

Ustekinumab treatment in severe atopic dermatitis: Down-regulation of T-helper 2/22 expression

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Background: It has recently been suggested that patients with moderate to severe atopic dermatitis (AD) may profit from anti-interleukin (IL)-12/-23 p40 therapy.

Objective: We sought to assess the immunologic effects of ustekinumab treatment on AD skin and to correlate them with the clinical efficacy of this drug.

Methods: We investigated the course of 3 patients with severe AD who were administered 45 mg of subcutaneous ustekinumab over a period of 16 weeks. Clinical scores and skin biopsy specimens, taken at baseline and at week 8, were used to assess changes in disease severity.

Results: All patients showed a gradual improvement of the disease, achieving a 50% reduction in the Eczema Area and Severity Index score by week 16. Immunohistology of skin biopsy specimens revealed a significant decrease in the degree of epidermal hyperplasia/proliferation and the number of infiltrating dermal T cells, dendritic cells, and mast cells after treatment. Using quantitative real-time polymerase chain reaction of lesional skin, we found a clear reduction of T-helper 2/22-associated molecules after therapy.

Limitations: The small number of patients (n = 3) limits efficacy analysis and warrants prospective placebo-controlled studies in larger patient cohorts.

Conclusion: Blocking IL-12/-23 p40 could be beneficial for a subgroup of patients with severely infiltrated AD. (J Am Acad Dermatol <http://dx.doi.org/10.1016/j.jaad.2016.07.047>.)

Key words: anti-interleukin-12/-23 p40; atopic dermatitis; cytokines; eczema; inflammatory; T helper 2; T helper 17; T helper 22; ustekinumab.

Atopic dermatitis (AD) is a stressful chronic inflammatory skin disease that affects about 15% to 30% of children and 2% of the adult population.¹ Genetic susceptibility, such as loss-of-

function mutations in the filaggrin gene, an aberrant innate immune response, and environmental factors lead to epidermal barrier impairment and progressive T-cell activation.¹⁻³ The mainstay of

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treatment consists of emollients, topical steroids, and various systemic immunosuppressive drugs with substantial side effects.³ Up to now there has been no effective and safe treatment for AD, but future biologic agents targeting specific T-cell pathways offer a promising perspective for disease management.^{4,5}

Currently, AD is regarded as a predominantly T-helper (Th)2-driven disease with a switch to Th1 in the chronic phase, but Th17 and Th22 cell subsets are increasingly recognized to contribute to cutaneous inflammatory responses.^{1,6-11}

Ustekinumab, a Food and Drug Administration–approved human monoclonal antibody for plaque psoriasis, targets the p40 subunit shared by interleukin (IL)-12 and IL-23.¹² Antigen-presenting cell–derived IL-12 has the capacity to skew

T-cell responses in a Th1 direction, whereas IL-23 is a key factor involved in Th17 cell differentiation.^{6,12,13}

The administration of ustekinumab in patients with AD has only been demonstrated in a handful of reports and results are conflicting.^{14,15} To assess the effects of anti-IL-12/-23 p40 treatment on atopic skin and correlate its immunologic effects with its clinical efficacy, we treated 3 individuals with severe recalcitrant AD with subcutaneous ustekinumab. By doing so, we sought to evaluate the impact of ustekinumab on clinical disease parameters and on quality and quantity of the inflammatory response operative in AD.

METHODS

Patients

An individual treatment attempt with ustekinumab (45 mg) was offered to 3 patients with severe therapy-refractory AD, diagnosed according to the criteria of Hanifin and Rajka.¹⁶ All patients had previously been subjected to various treatment modalities (Table I).

Written informed consent for off-label use of ustekinumab was sought from all subjects and approval for data analysis was obtained from the Ethics Committee of the Medical University of Vienna (EK 1418/2015). Clinically significant abnormal laboratory values and active infection (tuberculosis, HIV, hepatitis A/B/C) were ruled out before the first dose and routine electrocardiography tests were performed.

Contrary to the established dosing protocol for psoriasis, the patients were administered subcutaneous doses of 45 mg of ustekinumab according to their body weight (<100 kg) at weeks 0, 4, and 12 and subsequently every 8 weeks.

Clinical parameters

Clinical disease activity scores (Eczema Area and Severity Index [EASI],¹⁷ SCORAD index,¹⁸ 5-D itch scale¹⁹) and routine laboratory investigations including total serum IgE levels were performed at monthly intervals.

