outside-in” hypothesis⁴) is supported by recent reports of the presence of filaggrin (*FLG*) mutations in some AD patients,⁵ as well as wide down-regulation of terminal differentiation genes on chromosome 1q21, i.e. loricrin (*LOR*), late cornified envelope 2B, small proline-rich proteins, and the cornified envelope gene corneodesmosin (*CDSM*).^{6, 7} Mutations in additional candidate genes, such as *SPINK5* (serine proteinase inhibitor Kazal-type 5) and *KLK7* (kallikrein-related peptidase 7) were also suggested to predispose to the impaired epidermal barrier.⁸⁻¹¹ The “inside-out” hypothesis, favoring epidermal abnormalities secondary to underlying immune activation,¹² is suggested by broad cornification defects in patients without *FLG* mutations⁷ and the association of immune abnormalities in non-lesional AD (ANL) skin with disease severity.¹³

AD shares many features with psoriasis, including immune activation and epidermal hyperplasia. However, major differences in immune polarization exist between these diseases.^{2, 7, 14} While psoriasis is considered a Th1/Th17 disease, AD is predominantly a Th2/“T22”-polarized disease with some component of Th1 polarization in the chronic phase, and a relative impairment of the Th17 pathway.¹⁵ The “T22” cytokine IL-22 was found to induce epidermal hyperplasia and inhibit keratinocyte terminal differentiation.^{15, 16}

Whereas topical agents (i.e. corticosteroids and calcineurin inhibitors) are effective for mild AD, these options are insufficient to control more severe disease.¹⁷ Limited therapeutic options exist for moderate-to-severe AD: 1) oral steroids; 2) cyclosporine A; and 3) phototherapy (including ultraviolet (UV) B, UVA with psoralen (PUVA), and UVA-1.¹⁷ Both oral steroids and CsA have major adverse effects, prohibiting long-term use.¹⁷ Since UVA-1 phototherapy is expensive and not widely available in the U.S., narrowband-UVB (NB-UVB) has emerged as an effective alternative.^{18, 19}

Although prior studies have reported the clinical efficacy of NB-UVB in reducing the SCORing of AD (SCORAD) index,^{18, 20} its immunomodulatory effects are largely unknown in AD. Given that NB-UVB may be the only practical long-term treatment for moderate-to-severe AD, it is crucial to better understand its mechanistic properties in this disease. In psoriasis, NB-UVB treatment has been found to suppress the Th1/Th17 immune axes with subsequent reversal of epidermal hyperplasia.²¹⁻²⁴ These investigations defined parameters for psoriasis disease reversal and established an understanding of the pathogenic relationship between expression of immune-regulatory genes and disease activity.^{21-23, 25}

The present study analyzes the effects of NB-UVB on barrier and immune abnormalities in AD, aiming to establish reversibility of disease and, if reversed, to determine a specific set

of genomic and histological measures of disease reversal. We hypothesized that NB-UVB might reverse both the epidermal growth and differentiation defects as well as the underlying immune activation, lending support to the “inside-out” hypothesis. Conversely, persistence of the epidermal hyperplasia and terminal differentiation defects despite immune suppression with NB-UVB treatment would reject the “inside-out” hypothesis. Our study is the first to show clear genomic and tissue reversal of AD disease pathology and to highlight biomarkers of therapeutic response that could be implemented in testing of targeted therapeutics for AD.

