Effective Narrow-Band UVB Radiation Therapy Suppresses the IL-23/IL-17 Axis in Normalized Psoriasis Plaques

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Narrow-band UVB radiation (NB-UVB) therapy offers a well-established treatment modality for psoriasis. However, despite the common use of this form of treatment, the mechanism of action of NB-UVB is not well understood. We studied a group of 14 patients with moderate-to-severe psoriasis treated with carefully titrated and monitored NB-UVB for 6 weeks. Lesional plaques were classified as normalized ($n=8$) or nonresponsive ($n=6$) based on their histological improvement and normalization. We characterized lesional myeloid dendritic cells (DCs) and T cells and their inflammatory mediators using immunohistochemistry and real-time PCR. NB-UVB suppressed multiple parameters of the IL-23/IL-17 pathway in normalized plaques, but not in nonresponsive plaques. NB-UVB decreased the numbers of $CD11c^+$ DCs, specifically $CD1c^-CD11c^+$ "inflammatory" DCs, and their products, IL-20, inducible nitric oxide synthase, IL-12/23p40, and IL-23p19. Furthermore, effective NB-UVB suppressed IL-17 and IL-22 mRNAs, which strongly correlated with lesion resolution. Therefore, in addition to its known role in suppressing IFN-γ production, NB-UVB radiation therapy can also target the IL-17 pathway to resolve psoriatic inflammation.

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INTRODUCTION

Psoriasis, a chronic, debilitating skin disease affecting millions of Americans, is considered one of the most prevalent immune-mediated inflammatory diseases (Lebwohl, 2003). It has now become clear that infiltrating immune cells have very important roles in disease pathogenesis. T cells and dendritic cells (DCs) are significantly increased in lesional skin (LS) compared with uninvolved, ''non-lesional'' skin (NL) (Chamian *et al.*, 2005; Lowes *et al.*, 2005a). Along with the resident dermal myeloid DCs present in normal skin, an additional population of ''inflammatory'' DCs is detected in lesional psoriatic skin (Lowes *et al.*, 2005a; Zaba *et al.*, 2009a). DCs have an integral role in shaping the $CD4^+$ T-cell response, and the increased frequencies of IFN-γ and IL-17/IL-22-producing T cells observed in psoriatic lesional skin may be due to the production of IL-12 and IL-23 by inflammatory DCs, respectively (Zaba *et al.*, 2007b; Guttman-Yassky *et al.*, 2008; Lowes *et al.*, 2008; Pene *et al.*, 2008). Whereas IFN-γ and IL-17 induce chemokine expression and cellular recruitment to the skin, IL-22 leads to aberrant keratinocyte proliferation and epidermal hyperplasia (Boniface *et al.*, 2005; Sa *et al.*, 2007; Nograles *et al.*, 2008). Thus, the development of psoriatic lesions is likely due to the coordinated efforts of several inflammatory cytokine pathways and treatments targeting multiple facets of pathogenesis, and they are of great value.

UV radiation has been an effective treatment for psoriasis, as well as other skin diseases, for 85 years, since it was first used as a daily broad-band source (290–320 nm) combined with topical tar, known as the ''Goeckerman'' regimen (Goeckerman, 1925). Over the past 30 years, the introduction of fluorescent bulbs with a limited spectrum of 311–312 nm (narrow-band (NB)-UVB) has marked an advance in phototherapy, as this represents the wavelength with the optimal ''phototherapy index.'' When compared with conventional broad-band UVB therapy, treatment with NB-UVB has been found to have greater bioactivity (Walters *et al.*, 1999). However, we still do not fully understand the mechanism of action of this remarkably effective therapy.

At the cellular level, NB-UVB therapy has many immunosuppressive effects. It leads to a reduction of T cells (Carrascosa *et al.*, 2007; Erkin *et al.*, 2007) by inducing apoptosis (Krueger *et al.*, 1995; Ozawa *et al.*, 1999). Keratinocyte apoptosis also occurs as a result of *in vitro* NB-UVB (Aufiero *et al.*, 2006), but it is probably not

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Abbreviations: DC, dendritic cell; iNOS, inducible nitric oxide synthase; K16, keratin 16; LS, lesional; NB-UVB, narrow-band UVB; NL, non-lesional; PASI, psoriasis area and severity index

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contributing to disease resolution *in vivo* as it is not detected after treatment (Krueger *et al.*, 1995). In addition, some studies have found a decrease in the numbers of Langerhans cells after therapy (Murphy *et al.*, 1993; Tjioe *et al.*, 2003), although others have not found a significant decrease (Carrascosa *et al.*, 2007; Erkin *et al.*, 2007). Nevertheless, it is clear that NB-UVB impairs *in vitro* antigen presentation by both murine DCs and human Langerhans cells, rendering them tolerogenic rather than stimulatory (Noonan *et al.*, 1988; Baadsgaard *et al.*, 1990; Simon *et al.*, 1991; Murphy *et al.*, 1993; Goettsch *et al.*, 1998). Thus, NB-UVB can suppress a broad range of immune cells.

More recently, there have been efforts to understand the effect of NB-UVB on inflammatory cytokine production. NB-UVB specifically targets IFN-γ-producing Th1 cells as well as upstream cytokines, IL-12 and IL-23 (Piskin *et al.*, 2003; Walters *et al.*, 2003; Piskin *et al.*, 2004b). NB-UVB also has suppressive effects on additional inflammatory mediators, including IL-18, IL-8, IL-1β, and IL-6 (Walters *et al.*, 2003; Sigmundsdottir *et al.*, 2005). However, although it is clear that NB-UVB can suppress inflammatory cytokines, it is still unknown whether NB-UVB can similarly target pathogenic IL-17 production.

