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IL-4 Downregulates IL-1β and IL-6 and Induces GATA3 in Psoriatic Epidermal Cells: Route of Action of a Th2 Cytokine

Armanda J. Onderdijk,*† Ewout M. Baerveldt,*† Dorota Kurek,‡ Marius Kant,*† Edwin F. Florencia,*† René Debets,§ and Errol P. Prens*†

Clinical improvement of psoriasis induced by IL-4 treatment has been ascribed to changes in dermal inflammatory cells, such as activation of Th2 cells and tolerization of dendritic cells by suppressing IL-23 production. The pathologic epidermal alterations in psoriatic lesional skin include increased epidermal expression of IL-1β, IL-6, S100A7, and human β-defensin 2 (hBD2) and a downregulated expression of the epidermal transcription factor GATA3. Effects of IL-4 on the epidermal compartment of psoriasis lesions were not previously investigated. Therefore, we investigated whether IL-4 directly affects abovementioned psoriatic markers in the epidermal compartment. We cultured freshly isolated psoriatic epidermal cells, whole psoriatic and healthy skin biopsies, human keratinocytes and Langerhans cells with IL-4. The secretion of IL-1β and IL-6 by psoriatic epidermal cells was inhibited by IL-4 via transcriptional and posttranscriptional mechanisms, respectively. In normal skin, IL-4 inhibited IL-1β- and IL-17A–induced hBD2 expression in vitro. In addition, IL-4 reduced the protein expression of hBD2 in psoriatic skin biopsies and induced phospho-STAT6 protein. Epidermal GATA3 mRNA and protein were significantly upregulated by IL-4 in epidermal cells and keratinocytes. Our data argue that IL-4 improves psoriasis not only via modification/induction of Th2 cells and type II dendritic cells, but also via direct inhibition of inflammatory cytokines in resident IL-4R–expressing epidermal cells and thereby alters the psoriatic skin phenotype toward a healthy skin phenotype. *The Journal of Immunology, 2015, 195: 1744–1752.

Psoriasis is a chronic inflammatory skin disease characterized by red and scaly skin lesions. Although many aspects of the immunopathogenesis of psoriasis have been clarified, the exact sequence of pathogenic events remains unclear. The current concept is that psoriatic plaques (PP) arise as the result of interplay between inflammatory cells and genetically predisposed keratinocytes (KC) (1–4). Expression levels of antimicrobial peptides are highly upregulated in (non)lesional psoriatic skin, and they are considered to play a role in the induction of psoriasis via immune-modulation such as recruitment of leukocytes (5, 6). The activated epidermis is characterized by a high keratin 17 (K17) and a low GATA3 expression, high levels of antimicrobial peptides such as psoriasin (S100A7) and β-defensin-2 (hBD2), growth factors including nerve growth factor (NGF), and the proinflammatory cytokines IL-1β and IL-6 (2, 7–9). These molecules activate IL-23 producing dendritic cells (DC), resulting in the induction of skin T cells that produce cytokines such as TNF-α, IFN-γ, IL-17, and IL-22. This interplay is of key importance in inducing the regenerative epidermal hyperproliferative phenotype in psoriatic skin (6, 10).

Psoriasis is seen as the opposite of atopic dermatitis, because of the contrasting immune cell subsets involved. In psoriasis Th1/Th17 cells are the major players, and in early atopic dermatitis lesions Th2 cells and their cytokines are important (11). Psoriatic lesions are characterized by a relative absence of Th2 cells, and a strong Th1/Th17 signature (12, 13). The prototypic Th2 cytokine IL-4 is primarily regarded as a master switch essential for Th2 differentiation (14). IL-4 also has anti-inflammatory properties by downregulating IL-1, TNF-α, IL-6, IL-8, IL-12, and IL-17 production in many different cell types such as monocytes, DC, and macrophages (15–17). In addition, IL-4 can inhibit the production of antimicrobial peptides in KC (18). In psoriatic epidermal cells, the expression of the IL-4 receptor is increased (19). Fumarate treatment induces IL-4-producing Th2 cells in vivo and generates type II DC that produce IL-10 instead of IL-12 and IL-23 (20). Clinical improvement of psoriasis is accompanied by activation of IL-4 signaling pathways including upregulated expression of GATA3 (21). GATA3 is crucial in epidermal development, and its expression is strongly downregulated in active PP, whereas adding IL-4 to ex vivo healthy skin explants significantly enhances epidermal GATA3 expression (2). We hypothesize that IL-4 induces a shift away from Th1/Th17 inflammation, whereby the altered balance of proinflammatory cytokines and growth factors in PP may be reversed, and levels of epidermal GATA3 may be normalized.