METHODS

Patient characteristics and skin samples

Pre- and post-treatment lesional (AL) and non-lesional (ANL) (at least 10 cm from any active lesion) skin biopsies and blood samples were obtained from 12 patients with moderate-to-severe chronic AD (9 males, 3 females, ages 24-51, median 43) and from 10 healthy volunteers under an IRB-approved protocol (see Table E1 in the Online Repository). Patients received full-body NB-UVB 3 times weekly until clearance or up to 12 weeks (mean 23.5 sessions, range 9-48). Patients were allowed to use emollients only with no additional pharmacologic treatment during the study period. Pre- and post-treatment AL and ANL biopsies were obtained from the same skin area to evaluate therapeutic effect. Pre-treatment serum IgE was elevated in 9 of 12 patients (range, 1-6965, mean, 1387; reference range, 0-160 kU/L); serum eosinophil count was elevated in 1 patient (reference range, 0-7%). The SCORAD index was used to evaluate disease severity at enrollment and after completion of treatment²⁶ (see the Online Repository). Pre-treatment SCORAD ranged from 28 to 97.5 (mean 60); post-treatment SCORAD ranged from 0 to 21 (mean 10). A single-copy R501X mutation in the *FLG* gene was found in 1 patient (Table E1 in the Online Repository).

Immunohistochemistry

Biopsies were frozen in O.C.T. and immunohistochemistry (IHC) techniques were performed in a standard manner (see Table E5 in OR). Epidermal thickness and positive cells per millimeter were quantified using computer-assisted image-analysis software (ImageJ 1.42). “Pathologic” epidermal thickness was defined as pre-AL minus pre-ANL epidermal thickness.

Quantitative real time PCR and gene microarray analysis

RNA was extracted for real-time PCR (RT-PCR) and Affymetrix humanU133APlus2.0 arrays were used as previously described (see the Online Repository).¹⁵ The data in this publication have been entered in NCBI’s Gene Expression Omnibus (Series Accession number GSE27887) (see Tables E2 and E4 in the Online Repository).

Statistical Analysis

Analysis of IHC and RT-PCR data was carried out using a linear mixed model to account for the paired structure of AL and ANL and pre- to post-NB-UVB measures. Affymetrix CEL files were scrutinized for spatial artifacts using Harshlight.¹³ Expression values were obtained using the GCRMA algorithm. Probe-sets with >5 samples with expression >4 and standard deviation >0.2 were kept for analysis. P-values were adjusted for multiple hypotheses using the Benjamini-Hochberg procedure. Spearman rank correlations were used to correlate all variables measured by IHC and RT-PCR with clinical and histological response to NB-UVB using significance of $p < 0.1$.

RESULTS

After NB-UVB, all patients met the pre-defined criteria for response to therapy (a decrease of 50% in SCORAD index) with marked clinical improvement. Overall, a mean $81.1 \pm 8.7\%$ reduction in SCORAD was observed following an average of 23.5 NB-UVB sessions (see Table E1 in this article's Online Repository). Eleven of 12 patients were judged to be responders by histological (>40% reduction in pathologic epidermal thickness) criteria. Subsequent statistical analyses include the outcome of NB-UVB in the 11 responders.

To determine a specific set of cellular and molecular markers of disease reversal that correlate with a major reduction in the SCORAD index following NB-UVB, we have used the term "biomarker" and adopted its formal definition by the National Institutes of Health: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic response(s) to a therapeutic intervention."²⁷ We established biomarkers of disease activity in baseline AD, as well as biomarkers of disease reversal with therapy (variables for which a significant change with NB-UVB treatment correlated with the reduction in the SCORAD index).

Genomic reversal of epidermal hyperplasia and inflammatory markers with NB-UVB therapy

Gene array analysis was performed to compare genomic expression before and after phototherapy in both ANL and AL. We have defined the genomic differences in pre-ANL and pre-AL as the genomic phenotype of visible disease (Table E2 in this article's Online Repository).¹³ A heat-map illustrating this distinction between pre-ANL and pre-AL displays disease reversal following NB-UVB with post-ANL and post-AL skin showing similar expression levels (Figure 1A). By organizing patients in order of SCORAD index the partial retention of pre-AL expression levels can be appreciated in patients with higher SCORAD (Figure 1A) (gray-spectrum boxes at the top of each column represent an increase in SCORAD index from white to black). When represented in a scatterplot, the substantial nullification of the genomic disease phenotype is also observed (Figure 1B). Genomic expression differences in pre-AL versus pre-ANL expression (the AD disease phenotype) are graphed as an identity line (slope=1, line a, black, Figure 1B). In comparison, the linear regression line of genomic expression differences in post-AL versus post-ANL (line b, red) is reduced by 78%, representing the reduction in the AD disease phenotype (Figure 1B).