In this study, we sought to determine whether NB-UVB influences the IL-17/IL-23 axis, which is considered a critical pathogenic pathway in psoriasis (Blauvelt, 2008; Di Cesare *et al.*, 2009; Nestle *et al.*, 2009). Psoriatic skin tissue was analyzed in index plaques showing histological improvement after NB-UVB therapy (normalized) and those that did not respond (nonresponsive) by real-time PCR and immunohistochemistry. We found that NB-UVB suppressed multiple parameters of the IL-23/IL-17 pathway in normalized plaques, but not in nonresponsive plaques. Therefore, in addition to its known role in reducing IFN-γ production, NB-UVB therapy can also resolve psoriatic inflammation by targeting the IL-23/IL-17 axis.

RESULTS

Classification of response

In this study, 14 adult patients with moderate-to-severe psoriasis received a 6-week treatment regimen of regular, monitored NB-UVB radiation therapy. Psoriasis area and severity index (PASI) was decreased by over 50% (PASI 50) in the vast majority of patients (93%), whereas 28% of patients achieved over 75% improvement (PASI 75) by week 6 of treatment (Supplementary Table S1 online). Response of an index plaque to NB-UVB was further evaluated histologically by measuring epidermal thickness and keratinocyte differentiation by keratin 16 (K16) staining. Histological normalization in an index plaque does not always correlate with global clinical improvement as assessed by PASI. Plaques with epidermal thinning and normalization of K16 staining and mRNA expression by week 6 after treatment were classified as normalized (Figure 1a, c, and d), whereas nonresponsive plaques retained a thickened epidermis and strong K16 staining and mRNA (Figure 1b–d). Of the 14 plaques, 8 were histologically classified as normalized, whereas 6 were considered nonresponsive. Subsequent experiments presented in this study compare the outcome of NB-UVB therapy in both normalized and nonresponsive plaques.

We also used multivariate μ-scores (Wittkowski *et al.*, 2004) to generate a ''response score'' for each plaque, taking into account both percentage change in epidermal thickness and K16 mRNA. Plaques were ranked according to their response scores; the lower the response score, the better the improvement of the plaque. The six plaques classified as nonresponsive had the highest response scores, whereas the remaining normalized plaques were ranked with lower response scores (Supplementary Table S1 online). This analysis provided a nonbiased, quantitative method to correlate response with the percentage changes in gene expression or cell counts over the treatment period.

Inflammatory DCs were decreased in normalized plaques

To begin to dissect the immune mechanism of disease control by NB-UVB therapy, immunohistochemistry for DC markers was performed on skin sections before and after treatment. ''Resident'' myeloid DCs in normal dermis are identified by $CD11c^+$ and CD1c (Zaba *et al.*, 2009b). $CD1c^+$ cells were not present in the epidermis. Dermal $CD1c^+$ cells did not change in number during treatment with etanercept (Zaba *et al.*, 2007a), and similarly did not change during the course of NB-UVB in normalized plaques (Figure 2a and c). LS skin of nonresponsive plaques had a slight increase in $CD1c^+$ cells over NL skin, although this result was not statistically significant.

In psoriasis lesions, we have previously found a dramatic increase in a second population of myeloid ''inflammatory'' DCs (Lowes *et al.*, 2005a; Zaba *et al.*, 2009a) that express CD11c, but not CD1c. Treatment of psoriasis with efalizumab, alefacept, cyclosporine, or etanercept decreased these inflammatory myeloid DCs (Chamian *et al.*, 2005; Lowes *et al.*, 2005b; Zaba *et al.*, 2007a; Haider *et al.*, 2008). The effect of NB-UVB on inflammatory DCs was assessed using immunohistochemistry. Although fewer dermal $CD11c^+$ DCs were observed in NL skin compared with LS skin (Figure 2b and d), by week 6 of NB-UVB, dermal $CD11c^+$ DCs were significantly reduced in normalized plaques, but not in nonresponsive plaques. Similar results were observed in the epidermis (Supplementary Figure S1a online).

Currently, there is no positive marker to identify the inflammatory DCs. However, an approximation of the number of $CD11c^+CD1c^-$ inflammatory DCs can be calculated by subtracting the number of $CD1c^+$ cells from $CD11c^+$ cells. Normalized plaques had decreased dermal $CD11c^+CD1c^-$ inflammatory DCs, whereas nonresponsive plaques retained a population of these cells (Figure 2e). To assess the relationship between response and the downregulation of these cell subsets, we used multivariate μ-scores as described above. There was a strong correlation between epidermal $CD11c^+$ cells and response score ($r=0.863$, $P=0.002$; Supplementary Figure S1b online). Similarly, dermal $CD11c^+$ DCs correlated significantly with response ($r=0.675$, $P=0.015$), whereas dermal $CD1c^+$ cells did not ($r=0.255$, $P=0.362$; Supplementary Figure S2 online). Moreover, inflammatory DCs also had a significant