Skin biopsy specimens

We obtained 4-mm punch biopsy specimens from chronic lesions on corresponding body sites (ie, upper/lower extremities) at week 0 and week 8 according to an institutional protocol (EK 71/2005). Skin biopsy specimens from 5 healthy volunteers were used as control group (EK 351/2000).

Immunohistochemistry and immunofluorescence

The monoclonal antibodies used for all staining are shown in [Supplementary Table I](#). Immunohistochemistry on frozen tissue was performed as described previously using a staining kit (Vectastain Elite ABC kit, mouse IgG, Vector Laboratories, Burlingame, CA).²⁰ To determine the phenotype and number of infiltrating leukocyte subsets, double immunofluorescence staining was performed as described previously.²¹ In brief, after incubation with the primary antibody overnight at 4°C, an appropriate second step was applied (Rhodamine [TRITC]-conjugated AffiniPureF[ab']₂ fragment goat antimouse IgG [H+L], Jackson ImmunoResearch Laboratories, West Grove, PA; Streptavidin APC, Becton Dickinson, Franklin Lakes, NJ). Secondary fluorescence-labeled antibodies were then applied for at least 6 hours at 4°C. In all staining experiments appropriate negative controls were performed.

Evaluation of immunohistochemical and immunofluorescence results

Data acquisition and analysis were performed by using the TissueFAXS technology and HistoQuest

CAPSULE SUMMARY

- Atopic dermatitis is a chronic debilitating skin disease that is often refractory to conventional treatment.
- Blocking interleukin (IL)-12/-23 p40 reduces epidermal hyperplasia and down-regulates T-helper 2/22 expression, leading to a clinically relevant improvement of the disease.
- Anti-IL-12/-23 p40 therapy may be beneficial in a subgroup of patients with severely infiltrated skin lesions.

Abbreviations used:

AD:	atopic dermatitis
EASI:	Eczema Area and Severity Index
IL:	interleukin
mRNA:	messenger RNA
Th:	T-helper

and TissueQuest software (TissueGnostics, Vienna, Austria), respectively, as previously described.²⁰

Statistical analysis

Two-tailed paired *t* tests were performed to evaluate changes in parameters at baseline and after treatment. Data observed were displayed by dot plots showing individual measurements for all patients and control subjects, if applicable. Clinical disease activity was described by calculating the mean value and SD. Given the small sample size (*n* = 3) *t* tests are only valid under the assumption of normally distributed data.

RESULTS

Clinical efficacy and safety

The patients maintained their usual skin care regimen, which predominantly consisted of moisturizers. The mean EASI and SCORAD index before treatment were 39.5 ± 7.3 and 68.4 ± 16.1 , respectively. After ustekinumab initiation all patients experienced a gradual improvement of clinical disease activity with consecutive injections, achieving a 50% reduction in the EASI score by week 16 ($P < .0005$; 16.0 ± 6.6) (Fig 1, A and B). This was also reflected by a decrease in the SCORAD index to 38.3 ± 9.5 ($P = .0186$) and a substantial reduction of pruritus according to the visual analog scale and the multidimensional 5-D itch scale ($P < .05$) (Fig 1, C). Detailed analysis of distinct SCORAD parameters revealed predominant improvement of erythema, induration/papulation, and xerosis throughout the observational period.

The administration of ustekinumab was generally well tolerated and patient compliance was excellent. Reported adverse events were limited to a common cold of moderate intensity in 1 patient.

Epidermal effects of ustekinumab

Ustekinumab significantly reduced epidermal hyperplasia as determined by measuring epidermal thickness before and after therapy ($P < .05$) (Fig 2, A). Consistently, the abnormally expressed epithelial antigen keratin 16 and the elevated proliferation marker Ki-67 were substantially reduced after ustekinumab dosing (Fig 2, B to D). These findings are in

Table 1. Patient characteristics

	Patient 1	Patient 2	Patient 3
Age, y	27	55	46
Sex	Male	Female	Male
Onset of AD	Infancy	Infancy	Infancy
Family history for atopy	Positive	Positive	Negative
IgE level, kU/L	>5000	>5000	>5000
Allergic rhinitis	No	Yes	Yes
Childhood asthma	Yes	No	No
Asthma currently active	No	Yes	No
Filaggrin mutation	Yes	Yes	No
Previous therapies			
Oral corticosteroids	Yes	No	No
PUVA	No	Yes	Yes
Narrowband UVB	Yes	No	Yes
Cyclosporin A	Yes	No	No
MTX	Yes	No	No
Topical therapy	TCl, TS	TCl, TS	TCl, TS