To determine the differentially expressed genes (DEGs) between pre-ANL and post-ANL and pre-AL and post-AL, criteria of fold-change (FCH)>2 and false discovery rate (FDR)<0.1 were used. There was a down-regulation of 372 genes and an up-regulation of 192 genes in AL skin after phototherapy compared to pre-treatment expression levels (Figure E1 in the Online Repository), with a down-regulation of 35 and up-regulation of 23 genes in ANL skin (Figure E1). Markers of epidermal proliferation (Ki67, keratin 16 (K16), keratin 6B) as well as many inflammatory mediators were within this set of genes (see Tables E3 (the top 50 DEGs) and E4 (the complete list of DEGs) in this article's Online Repository). Among the most significantly down-regulated immune genes were markers of T-cells (CD2), T-cell activation (CD69), Th2-associated products (CCL13, CCL18, CCL26 and IL-10), Th1-related products (TNF α and IL-12), interferon-induced genes (MX-1 and OAS1), and dendritic cell (DC) antigens (ITGAM/CD11b, CD209, CD1c, Fc ϵ R1, and CD1a) and several keratinocyte-associated immune mediators (CCL2, TNF α -induced proteins, and CCL5, and the IL-7 receptor) (Tables E3 and E4 in the Online Repository).

Reduction of pathologic epidermal proliferation with NB-UVB

We evaluated tissue response by comparing epidermal thickness and keratinocyte proliferation (K16 staining) in pre-ANL, pre-AL, post-ANL, and post-AL samples (Figure 2A-B). Pre-AL epidermal thickness was 141% greater (on average) than pre-ANL and was significantly reduced after treatment ($p < 0.005$), with a mean decrease of $115.6 \pm 125.8 \mu\text{m}$ (a reduction of pathologic epidermal thickness of $86 \pm 23.6\%$) (Figure 3). A normalization of keratinocyte proliferation was seen following therapy: suprabasilar K16 expression, not typically expressed in normal skin, was present in only 1 post-AL sample (versus K16-positivity in all 12 pre-AL skin samples). These data were confirmed by a significant reduction in K16 mRNA expression from pre-AL to post-AL ($p < 0.001$) (Figure 4).

Suppression of inflammatory-cell infiltrates following NB-UVB

The NB-UVB effect on immune cell infiltrates in AD skin was most evident in the papillary dermis, with some decrease in cell density in the reticular dermis (Figure 2C-E). Comparison of cell counts in pre-AL and post-AL skin revealed significant decreases in CD3^+ and CD8^+ T-cells ($p < 0.001$) and significant reductions in DC subsets, including myeloid (CD11c^+) ($p < 0.05$), “resident” (CD1c^+)²⁸ ($p < 0.001$), “inflammatory” (TRAIL^+)²⁸ ($p < 0.05$), plasmacytoid (BDCA-2^+) ($p < 0.01$), and mature (CD83^+) ($p < 0.01$) DCs (Figure 3). Inflammatory dendritic epidermal cells (IDECs), quantified by CD1b^+ , $\text{Fc}\epsilon\text{R1}^+$, and CD206^+ cells, also significantly decreased following NB-UVB therapy (Figure 3). CD1a , a marker of Langerhans cells and IDECs, was also significantly reduced ($p < 0.001$) (Figure 2E, Figure 3), as was OX40-ligand (OX-40L), considered a marker of “atopic” DCs²⁹ (Figure 3). Low numbers of eosinophils (major basic protein⁺ (MBP^+)) (Figure 3) and mast cells (tryptase⁺) (Figure E2A in the Online Repository) were observed in pre-AL skin; these cells were significantly reduced following NB-UVB ($p < 0.05$ and $p < 0.01$, respectively).