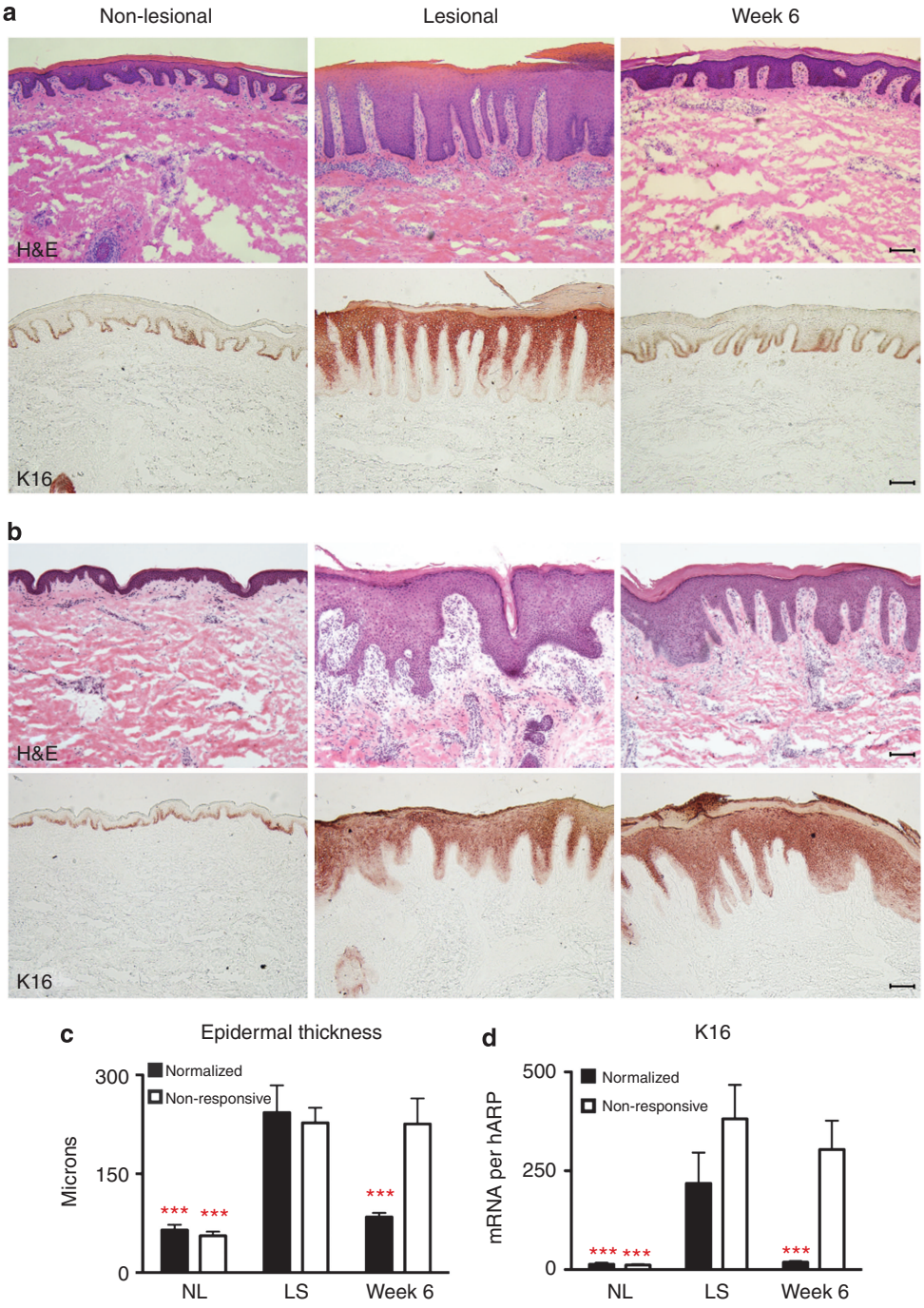

Figure 1. Classification of normalized and nonresponsive plaques. (**a**, **b**) Histological response was measured at baseline in non-lesional (NL) and lesional (LS) skin and skin at 6 weeks after treatment (week 6). Representative histology and immunohistochemistry showing hematoxylin and eosin (H&E) and K16 expression in (**a**) normalized plaques and (**b**) nonresponsive plaques. Scale bar = 100 μm. (**c**) Epidermal thickness of normalized (black bars, $n = 7$) and nonresponsive plaques (white bars, $n = 6$) before and after NB-UVB therapy. Error bars represent the mean ± SEM. NL and week 6 values are compared with LS. ***$P < 0.001$. (**d**) K16 mRNA expression normalized to *hARP* in both normalized (black bars) and nonresponsive plaques (white bars). Error bars represent the mean ± SEM.

correlation with response score ($r = 0.664$, $P = 0.017$; Supplementary Figure S2 online). To further characterize these DCs, two-color immunofluorescence was performed using antibodies against CD11c and CD1c. The majority of cells in NL skin costained for these two markers (Supplementary Figure S3 online), whereas in LS skin, there was a higher proportion of cells staining for CD11c, but not CD1c (Figure 2f). After 6 weeks of NB-UVB, most cells were $CD11c^+CD1c^+$ in normalized plaques, suggesting a decrease in inflammatory DCs with effective treatment. Nonresponsive plaques maintained a population of $CD11c^+$ $CD1c^-$ DCs after treatment (data not shown). These data