AD, Atopic dermatitis; MTX, methotrexate; PUVA, psoralen plus ultraviolet A; TCl, topical calcineurin inhibitors; TS, topical steroids; UV, ultraviolet.

line with our clinical observation of a predominant reduction in cutaneous induration and xerosis. Although ustekinumab treatment led to a clear reduction of epidermal hyperproliferation and hyperplasia, these parameters could not be completely converted back to the state of healthy control skin.

Alterations of the inflammatory infiltrate after anti-IL-12/-23 p40 treatment

Further characterization revealed a stable number of epidermal Langerhans cells throughout the treatment period, which was comparable with healthy skin (data not shown). CD11c⁺/HLA-DR⁺ inflammatory dendritic epidermal cells, which are considered to be a hallmark of AD and have the capacity to skew the atopic immune response in a Th1 direction,²² were significantly reduced after ustekinumab therapy ($P < .05$) (Fig 3, A). Dermal mast cells, as quantified by CD117 and IgE immunoreactivity, also demonstrated a statistically significant decrease upon inhibition of IL-12/-23 p40 ($P < .05$) (Fig 3, B).

Interestingly, the total number of IgE-bearing cells remained fairly stable within atopic skin before and after ustekinumab treatment. Likewise, total serum IgE levels in our cohort of patients with extrinsic AD remained unaltered over the treatment period (data not shown).

We further observed a statistically significant decrease in epidermal CD4⁺ helper and CD8⁺ cytotoxic T cells ($P < .005$, data not shown), and dermal CD4⁺ Th cells ($P < .05$) (Fig 3, C, E, and F), although not completely to the level of healthy