NB-UVB suppresses inflammation in AD

We validated the UVB effects on inflammatory pathways with RT-PCR. Th2-associated products (IL-13, CCL11, CCL17, CCL18, CCL22), which perpetuate Th2-associated inflammation, showed significantly reduced mRNA expression levels (Figure 4). Reductions of IL-10 and the thymic stromal lymphopoietin receptor (TSLP-R) approached significance ($p = 0.346$ and $p = 0.097$, respectively) (Figure 4). The “T22” cytokine IL-22, which was significantly increased in pre-AL compared to normal skin ($p < 0.05$), decreased significantly with NB-UVB ($p < 0.05$) (Figure 4). A decrease was also observed in mRNA expression of the S100A7 and S100A8 antimicrobial proteins (AMPs), which are induced by IL-22,¹⁵ (Figure 4).

The mRNA expression of the Th1-associated cytokine IFN- γ and of the interferon-induced proteins MX-1 and CXCL-10 was increased in pre-AL skin compared to normal ($p < 0.05$, $p < 0.01$ and $p = 0.097$; respectively) and was down-regulated with treatment ($p = 0.119$, $p = 0.186$, and $p = 0.134$; respectively) (Figure 4, Figure E2B in the Online Repository).

We detected decreases in the mRNA expression of IL-17A, IL-23p19 (Figure 4) and IL-23p40 (Figure E2C in the Online Repository) with treatment ($p < 0.05$ for p19 alone). Finally, mRNA expression of the regulatory T-cell marker FOXP3 was significantly increased in pre-AL compared to normal skin ($p < 0.001$) and decreased with NB-UVB ($p < 0.05$) (Figure 4).

Normalization in barrier proteins with NB-UVB

Histological evaluation of the terminal differentiation proteins loricrin (LOR), FLG, and involucrin (IVL) revealed clear increases in granular layer expression (Figure 5). We observed a more continuous expression and a greater depth of expression (into the stratum

spinosum layer) of LOR (10/10 patients) (Figure 5A), FLG (8/10 patients) (Figure 5B) and IVL (5/8 patients) (Figure 5C) in post-ANL and post-AL compared to before NB-UVB, representing a relative normalization. The normalization in granular layer proteins correlated, in general, with reversal of hyperplasia markers (suprabasilar K16 staining and epidermal thickness). Likewise, mRNA expression of terminal differentiation proteins (LOR and periplakin (PPL)) was significantly inversely correlated with a reduction in SCORAD, suggesting increased expression of these proteins with greater therapeutic response (Table 1B).

Suppression of inflammatory cells and their products correlates with clinical improvement

Several immune and terminal differentiation markers significantly correlate with disease severity (SCORAD index) in baseline pre-AL skin (prior to treatment). These include T-cell (CD8⁺) and DC (CD1a⁺, CD206⁺ and OX-40L⁺) markers, Th2-associated products (IL-13 and CCL22), “T22”-associated IL-22, and the anti-microbial protein S100A7, as well as the terminal differentiation proteins LOR and PPL (inversely correlated with disease severity) (Table 1A).

We also have evaluated which immune, terminal differentiation, and hyperplasia markers are associated with clinical and histological disease reversal by determining the correlations of each variable measured by IHC and RT-PCR with 1) reduction in SCORAD (clinical disease reversal) (Table 1B) and 2) reduction in epidermal hyperplasia and K16 expression (histological disease reversal) (Table 1C).

Immune cell subsets were the most highly correlated variables with clinical improvement. These included CD8⁺ T-cells and several DC subsets (CD1a⁺ IDECs, inflammatory (CD1b⁺ and FcεR1⁺) DCs, and mature (CD83⁺) DCs) (Table 1B). The Th2-associated chemokines (CCL17, CCL18, CCL22 and CCL5), which are produced by myeloid and plasmacytoid DCs,² were highly correlated with histological improvement (Table 1C).