Studies in murine psoriasiform models have indeed demonstrated that transdermal IL-4 gene therapy partially prevents the “psoriasiform-like” phenotype (22) and these models confirmed the role of IL-4 in Th2 differentiation (14, 22, 23). Psoriasis patients treated with recombinant human IL-4 also showed impressive clinical improvement, up to 68% psoriasis area and severity index reduction in 6 wk (24), which equals clinical improvement seen.

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Address correspondence and reprint requests to Dr. Armanda J. Onderdijk, Erasmus MC, University Medical Center Rotterdam, Departments of Dermatology and Immunology, P.O. Box 2040, 3000 CA Rotterdam, the Netherlands. E-mail address: a.onderdijk@erasusmc.nl

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with anti-IL-12/23p40 therapy (ustekinumab) (25). This IL-4 induced improvement was initially attributed to its effects on the Th1/Th2 balance in the dermal infiltrate (24). Later, it was shown that IL-4 treatment reduces the cutaneous expression of IL-23p19 and IL-17, and reduces the expression of IL-1β, IL-6, and IL-23 in dermal DC (26).

Despite the importance of the epidermal compartment in the pathogenesis of psoriasis, previous studies focused merely on the effect of IL-4 on the dermal infiltrate. So far, the direct effects of IL-4 on human KC and especially freshly isolated psoriatic epidermal cells (EC) are largely unknown.

We investigated whether IL-4 could inhibit epidermal inflammatory responses analogous to its dermal or systemic anti-inflammatory effects. The aim of this study was to further explore the function of IL-4 in epidermal inflammation and especially on the expression of typical psoriasis markers such as IL-1β, IL-6, IL-23p19, S100A7, hBD2, NGF, K17, and GATA3, using 1) PP biopsies and healthy skin explants, 2) freshly isolated EC from PP, 3) cultured HaCaT cells and normal human keratinocytes, 4) Langerhans cells (LC), and 5) activated PBMC as a representative of the dermal infiltrate.

Materials and Methods

Patients and controls

All patients were included following informed consent. Skin shaves or biopsies (depending on the experiment) were taken from plaques of 25 patients with moderate to severe psoriasis. Patients did not receive any local therapy in the 2 wk preceding biopsy, nor systemic therapy in the previous 2 mo before biopsy. Healthy control skin specimens were obtained from 15 healthy patients undergoing plastic breast or abdominal surgery at the Erasmus MC, or Sint Franciscus Hospital Rotterdam. The study was approved by the local medical ethical committee (registration number 104.050/SPO/1990/30 – MEC 99.785 version April 19, 2011) and conducted according to the Declaration of Helsinki principles.

Epidermal cell suspensions and PBMC

Skin shaves were obtained using a Davies Gold Series dermatome (Stolper Medical, Utrecht, the Netherlands). Briefly, split skin dermatome specimens were left floating in a solution of 0.25% trypsin and 0.1% EDTA in PBS for 15 min to separate the epidermis from the dermis, followed by preparation of single epidermal cell suspensions as described previously (27). Previous immunostainings have shown that epidermal cell suspensions contain mainly keratinocytes (95%), 3–4% melanocytes and the remaining 1–2% consists of Langerhans cells (LC). Epidermal cells were incubated for 1–2 h at 37°C and 5% CO2 in medium containing 0.5% AB serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.1% human serum. PBMC were cultured at 5 × 10^6 in RPMI 1640 medium with HEPES, glutamax-I, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.1% human serum. The culture medium was aspirated and replaced by medium RPMI 1640 medium, 5% FCS, without HEPES and antibiotics. After 24 h of adaptation, the culture medium was replaced by medium with or without cytokines. For immunofluorescent stainings, cryosections were fixed with 0.25% trypsin and 0.1% EDTA in PBS and then washed in dH2O. The slides were put in Tris-EDTA buffer (pH 9) at 4°C for 10 min to separate the epidermis from the dermis for independent analysis of epidermal and dermal mRNA. Whole skin explants were placed in lymphosarin buffer containing 2.5% 2-ME for whole biopsy mRNA analysis.

Quantitative PCR analysis

Total mRNA was extracted using the GeneElute Mammalian Total RNA kit (Sigma-Aldrich). cDNA was made using 1 µg total RNA template, with SuperScript II reverse transcriptase (Invitrogen) and oligo(dT). PCR was performed using the ABI PRISM 7900 sequence-detection system (Applied Biosystems). ABL1 was chosen as a reference housekeeping gene (29). The PCR primer sequences and probe numbers are specified in Table I.