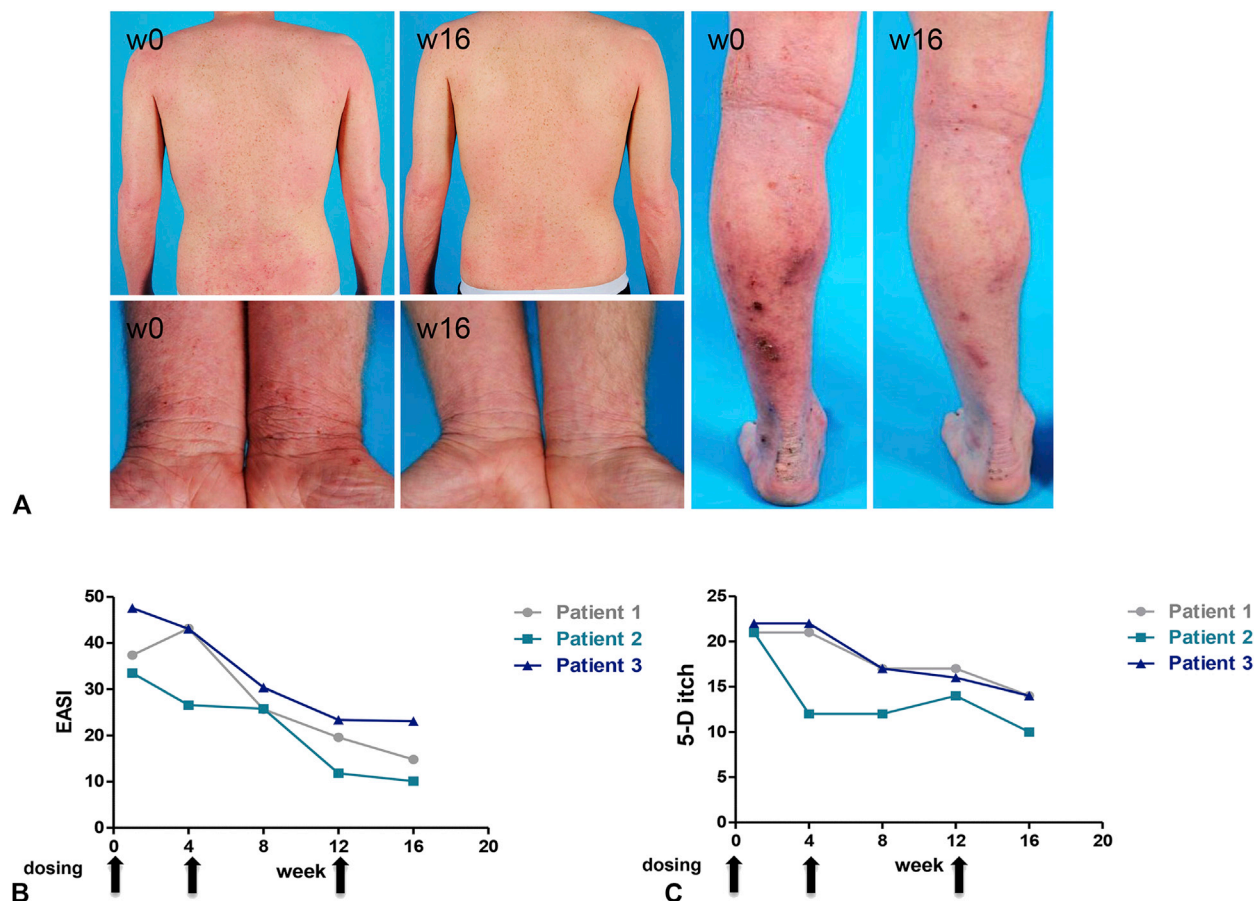


Fig 1. Ustekinumab therapy partially improves clinical disease activity in patients with atopic dermatitis. **A**, Representative clinical photographs from 2 different patients demonstrate clinical improvement over the course of treatment with ustekinumab from week 0 (*w0*) to week 16 (*w16*). **B**, Continuous improvement of clinical severity in all 3 patients after 16 weeks of treatment as quantified by the Eczema Area and Severity Index (*EASI*) score. Dosing of 45 mg of subcutaneous ustekinumab was performed at week 0, 4, and 12. The *EASI* score can range from 0 to 72, with higher numbers indicating more extensive disease. **C**, Reduction of itch after ustekinumab treatment according to the 5-D itch scale. Possible scores range from 5 to 25, with higher numbers indicating greater itch.

control skin. Dermal CD8⁺ cytotoxic T cells were substantially reduced upon treatment (Fig 3, D to F).

Down-regulation of Th2- and Th22-related cytokines

We next sought to determine the effects of ustekinumab on the molecular fingerprint of AD lesions by measuring messenger RNA (mRNA) levels of chemokines and cytokines involved in distinct T-cell pathways (Supplementary Fig 1). A clear reduction in gene expression levels of Th2- and Th22-associated products was found in week 8 after treatment initiation (Supplementary Fig 1, A and D). In contrast, amplification of Th1- and Th17-related mRNA remained fairly stable over the treatment period (Supplementary Fig 1, B and C).

Noteworthy, IL-12/-23 p40 mRNA was significantly reduced with treatment ($P < .05$) (Supplementary Fig 1, B).

DISCUSSION

The pathophysiological mechanisms implicated in the initiation and perpetuation of AD are complex and, because of a lack of effective treatment, targeted therapeutics are urgently needed.^{1,4} Here, we treated patients with severe AD by specifically targeting the IL-12/Th1 and IL-23/Th17 cell pathway with ustekinumab and evaluated both its clinical efficacy and impact on the inflammatory infiltrate and the molecular signature of the skin. Contrary to the established dosing protocol for psoriasis, all patients received subcutaneous doses of 45 mg of ustekinumab at