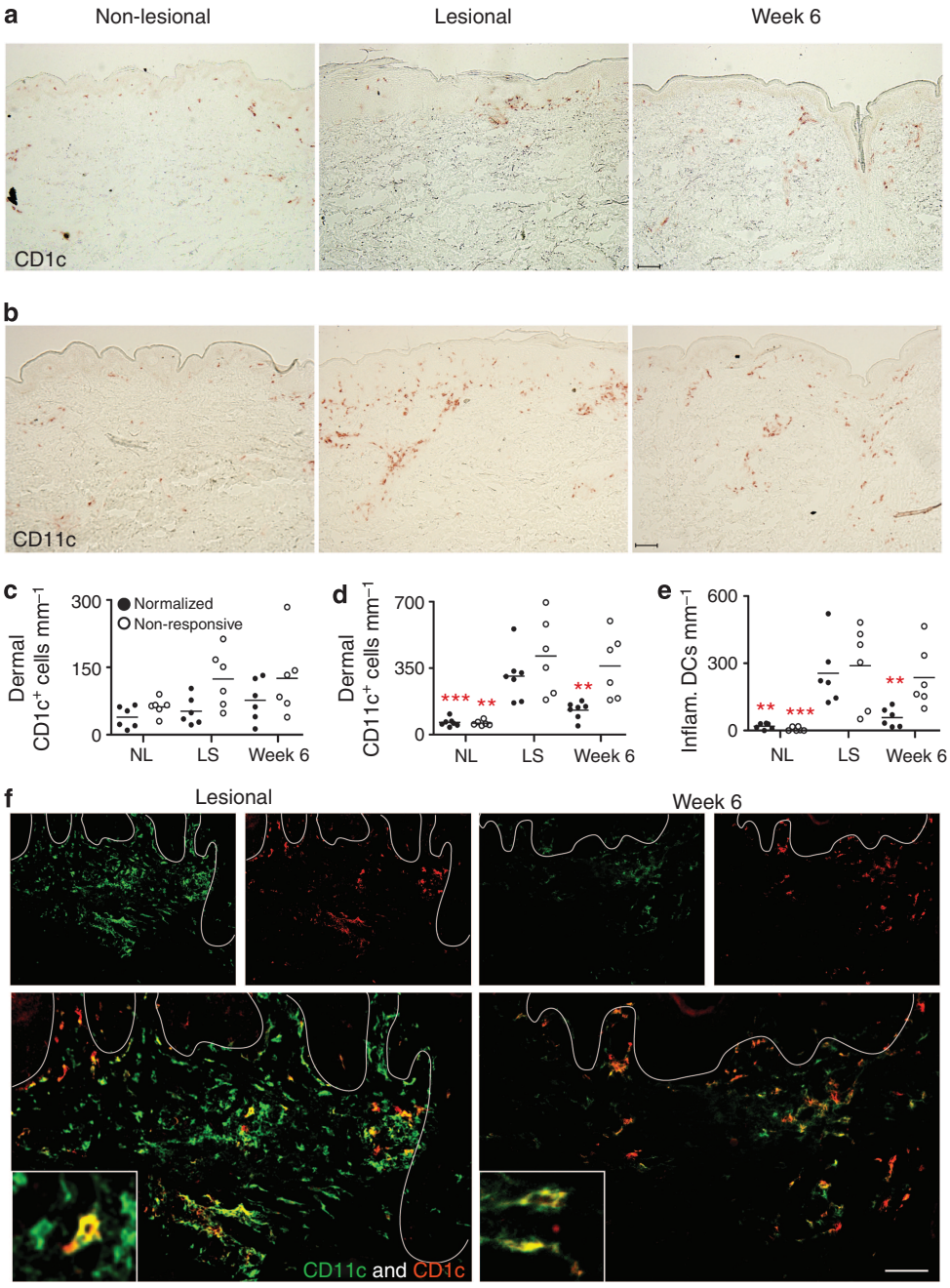

Figure 2. Inflammatory myeloid dendritic cells (DCs) are reduced in normalized plaques. (**a**, **b**) Representative immunohistochemistry of $CD1c^+$ and $CD11c^+$ cells. Bar = 100 μm. (**c**, **d**) Quantification of dermal $CD1c^+$ or $CD11c^+$ cells in normalized (black) and nonresponsive plaques (white). Each circle represents a plaque. Non-lesional (NL) and week 6 values are compared with lesional (LS) skin values. $^{**}P<0.01$, $^{***}P<0.001$. (**e**) $CD11c^+CD1c^-$ cell numbers were calculated by subtracting $CD1c^+$ counts from $CD11c^+$ counts. (**f**) Two-color immunofluorescence of CD11c (green) and CD1c (red) of normalized plaque. Cells coexpressing both markers appear yellow because of the superimposition of both green and red signals. Inset, high-power magnification of double-positive cells. The white line delineates the dermoepidermal junction. Scale bar = 100 μm.

support our concept that inflammatory DCs may be an important pathogenic cell population in psoriasis.

Decreased DC cytokines in normalized plaques

We have previously shown that inflammatory myeloid DCs produce tumor necrosis factor, inducible nitric oxide synthase (iNOS), IL-20, and IL-23 (Lowes *et al.*, 2005a; Zaba *et al.*, 2007a; Guttman-Yassky *et al.*, 2008). To evaluate the expression of these cytokines during NB-UVB treatment, we performed quantitative real-time PCR on pre- and post-treatment skin RNA. There were significantly increased iNOS, IL-20, IL-12/23p40 and IL-23p19 in LS tissue compared with NL baseline skin ($P<0.05$ for most comparisons; Figure 3a). However, significant decreases were only observed in plaques that normalized after NB-UVB treatment ($P<0.001$ for all groups). In contrast, expression of these