IL-22 was the only cytokine for which its reduction in gene expression with NB-UVB was significantly correlated with the reduction in SCORAD, suggesting the importance of this cytokine in disease activity (Table 1B). The reductions in expression of the IL-22-induced AMPs, S100A7 and S100A8,³⁰ were similarly highly correlated with clinical and histological improvement (Table 1B). Moreover, considering all variables, the cytokine IL-22 appeared the most highly correlated across several characteristics of disease activity (Th2-related products, SCORAD, and inflammatory cell infiltrates) (Table 1D). In comparison, the reduction in IL-13 mRNA expression was not strongly correlated with either clinical or histological improvement, although it was highly correlated with expression of several terminal differentiation proteins, including LOR, TGM1, and CDSN (Spearman correlations of $r=0.720$, $r=0.621$, and $r=0.569$, respectively). Th1- and Th17-related products were not correlated with disease improvement, but reduction in expression of the p40 cytokine subunit was correlated with the reduction in hyperplasia (Table 1C).

Taken together with the variables correlated with disease activity at baseline (Table 1A), our data show that the reversal of disease activity with NB-UVB is most strongly associated with elimination of CD8⁺ T-cells, several DC subsets, the cytokine IL-22, the Th2-chemokines (CCL17, CCL18, CCL22), the AMPs S100A7 and S100A8, and a normalized expression of barrier proteins (Table 1B-D).

Discussion

Few studies have evaluated the mechanism of action of NB-UVB in AD and these have mostly concentrated on clinical response.³¹ There is also limited data on molecular effects of

UV-radiation in AD.³²⁻³⁷ In a mouse model, UVB has been shown to improve barrier function through increased expression of terminal differentiation proteins (FLG and IVL) and AMPs; with altered expression of the AMPs human beta-defensins-1 and -2 described in AD patients treated with NB-UVB.³² Other studies on UV in AD have found reductions in cutaneous T-cells^{34, 36} with no significant changes in mast cells or FOXP3⁺ cells.^{18, 34, 37} The lack of mechanistic studies in therapeutic trials in AD extends to other treatments including cyclosporin A, corticosteroids, and calcineurin inhibitors³⁸⁻⁴⁸ and partially stems from an incomplete understanding of pathogenic mechanisms underlying the disease.

However, recent reports have promoted the understanding of AD pathogenesis,^{7, 14} establishing the following features: 1) Regenerative hyperplasia^{7, 13} and 2) abnormal TD in both AL and ANL skin,¹³ and 3) activation of Th2 and “T22” subsets in AL.¹⁴ Thus, a complex immune pathogenesis characterizes chronic AD with a relative deficiency of the Th17 immune axis and an up-regulation of the “T22” axis in the context of ongoing Th2-pathway dominance.^{2, 7, 15} Although the Th2 cytokines IL-4/IL-13 have been reported to inhibit Th17 activation,⁴⁹ terminal differentiation proteins,⁵⁰ and AMPs,⁵¹ Th2-products have not previously been reported to promote epidermal hyperplasia.

There is increasing evidence linking IL-22 to many aspects of epidermal pathology in AD, including inhibition of epidermal differentiation and induction of hyperplasia, and also associating it with disease activity in AD.^{15, 16, 52, 53} Furthermore, the cytokine has been reported to strongly up-regulate the AMPs S100A7 and S100A8,^{14, 16, 49} although it is a much less potent inducer of AMPs than IL-17, which is relatively deficient in AD.¹⁵ Finally, staphylococcal exotoxins induce IL-22 secretion in keratinocytes, supporting an IL-22-dependent role of *S. aureus* in AD severity.⁵⁴

These advances allowed us to analyze the NB-UVB effects in AD and to correlate the normalization of immunological and histological parameters with clinical improvement. We evaluate how Th2/“T22” immune suppression induced by NB-UVB influences reversal of the genomic and epidermal disease phenotype of AD and create a set of measures for the assessment of disease reversal.