ELISA

IL-1β (full-length and processed) and IL-6 were measured with commercially available ELISAs (Invitrogen) using the protocols provided by the manufacturer. The cutoffs of the ELISA system were defined as negative control + 3 SD.

Ex vivo short-term skin culture in the Transwell system

Whole skin biopsy explants were cultured as described previously (30). Briefly, 4-mm punch biopsies were placed in punched-out holes in a Transwell membrane placed in a 12-well plate. Biopsies were cultured in medium with or without IL-4 (100 ng/ml; PeproTech) and IL-1β (10 ng/ml; R&D Systems) and IL-17A (100 ng/ml; R&D Systems). Medium consisted of IMDM supplemented with 0.5% PenStrep, HEPES glutamate and 0.5% human AB serum. The well plates were placed at 5% CO2, 37°C for 24 h. After culture, one-quarter was placed in thermolysin for 1 h to separate the dermis from the epidermis for independent analysis of epidermal and dermal mRNA. The other whole placers were placed in lysin buffer containing 2.5% 2-ME for whole biopsy mRNA analysis.

hBD2 staining

Sections on glass slides were dipped in xylene and incubated in ethanol and then washed in dH2O. The slides were put in Tris-EDTA buffer (pH 9) at 96°C for 30 min, and transferred into a blocking solution (PBS-0.5% Tween, 2% BSA, and 1% NMS) for 30 min. The primary Ab (goat-anti-human anti-hBD2 1:250; Abcam, Cambridge, U.K.) was added in PBS-0.5% Tween and 1% BSA and incubated for 60 min at room temperature. The slides were rinsed, and the secondary biotinylated rabbit-anti-goat Ab (1:400) was added followed by streptavidin poly-HRP (1:500) and incubated for 45 min. Staining was visualized using 3-amin-9-ethylcarbazole as the substrate. Sections were counterstained with hematoxylin.

Immunofluorescence

HaCaT were cultured on Teflon-coated diagnostic slides (De Beer Medicals, Diessen, the Netherlands) at a density of ~1 × 10^5 cells/well in IMDM 1640 medium, 5% FCS, without HEPES and antibiotics. After 24 h of adaptation, the culture medium was aspirated and replaced by medium with or without cytokines. For immunofluorescent stainings, cryosections from PP skin biopsies and slides with cultured HaCaT KC were fixed with 4% paraformaldehyde in PBS for 10 min at 4°C. The slides were rinsed, and the secondary biotinylated rabbit-anti-goat Ab (1:400) and streptavidin poly-HRP (1:500) were added. The slides were rinsed and the antibody was detected with DAPI (Thermo Fisher Scientific). Pictures were taken with an Axio Imager fluorescence microscope (Carl Zeiss Microimaging, Jena, Germany).

Statistical analysis

Wilcoxon signed-rank test and one-way ANOVA with Bonferroni’s posttest were used for statistical analysis using GraphPad Prism version 5.04
IL-4 inhibits the mRNA expression of IL-1β and the secretion of IL-6 by psoriatic epidermal cells

IL-4 is known as a negative regulator of proinflammatory gene expression and is capable of downregulating cytokine production in many different cell types (16, 17). IL-1β is produced by monocytes, macrophages, and NK cells, and IL-6 is produced by T cells, fibroblasts, and macrophages. To investigate whether proinflammatory cytokine production is inhibited by IL-4, we cultured activated PBMC in the presence of IL-4 and analyzed the mRNA expression (Fig. 1A, Table I). Because the baseline expression and release of IL-1β and IL-6 is normally low in PBMC, we induced a proinflammatory situation and show that in proinflammatory conditions (triggered by LPS) IL-4 inhibits the induced expression of IL-1β and IL-6. IL-4 appears to be a potent inhibitor of IL-1β, to a degree comparable with dexamethasone (Fig. 1A). By using epidermal cell suspensions we tried to come close to the effect of IL-4 in vivo, by studying this ex vivo. In freshly isolated PP EC, IL-4 inhibited IL-1β mRNA expression but not IL-6 mRNA (Fig. 1B). To check whether these findings would also result in a reduced protein secretion,
we cultured PBMC for 16 h and PP EC for 4 and 24 h, with or without IL-4 and measured the cytokine release with ELISA. As measured by ELISA, PBMC incubated with LPS for 16 h with or without IL-4 or 10−7 M dexamethasone for 4 h n = 9 (PBMC). (B) PP EC without or with IL-4 (100 ng/ml), supernatants were harvested at 4 and 24 h and tested by ELISA (n = 5). Dots connected by a line represent cells from the same individual under different conditions. One-way ANOVA with Bonferroni’s posttest and Wilcoxon signed-rank test. Results are displayed with median; *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 2.** IL-4 inhibits the release of LPS-induced IL-1β and IL-6 by PBMC and the release of IL-6 in psoriatic epidermal cells. (A) ELISA using supernatants of the same stimulated PBMC (with two additional samples) incubated with LPS for 16 h with or without IL-4 or 10−7 M dexamethasone for 4 h n = 9 (PBMC). (B) PP EC without or with IL-4 (100 ng/ml), supernatants were harvested at 4 and 24 h and tested by ELISA (n = 5). Dots connected by a line represent cells from the same individual under different conditions. One-way ANOVA with Bonferroni’s posttest and Wilcoxon signed-rank test. Results are displayed with median; *p < 0.05, **p < 0.01, ***p < 0.001.