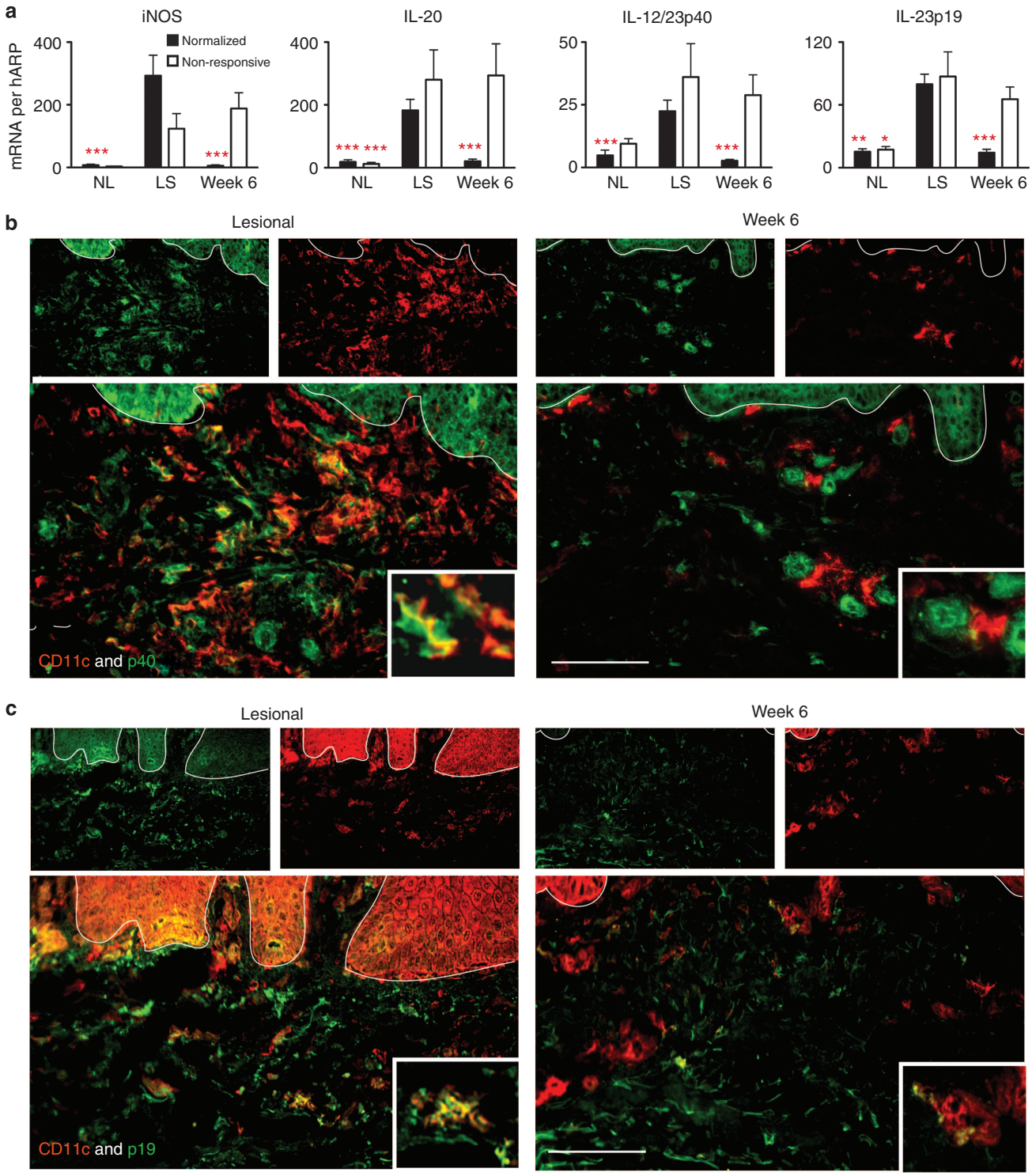

Figure 3. Decreased dendritic cell (DC) products in normalized plaques. (**a**) mRNA expression levels normalized to *hARP* for the inflammatory DC products, inducible nitric oxide synthase (iNOS), IL-20, IL-12/23p40, and IL-23p19, in both normalized (black bars, $n=8$) and nonresponsive plaques (white bars, $n=6$). Error bars represent the mean ± SEM. Non-lesional (NL) and week 6 levels are compared with lesional (LS) skin levels. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. (**b**, **c**) Two-color immunofluorescence of $CD11c^{+}$ myeloid DCs (red) with IL-23 subunits in a normalized plaque, (**b**) p40 and (**c**) p19 (green), showing coexpression in baseline LS skin (yellow cells), which is diminished by week 6 after therapy. Antibodies conjugated with a fluorochrome gave background epidermal fluorescence. The white line delineates the dermal epidermal junction. Inset, high-power magnification of double-positive cells. Scale bar = 100 μm.

cytokines remained high in nonresponsive plaques. Response scores significantly correlated with IL-23p19 mRNA expression ($r=0.743$, $P=0.008$) and IL-20 ($r=0.88$, $P=0.002$). We further confirmed the expression of IL-23 subunits at the protein level by performing two-color immunofluorescence with CD11c versus p40 or p19. Little p40 and p19 proteins

were observed in NL skin (Supplementary Figure S4 online). LS skin showed $CD11c^+$ cells that costained for both IL-23 subunits, and by week 6 there was a reduction in both $CD11c^+$ cells and the IL-23 subunits in normalized plaques (Figure 3b and c). Some cells expressed p40 or p19, but did not costain with CD11c, and are most likely macrophages (Lowes, NA; unpublished data). Furthermore, in nonresponsive plaques, p19 and p40 protein expression was not reduced by NB-UVB at week 6 (Supplementary Figure S5 online). These findings indicate that after NB-UVB therapy, normalization of psoriasis lesions is associated with a reduction in these inflammatory mediators, further implicating their potential role in psoriasis (summarized in Supplementary Figure S6 online).

Decreased T cells in normalized plaques

We have previously shown that NB-UVB therapy effectively depletes T cells by inducing apoptosis (Ozawa *et al.*, 1999). We confirmed this finding showing a significant increase in LS $CD3^+$ T cells compared with NL skin in both dermis (Figure 4a and b) and epidermis (Supplementary Figure S1c online) that were reduced after NB-UVB ($P<0.01$). However, we extend this observation to show that a significant reduction in $CD3^+$ T cells only occurs in plaques responding to NB-UVB ($P<0.001$; Figure 4b). To access the biological significance of the varying degrees of $CD3^+$ T-cell depletion with treatment, we correlated $CD3^+$ T cells with response scores. There was a strong, statistically significant correlation between dermal $CD3^+$ T cells and response scores ($r=0.791$, $P=0.004$; Figure 4c), as well as epidermal $CD3^+$ T cells versus response scores ($r=0.708$, $P=0.011$; Supplementary Figure S1d online). These results indicate that a positive response to NB-UVB is associated with a profound decrease in T cells, in addition to the myeloid inflammatory DCs discussed above.