UV radiation has immunomodulatory effects that lead to improvement of inflammatory skin diseases.³⁶ In animal and human models, UVB inhibits immune responses,^{23, 55, 56} likely related to induction of T-cell apoptosis.^{21, 23, 57} In psoriasis, NB-UVB was initially shown to induce immune suppression followed by normalization of epidermal hyperplasia, supporting an immune regulation of the epidermal pathology in this disease.⁵⁸ Subsequently, NB-UVB was found to suppress major T-cell pathways involved in disease pathogenesis, namely the Th17/IL-23 and Th1 pathways.^{22, 24, 59, 60} In these psoriasis studies, there was a significant reduction in IFN- γ gene expression with NB-UVB, although the reductions in IL-17 and IL-22 were found to better correlate with the clinical response.²⁴ Similarly, our study shows strong suppression of the Th2 and “T22” axes in AD patients following NB-UVB, together with normalization of epidermal barrier function. We also found significant reductions in inflammatory cell subsets (including T-cells, IDECs, and “atopic” DCs).

Our study extends the mechanism of action of NB-UVB to include suppression of Th2 immune activation in chronic AD. Thus, NB-UVB can suppress all activated polar T-cell immune pathways within inflammatory diseases. Overall, we show that in chronic AD patients, NB-UVB induces significant immune suppression of the Th2 and “T22” axes and a less-significant suppression of the Th1 axis. Thus, in both AD and psoriasis,²⁴ the immune suppression changes seen with NB-UVB treatment reflect the major inflammatory pathways that characterize each disease and are correlated with the therapeutic response.

Interestingly, concordant with prior reports,^{51, 61} the immunoregulatory cytokine IL-10 showed up-regulated mRNA expression in both AL and ANL as compared with normal skin, which decreased with NB-UVB. Increased levels of IL-10 have been postulated to indirectly contribute to the AMP deficiency in AD, potentially accounting for an increased propensity for infections.⁶² Higher IL-10 expression levels were reported in chronic AD skin compared with the acute stage, possibly due to an up-regulation of the IL-10 receptor by IFN- γ .^{63, 64}

We present data for reversal of epidermal hyperplasia and aberrant terminal differentiation with NB-UVB, and the correlation of these variables with Th2 and “T22” immune pathways. The suppression of Th2-chemokines was highly correlated with reversal of hyperplasia following NB-UVB therapy. We also found a high correlation between the reduction in IL-22 expression with the reduction in epidermal hyperplasia and with the reduction in expression of S100A7 and S100A8. The reduction in IL-22 expression following NB-UVB was also inversely correlated with the change in expression of terminal differentiation proteins. Since our observations link suppression of IL-22 to: 1) a reduction in SCORAD; 2) reversal of epidermal hyperplasia; 3) depletion of immune cell subsets, including Langerhans cells and plasmacytoid DCs, (both recently reported to induce “T22” cells);^{65, 66} and 4) normalization of terminal differentiation proteins, we hypothesize that IL-22 is critical in AD pathogenesis. Up-regulation of the “T22” axis may be the link between the immune and epidermal abnormalities seen in AD.¹⁴

The novel approach we have taken to evaluate NB-UVB effects in AD allows us to define a set of biomarkers of disease response that associate resolved Th2 and “T22” inflammation with reversal of epidermal barrier pathology. We clearly show reversal of the AD epidermal phenotype with a broad-based immune targeted therapy, which argues against a fixed genetic phenotype. Thus, although not providing absolute proof for the “inside-out hypothesis,” we cannot refute this assumption. However, since NB-UVB may have direct effects on epidermal keratinocytes,⁶⁷ some therapeutic effects could also be modulated through keratinocytes. Future studies with specific immune antagonists coupled with the disease markers shown here are warranted to critically test the alternate hypotheses of disease pathogenesis: “inside-out” (immune-generated epidermal dysfunction) versus “outside-in” (defined by barrier-initiated immune activation).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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None