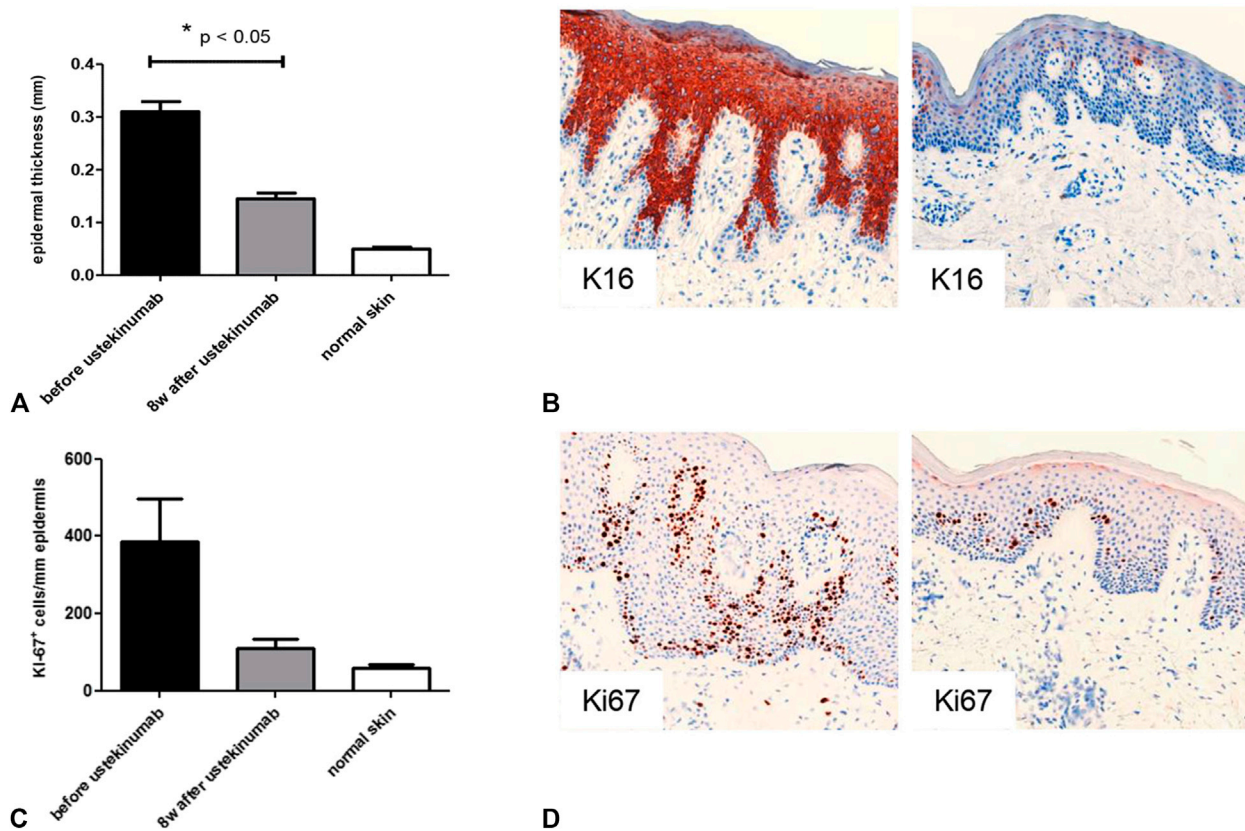


Fig 2. Ustekinumab treatment ameliorates pathologic epithelial changes in patients with atopic dermatitis (AD). Lesional biopsy specimens from all patients obtained before therapy and at week 8 (8w) and skin from 5 healthy control subjects were subjected to immunohistochemical analysis of epidermal hyperplasia and proliferation. **A**, Significant reduction of epidermal thickness ($P < .05$) at 8w as compared with untreated AD skin. **B**, Anti-keratin 16 (K16) staining was completely normalized at 8w (right). **C** and **D**, Epidermal proliferation is clearly reduced at 8w as demonstrated by anti-Ki-67 staining. Data are mean $\pm$ SD. (**B** and **D**, Original magnification: $\times 20$.) *Demonstrates significance $P < .05$.

weeks 0, 4, and 12 and subsequently every 8 weeks. Ustekinumab administration resulted in a significant reduction of clinical disease activity (50% reduction in the EASI score) and pruritus after 16 weeks.

Improvements in the parameters erythema, induration/papulation, and xerosis were observed most consistently according to the SCORAD. Immunohistologically, this clinical observation was confirmed by a reduction of epidermal hyperplasia and proliferation.

On a cellular level, the amount of Langerhans cells remained unaltered upon inhibition of IL-12/-23 p40, whereas the population of pathogenically relevant cells such as inflammatory dendritic epidermal cells, mast cells, and epidermal/dermal CD4⁺ and CD8⁺ T cells were clearly reduced upon treatment. The number of total IgE⁺ cells in the dermis and serum levels of IgE remained stable despite clinical improvement. This finding indicates limited impact of IgE-mediated responses on the pathogenesis of

AD consistent with former reports of placebo-controlled trials.²³

Interestingly, on a molecular level, ustekinumab led to a substantial down-regulation of Th2- and Th22-related products. It is conceivable that a modifying effect of ustekinumab on Th22-related pathways in chronic AD lesions results in a down-regulation of Th2-associated proinflammatory processes.