IL-17 and IL-22 were reduced in normalized plaques

Recent studies have implicated IL-17 and IL-22 in psoriatic inflammation (Lowes *et al.*, 2008; Harper *et al.*, 2009). We performed real-time PCR of skin tissue RNA to evaluate pathogenic cytokine expression. We confirmed that IL-17, IFN-γ, and IL-22 were increased in LS tissue compared with NL tissue, and there was a significant reduction in these cytokines in normalized plaques ($P<0.001$ for all; Figure 5a). In contrast, there was no reduction in these three cytokines in nonresponsive plaques at week 6. We also found increased LS expression of β-defensin-4 ($P<0.01$) and myxovirus resistance-1 ($P<0.01$), the downstream target genes of IL-17 and IFN-γ, respectively, which decreased with NB-UVB treatment only in normalized plaques (data not shown). When cytokine mRNA was correlated with response scores, a significant correlation was found with IL-22 ($r=0.932$, $P=0.001$) and IL-17 ($r=0.868$; $P=0.002$; Figure 5b). On the other hand, there was no correlation between IFN-γ mRNA expression and response score ($r=0.214$, $P=0.445$), which may indicate that expression of IFN-γ mRNA is not necessarily associated with lesion resolution in response to NB-UVB. These cytokine data support earlier studies showing the importance of IL-17 and IL-22 in psoriatic inflammation, but to our knowledge, it is previously unreported that suppressing this axis is associated with histological normalization after NB-UVB.

It is difficult to ascertain whether cytokine-related changes observed in the skin at the end of therapy are a direct result of NB-UVB radiation or simply a reflection of the improvement of psoriasis in general. Therefore, using intracellular cytokine staining techniques, we examined the ability of a single irradiation of 312 nm NB-UVB to modulate cytokine expression *in vitro*. As serial shave biopsies are difficult to obtain for isolation of lesional T cells, we used peripheral blood mononuclear cells as a surrogate source of T cells. Peripheral blood mononuclear cells of normal volunteers were irradiated with different doses (25 or 50 mJ) of NB-UVB, and cytokine synthesis was measured after a 4-hour stimulation. As previously observed, IFN-γ was decreased by an average of 85% (Supplementary Figure S7a online). Furthermore, $CD3^+$ T-cell production of IL-22 and IL-17 was significantly decreased after NB-UVB by an average of 45 and 89%, respectively (Supplementary Figure S7b online). These data suggest that NB-UVB directly modulates production of these pathogenic cytokines. Taken together, our results indicate that NB-UVB radiation can target the IL-23/IL-17 pathway to resolve inflammation.

DISCUSSION

In this paper, we have focused on the effects of NB-UVB at the cellular and cytokine/inflammatory mediator levels to define a set of outcomes that were necessary for histological normalization after NB-UVB. Specifically, for lesion resolution there must be a reduction in myeloid inflammatory DCs and T cells and inflammatory products (iNOS, IL-23, IL-20, IFN-γ, IL-17, and IL-22). Previous studies characterizing the effects of NB-UVB in psoriasis have shown a downregulation of IFN-γ (Piskin *et al.*, 2003, 2004a, b), but this study demonstrated that there is a greater correlation between decreased IL-22/IL-17 expression and histological improvement. We have previously shown that this general set of cell and cytokine alterations is also required for response to other therapies for psoriasis, including alefacept, efalizumab, etanercept, and cyclosporine (Chamian *et al.*, 2005; Lowes *et al.*, 2005b; Zaba *et al.*, 2007a; Haider *et al.*, 2008). Despite the fact that all of these agents work in such different ways—targeting CD2, CD11a, tumor necrosis factor, and T cells, respectively—their common feature is that they are all immunosuppressive. However, it is still unclear whether suppressing the IL-23/IL-17 axis is simply because of the depletion of immune cells by a particular therapy, or whether the therapy itself can specifically inhibit cytokine production. Previous studies using flow cytometry have shown that production of IFN-γ by dermal T cells is decreased after UVB irradiation, indicating that UVB can directly inhibit cytokine production by T cells (Piskin *et al.*, 2003). In this study we also report that, *in vitro*, NB-UVB can directly suppress T-cell production of IL-17 and IL-22.

Some studies have suggested that the clearing of the inflammatory cells is specifically related to how far the