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ABBREVIATIONS

AD	Atopic dermatitis
AL	Lesional atopic dermatitis
AMP	Antimicrobial protein
ANL	Non-lesional atopic dermatitis
CDSN	Corneodesmosin
DC	Dendritic cell
DEG	Differentially expressed genes
EDC	Epidermal differentiation complex
FCH	Fold change
FDR	False discovery rate
FLG	Filaggrin
IDEC	Inflammatory dendritic epidermal cell
IVL	Involucrin
K16	Keratin 16

KLK 7	kallikrein-related peptidase 7
LCN2	Lipocalin-2
LOR	Loricrin
MBP	Major basic protein
NB-UVB	Narrow-band ultraviolet B
OX-40L	OX-40 ligand
PPL	Periplakin
PUVA	Psoralen with ultraviolet-A
RT-PCR	Real-time PCR
SCORAD	SCORing of Atopic Dermatitis index
SPINK5	serine protease inhibitor Kazal-type 5
TGM1	Transglutaminase-1
TRAIL	TNF-related-apoptosis-inducing ligand
TSLP-R	Thymic stromal lymphopoietin receptor
UV	Ultraviolet

Figure 1. Genomic differences in atopic dermatitis (AD) before and after narrowband-UVB (NB-UVB) phototherapy

A, Differentially expressed genes (DEGs) in non-lesional (ANL) and lesional (AL) AD skin before and after NB-UVB (red: gene up-regulation; blue: gene down-regulation). **B**, A scatterplot of gene-expression values in pre-NB-UVB (x-axis) and post-NB-UVB (y-axis) samples illustrates the substantial reduction (78%) in the AD disease phenotype with NB-UVB.

Figure 2. Immunohistochemistry of non-lesional (ANL) and lesional (AL) atopic dermatitis skin before (pre-) and after (post-) NB-UVB. Staining of hematoxylin and eosin (H&E) (**A**) and proliferation marker K16 (**B**) display reductions in epidermal hyperplasia and abnormal proliferation following NB-UVB. **C-E**; Reductions in dermal (CD3+) T-cells, myeloid (CD11c+) dendritic cells and Langerhans (CD1a+) cells. Scale bar=100 μ m.

Figure 3.

Reductions in epidermal thickness and immune cell infiltrates, quantified by immunohistochemistry, in lesional (AL) (red lines) and non-lesional (ANL) (blue lines) atopic dermatitis following NB-UVB (asterisks above lines: significance of the change). Black error bars and asterisks represent standard error of the mean in normal skin samples and significance of difference in AL or ANL versus normal (when available), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4.

RT-PCR expression of selected genes in lesional (AL) (red lines) and non-lesional (ANL) (blue lines) atopic dermatitis following NB-UVB UVB (asterisks above lines: significance of the change). Associated inflammatory pathways (Th1, Th2, Th17, or "T22") are indicated for each cell type. Black error bars and asterisks represent standard error of the mean in normal skin samples and significance of difference in AL or ANL versus normal (when available), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 5. Immunohistochemistry of non-lesional (ANL) and lesional (AL) atopic dermatitis skin before and after NB-UVB. Staining of the terminal differentiation proteins loricrin (LOR) (A), filaggrin (FLG) (B) and involucrin (IVL) (C) displays increased and more continuous granular layer staining following NB-UVB therapy. Scale bar=100 μ m.