Besides Th22 and Th17 cells, human mast cells have recently been recognized to be a major source of IL-22 production.^{10,24} Consequently, we speculate that the observed reduction of mast cells in ustekinumab-treated lesions together with the suppression of IL-23 production contributes to a decreased IL-22 mRNA expression.²⁵

IL-22 is known to mediate epidermal hyperplasia, modulate terminal keratinocyte differentiation by up-regulating the expression of S100A8 and S100A9,²⁶ and promote dermal fibroblast activity

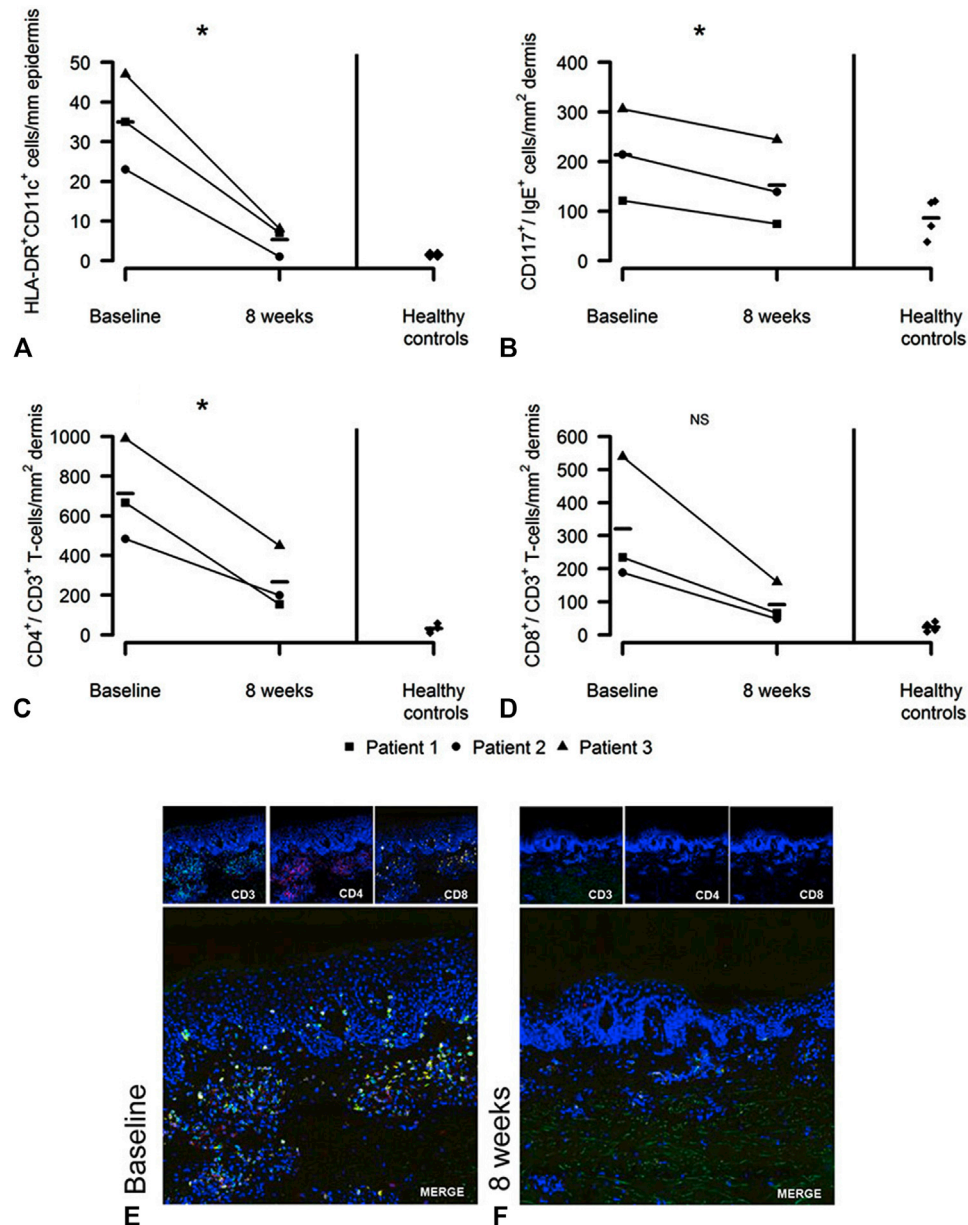


Fig 3. T cells, mast cells, and inflammatory dendritic epidermal cells (IDEC) are significantly reduced after ustekinumab treatment. Immunofluorescence analysis of lesional biopsy specimens from all patients before and 8 weeks after therapy and of skin samples from 5 healthy control subjects. Significant reduction of IDEC (CD11c⁺/HLA-DR⁺ cells) (**A**) and mast cells (CD117⁺ cells) (**B**) after therapy. Significant reduction of infiltrating CD4⁺/CD3⁺ T-helper cells (**C**, **E**, and **F**) and a clear decrease of CD8⁺/CD3⁺ cytotoxic T cells (**D**, **E**, and **F**) after ustekinumab therapy. Results are depicted for each patient. (**E** and **F**, Original magnification: ×20.) *Demonstrates significance $P < .05$.