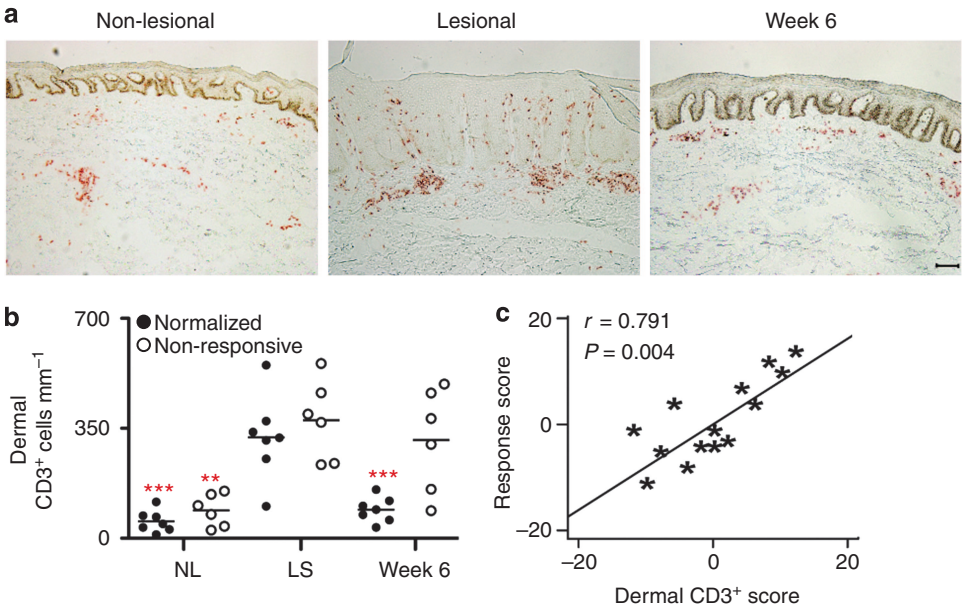

Figure 4. T cells are reduced in normalized plaques. (**a**) Representative immunohistochemistry of $CD3^+$ cells in non-lesional (NL), lesional (LS), and week 6 skin of a normalized plaque. Scale bar = 100 μm. (**b**) Quantification of $CD3^+$ T-cell counts per mm of skin in both normalized (black circles) and nonresponsive plaques (white circles). NL and week 6 counts are compared with LS skin counts. $^{**}P<0.01$, $^{***}P<0.001$. (**c**) Response scores were correlated with μ-scores for $CD3^+$ cell counts. Spearman's correlation coefficients (*r*) and *P*-values are shown.

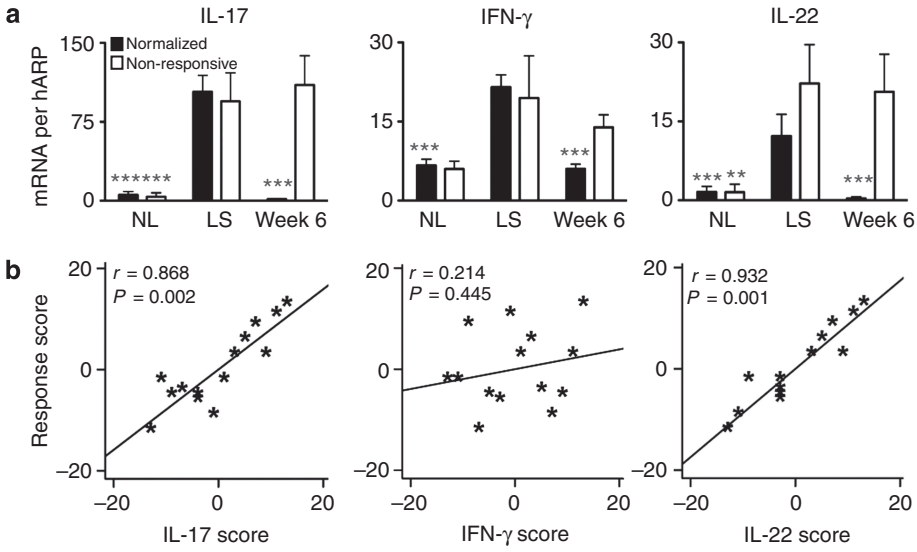

Figure 5. Decreased IL-17 and IL-22 after therapy. (**a**) mRNA expression levels normalized to *hARP* for the T-cell products, IL-17, IFN-γ, and IL-22, in both normalized (black bars, $n=8$) and nonresponsive plaques (white bars, $n=6$) in non-lesional (NL), lesional (LS), and week 6 skin. Error bars represent the mean ± SEM. NL and week 6 levels are compared with LS skin levels. $^{**}P<0.01$, $^{***}P<0.001$. (**b**) Response scores were correlated with μ-scores for mRNA expression. Spearman's correlation coefficients (*r*) and *P*-values are shown.

UV radiation can penetrate (Bruls *et al.*, 1984; Krueger *et al.*, 1995). For example, psoralen plus ultraviolet light A therapy, which uses psoralen and UVA radiation, penetrates much deeper than UVB radiation and is an even more effective therapeutic agent for psoriasis (Yones *et al.*, 2006; Mahmoud *et al.*, 2008), although it is no longer a preferred method of treatment as it seems to increase the risk of skin cancer (Patel *et al.*, 2009). At the cellular level, sufficient penetration of NB-UVB into the upper dermis results in the clearing of myeloid DCs and T cells, which seems necessary for reduction of inflammatory cytokine production. Furthermore, previous studies have suggested that the dermal T cells that remain after NB-UVB produce less inflammatory cytokines, such as IFN-γ (Piskin *et al.*, 2004a). Although we did not directly assess IL-17 and IL-22 production by lesional T cells, our *in vitro* results suggest that NB-UVB can also alter production of proinflammatory IL-17 and IL-22.

Although some studies have shown that NB-UVB therapy can deplete $CD1a^+$ Langerhans cells (Murphy *et al.*, 1993; Tjioe *et al.*, 2003), to our knowledge, it is previously

unreported that it can also deplete dermal inflammatory myeloid $CD11c^+$ DCs, both in the epidermis and the dermis. Nonresponsive plaques retained a population of $CD11c^+CD1c^-$ DCs and concurrent mRNA expression of inflammatory DC products, indicating that depletion of these cells is necessary for resolution of psoriatic lesions. As DCs are the bridge between the innate and adaptive immune systems driving subsequent T-cell responses, it seems logical that they need to be eliminated first to prevent further T-cell activation.