Table 1

Correlation of epidermal and immune variables with atopic dermatitis (AD) severity at baseline and with disease improvement and reductions of inflammatory cytokines following narrow-band UVB (NB-UVB) phototherapy.*

A. Variables significantly correlated with disease severity (SCORing of Atopic Dermatitis (SCORAD) index) in baseline lesional AD skin.		
Disease Variable	Spearman Correlation	P-value
CD206 ⁺ cells (I)	0.87	0.003
CD1a ⁺ cells (I)	0.57	0.026
CCL22 (R)	0.62	0.028
CD8 ⁺ cells (I)	0.68	0.032
OX-40L ⁺ cells (I)	0.64	0.044
TRAIL ⁺ cells (I)	0.51	0.045
IL-13 (R)	0.43	0.081
S100A7 (R)	0.43	0.081
IL-22 (R)	0.42	0.087
PPL (R)	-0.55	0.032
LOR (R)	-0.51	0.098

B. Variables significantly correlated with reduction of SCORAD index following NB-UVB.		
Disease Variable	Spearman Correlation	P-value
CD1a ⁺ cells (I)	0.729	0.004
CD1b ⁺ cells (I)	0.688	0.007
FcεR1 ⁺ cells (I)	0.665	0.009
CD8 ⁺ cells (I)	0.740	0.018
CD83 ⁺ cells (I)	0.663	0.037
IL-22 (R)	0.532	0.038
S100A8 (R)	0.583	0.065
LOR (R)	-0.804	0.008
PPL (R)	-0.755	0.015

C. Variables significantly correlated with reduction of epidermal hyperplasia following NB-UVB.					
Epidermal Thickness			K16 Expression by RT-PCR		
Disease Variable	Spearman Correlation	P-value	Disease Variable	Spearman Correlation	P-value
CD83 ⁺ cells (I)	0.638	0.044	BDCA-2 ⁺ cells (I)	0.992	<0.001
TRAIL ⁺ cells (I)	0.467	0.063	LCN2 (R)	0.93	<0.001
CCL17 (R)	0.487	0.077	Elafin (R)	0.913	<0.001
CD3 ⁺ cells (I)	0.430	0.081	CCL18 (R)	0.903	<0.001
CCL5 (R)	0.526	0.090	CCL5 (R)	0.883	<0.001
BDCA-2 ⁺ cells (I)	0.522	0.092	CCL22 (R)	0.848	0.001
CCL18 (R)	0.456	0.093	S100A7 (R)	0.806	0.002

CD1a ⁺ cells (I)	0.395	0.102	S100A8 (R)	0.729	0.008
			IVL (R)	0.536	0.055
			CCL17 (R)	0.468	0.086
			Thickness (I)	0.428	0.083

D. Variables significantly correlated with reduction in mRNA expression of the cytokine IL-22 following NB-UVB.		
Disease Variable	Spearman Correlation	P-value
BDCA-2 ⁺ cells (I)	0.752	0.016
S100A8 (R)	0.678	0.016
SCORAD	0.532	0.038
OX-40L ⁺ cells (I)	0.655	0.039
CD1b ⁺ cells (I)	0.454	0.069
S100A7 (R)	0.464	0.088
CDSN (R)	-0.591	0.036
LOR (R)	-0.590	0.036

AD: Atopic Dermatitis; CDSN: Corneodesmosin; IVL: Involucrin; K16: Keratin 16; LCN2: Lipocalin-2; LOR: Loricrin; MBP: Major basic protein; NB-UVB: Narrow-band UVB; OX-40L: OX-40 ligand; PPL: Periplakin; SCORAD: SCORing of Atopic Dermatitis index; TRAIL: TNF-related apoptosis inducing ligand.

* Spearman rank correlations were determined for all evaluated variables quantified by immunohistochemistry (denoted by “I”) and real-time PCR (RT-PCR) (denoted by “R”) with baseline disease severity (1A) and reductions in SCORAD (1B), epidermal hyperplasia (1C), and IL-22 (1D) following NB-UVB with results in order of significance. Variables with significant inverse correlations are listed after all positive correlations.