during skin wound repair.²⁷ These pathogenic correlations might explain our findings of diminished epidermal hyperplasia/proliferation and dermal lichenification after blocking IL-12/-23 p40.

Of note, expression of Th1- and Th17-associated molecules were not clearly altered upon IL-12/-23 p40 inhibition, indicating ongoing amplification of

Th1 and Th17 mRNA with a delayed or nonexistent inhibition of these pathways.

Taken together, our results demonstrate a distinct clinical and immunologic response to ustekinumab in a small patient cohort. Although one must be aware of the high response rate to placebo in patients with AD when interpreting clinical data

that lack a placebo-controlled design,^{5,28} we suggest that the IL-12/-23 pathway might play a role in the pathogenesis of AD. Because of the prominent effects of ustekinumab on keratinocyte proliferation/differentiation, it is conceivable that at least a subgroup of patients with predominant severely infiltrated disease characteristics might benefit from IL-12/-23 p40 inhibition.

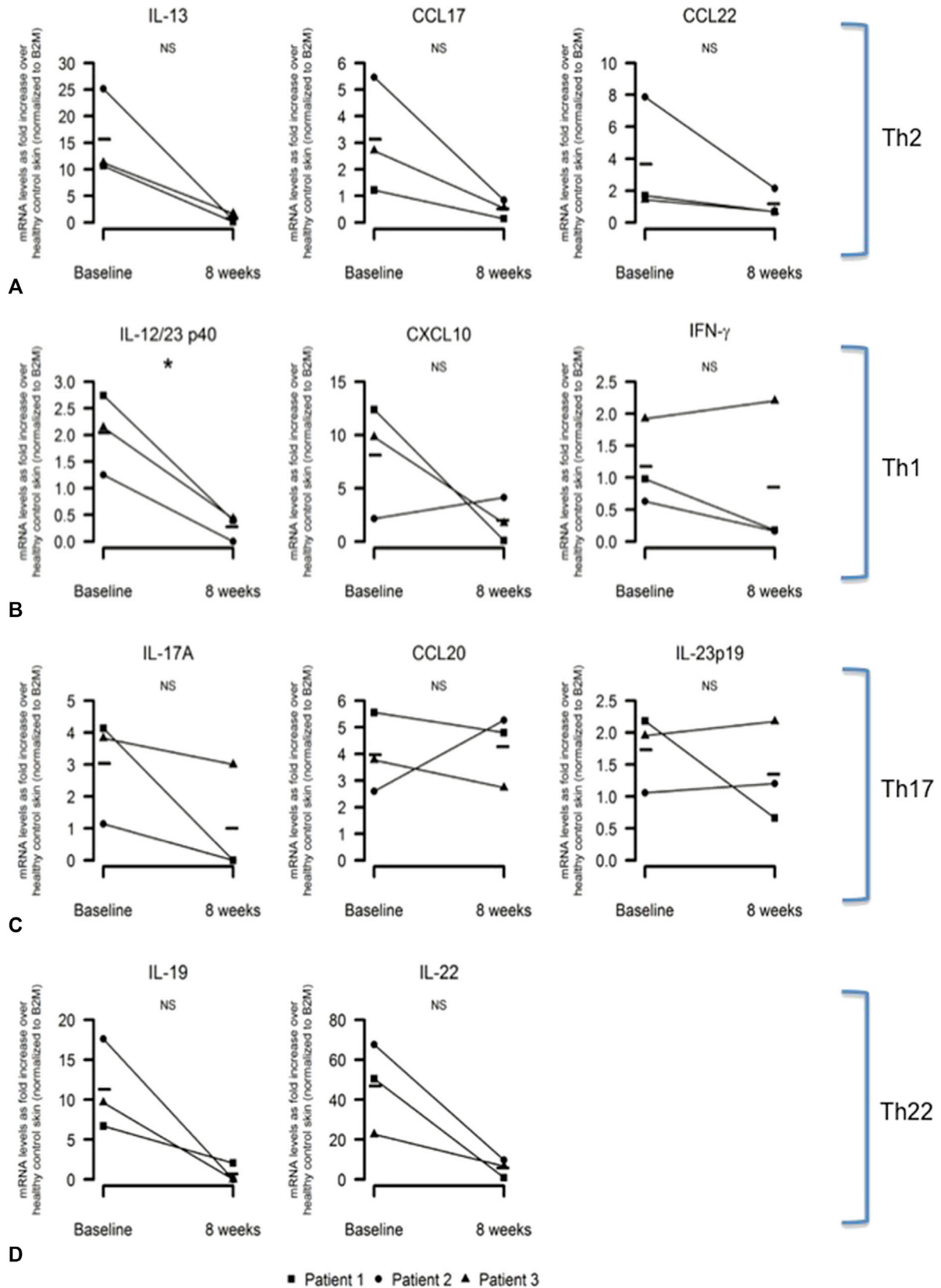
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SUPPLEMENTARY METHODS**Quantitative real-time polymerase chain reaction**