There have been some concerns stemming from data in animal models that targeting IL-12 and IL-23 with monoclonal antibodies will potentially increase overall cancer risk (Maeda *et al.*, 2006). In humans, systemic immunosuppression clearly increases the risk of skin carcinomas and alters their aggressiveness (Berg and Otley, 2002). Hence, it is important to establish the potential relationship between immunosuppression in human skin and associated cancer risk. Suppression of p40 cytokine synthesis in the skin during NB-UVB raises the same potential concerns as blocking p40 cytokines using monoclonal antibodies. Recent studies have shown that careful, long-term administration of NB-UVB is not associated with increased risk in any skin cancers, including basal and squamous cell carcinomas and melanoma (Weischer *et al.*, 2004; Hearn *et al.*, 2008; Patel *et al.*, 2009). Neither is there any reported risk of internal cancer from NB-UVB therapy. Thus, blocking the IL-23/IL-17 pathway in the skin with NB-UVB seems to be relatively safe and noncarcinogenic, suggesting that targeting this axis *per se* does not lead to cutaneous cancer. Another factor to consider is IL-22, as it has been shown to promote some cancers, probably because of its trophic effects on epithelia (Bard *et al.*, 2008; Zhang *et al.*, 2008). This procarcinogenic factor is also reduced by UVB therapy.

In conclusion, this study clearly shows the set of changes that are necessary for resolution of psoriasis lesions: a reduction in myeloid inflammatory DCs and their products (iNOS, IL-23, and IL-20) associated with a decrease in T cells and additional pathogenic cytokines (IL-17, IFN-γ, and IL-22). These changes were not observed in nonresponsive plaques. These results establish a group of cells and products that must be effectively targeted for clearing of psoriasis, and confirm the IL-23/IL-17 pathway as an essential therapeutic target.

MATERIALS AND METHODS

Study design and skin biopsies

We conducted a therapeutic clinical trial under the Rockefeller University institutional review board-approved protocol in which 14 psoriasis patients received treatment with regular, monitored NB-UVB radiation therapy (Supplementary Table S1 online). This trial is registered at clinicaltrials.gov under NCT00220025. Informed consent was obtained and the study was performed in adherence with the Declaration of Helsinki Principles. Patients were initially treated with 50% of their minimal erythemal dose, with increments of 5–10% 3 to 4 times per week, for up to 6 weeks. Skin punch biopsies, 6 mm in diameter, from NL skin were taken before treatment as a baseline; pre- and post-treatment lesional biopsies were taken from an index plaque. Each biopsy was cut in two: half was stored in optimal cutting temperature compound for cryosections, and half snap-frozen in liquid nitrogen for RNA extractions. Skin tissue from one plaque (normalized) was only used for RNA.

Antibodies

All antibodies used for immunohistochemistry, immunofluorescence, and flow cytometry are listed in Supplementary Tables S2–S4 online.

Immunohistochemistry

Skin sections were stained as previously described (Zaba *et al.*, 2007b). Positive cells per mm were counted manually using computer-assisted image analysis software (ImageJ 1.42, National Institutes of Health, Bethesda, MD).

Immunofluorescence

Skin sections ($n=3$ for normalized and nonresponsive plaques) were stained as previously described (Zaba *et al.*, 2007b). Images were acquired using appropriate filters of a Zeiss Axioplan 2I microscope with Plan Apochromat 20×0.7 numerical aperture lens and a Hagamatsu orca ER-cooled charge-coupled device camera, controlled by METAVUE software (MDS Analytical Technologies, Dowingtown, PA). Dermal collagen fibers gave green autofluorescence. Antibodies conjugated with a fluorochrome often gave background epidermal fluorescence. Cells coexpressing both markers appear yellow because of the superimposition of both green and red signals. The white line delineates the dermal epidermal junction.

mRNA extraction and real-time PCR

RNA extraction and real-time PCR using Taqman gene expression assays (Supplementary Table S5 online) were performed as previously described (Chamian *et al.*, 2005). Custom primers for IFN-γ, K16, and IL-12/23p40 were generated as previously described (Chamian *et al.*, 2005). Data normalized to *hARP* housekeeping gene were quantified by software provided with Applied Biosystems (Foster City, CA) PRISM 7700 (Sequence Detection Systems, version 1.7). The normalized PCR data were $\log_2$ transformed before statistical analysis.

In vitro NB-UVB irradiation and flow cytometry

Peripheral blood mononuclear cells were prepared from heparinized venous blood of healthy volunteers by Ficoll sedimentation ($n=3$). Cells (10^6 cells ml^{-1}) were irradiated with NB-UVB (TL-01) at varying doses (25 and 50 mJ cm^{-2}) in uncovered tissue culture plates in phosphate-buffered saline, then stimulated for 4 hours with phorbol myristate acetate (25 ng ml^{-1}), ionomycin (1 μg ml^{-1}), and brefeldin A (10 μg ml^{-1}) (all from Sigma-Aldrich, St Louis, MO). Unactivated controls were treated with brefeldin A only. Cells were stained as previously described (Zaba *et al.*, 2009a). Samples were acquired by an LSR-II flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Treestar, Ashland, OR).

Statistical analysis

A repeated measures analysis of variance with Tukey's multiple comparison test was used to compare reverse transcriptase PCR data and cell counts from LS skin versus respective NL skin or post-treatment skin pairs. A P-value of <0.05 was considered significant.

Generation of response score. The percentage change of each gene or leukocyte count was calculated using the following formula: ((week 6−LS)/(LS−NL) × 100). Multivariate μ-scores (Wittkowski *et al.*, 2004) were then used to produce an overall histological response score (''response score'') for each plaque, combining the percentage change in epidermal thickness and K16 mRNA. We have used this multi-variant method previously (Haider *et al.*, 2008). The lower the response score, the better the improvement of the index plaque. The μ-scores were also calculated for the percentage change in expression for each gene or cell count (e.g., IL-17 score) and were then correlated with response score by Spearman-type correlation coefficients (*r*).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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

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