RNA was isolated with TRI Reagent (Sigma-Aldrich, St. Louis, MO) and RNA concentrations were measured using the NanoDrop ND-100 Spectrophotometer (Peqlab Biotechnology, Erlangen, Germany). For further quantification RNA was transcribed to cDNA

and analysis was performed as previously described.²⁹ Results were normalized to the housekeeping gene human β_2 -microglobulin and results are shown as fold increase over healthy control skin. Primers and probes for the TaqMan quantitative real-time polymerase chain reaction assays were used from TaqMan gene expression assays (Applied Biosystems, Foster City, CA).



Supplementary Table I. List of antibodies used for immunohistochemistry and immunofluorescence

Antigen	Supplier	Clone	Isotype
IgE UNLB	Southern Biotech, Birmingham, AL	B3102E8	mIgG1
CD4 UNLB	Dako, Glostrup, Denmark	MT310	mIgG1
S100A8 UNLB	Acris antibodies, Rockville, MD	8-5C2	mIgG1
S100A9 UNLB	Abcam, Cambridge, UK	47-8D3	mIgG1
Ki67 UNLB	Dako	MIB-1	mIgG1
Keratin 16 UNLB	NeoMarkers, Fremont, CA	LL025	mIgG1
CD3 FITC	Becton Dickinson, Franklin Lakes, NJ	SK7	mIgG1
HLA-DR FITC	Becton Dickinson	L243	mIgG2a
CD117 APC	Beckman Coulter, Brea, CA	104D2D1	mIgG1
CD8 APC	Beckman Coulter	B9.11	mIgG1
CD207 PE	Beckman Coulter	DCGM4	mIgG1
CD11c biot	Biolegend, San Diego, CA	3.9	mIgG1

Supplementary Fig 1. Changes of inflammation-associated skin products in lesional atopic dermatitis skin after ustekinumab treatment as quantified by using quantitative real-time polymerase chain reaction. Changes in messenger RNA (*mRNA*) levels are depicted as fold increases of pretreatment and posttreatment mRNA expression normalized to beta-2-microglobulin expression in healthy control skin. Results are depicted for each patient. **A**, Clear reduction of T-helper (*Th*)2-related gene products (ie, interleukin [*IL*]-13, CCL17, CCL22) after 8 weeks of ustekinumab treatment. **B**, Significant decrease of IL-12/-23 p40 mRNA levels, but heterogeneous expression of other Th1-associated products (ie, CXCL-10, IFN- γ) and **C**, Th17-related (ie, IL-17A, CCL20, IL-23 p19) gene products. **D**, Substantial decline of Th22-associated cytokines (ie, IL-19, IL-22) after therapy. *Demonstrates significance $P < .05$.