IL-4 inhibits IL-1β mRNA expression induced by proinflammatory cytokines in cultured keratinocytes

To confirm our results, we stimulated normal human keratinocytes with various proinflammatory conditions. At the mRNA level, IL-4 significantly reduced the IL-1β expression that was induced by a proinflammatory cytokine mixture consisting of IL-1β+TNF-α or IL-17A (Supplemental Fig. 1A). The mRNA expression of IL-6 was not inhibited, but from the literature and our previous experiments, it is known that IL-6 is regulated at the posttranscriptional level (31). At the protein level IL-4 did not significantly reduce the release of IL-1β induced by the proinflammatory cytokines, but IL-4 did reduce in all cases the release of IL-6 induced by the proinflammatory cytokine; however without reaching statistical significance (Supplemental Fig. 1B).

IL-4 reduces the expression of IL-1β induced by proinflammatory cytokines in LC

Because the inhibitory effect of IL-4 on proinflammatory cytokine production in epidermal cell suspensions was stronger than in single keratinocytes, we wondered whether epidermal LC were involved in the observed results in the epidermal cell suspensions. We therefore purified LC from healthy skin using paramagnetic
beads up to a purity of 99% and stimulated them in culture. Also in LC, IL-4 significantly reduced the expression of IL-1β, induced by proinflammatory cytokines, but IL-6 mRNA was not reduced (Supplemental Fig. 2). The cytokine release of IL-1β and IL-6 was not significantly reduced by IL-4 (data not shown). Our results are largely in agreement with findings of Matsue et al. (32) who showed that LC are the major source of IL-1β mRNA in mouse skin.

IL-4 inhibits IL-1β– and IL-17–induced hBD2 expression and IL-1β–induced S100A7 expression

The expression of the antimicrobial peptides S100A7 and hBD2 is increased in PP, which reflects the altered state of epidermal activation and barrier function in psoriasis. The baseline expression of hBD2 and S100A7 in normal skin is low (33). However, in culture, with increasing time of culture, NN skin begins to show a more inflammatory profile, with increased mRNA levels of hBD2 and S100A7 and reduced levels of GATA3, which represents a wound healing effect (data not shown). We checked whether IL-4 could reduce the expression levels of these antimicrobial peptides in psoriatic epidermis. Quantitative PCR using mRNA derived from PP epidermis and whole PP skin revealed no significant differences in S100A7 and hBD2 expression after culturing for 24 h with IL-4 (Supplemental Fig. 3A). However, a significant decrease in both markers was detected after 24 h in whole NN biopsies (Supplemental Fig. 3B). In addition, IL-4 significantly inhibited IL-1β– and IL-17–induced hBD2 expression (Fig. 4A) and IL-1β–induced S100A7 expression (Supplemental Fig. 3C). We performed immunohistochemical staining for hBD2 in psoriatic skin biopsies, and this shows a clear reduction in hBD2-staining by IL-4 (Fig. 4B). Note the absence of staining in the dermis, as it is known that hBD2 in serum is originated from the epidermis and migrates through the dermis into the vasculature (34).

IL-4 downregulates gene expression of NGF but not IL-23p19 and upregulates STAT6 expression in psoriatic skin

K17 is not expressed in healthy skin, but is expressed in hyperproliferative skin conditions such as psoriasis. Stimulation of biopsies with IL-4 did not result in a significant change in K17 expression in epidermal psoriatic as well as in whole PP skin (Supplemental Fig. 4). IL-4 stimulation did not affect IL-23p19 mRNA expression in epidermal nor whole PP skin. Lesional epidermal NGF mRNA expression was not significantly reduced after culturing for 24 h in presence of IL-4, but NGF mRNA was significantly reduced in both whole PP and NN cultured biopsies (Supplemental Fig. 4). IL-4 exerts its function via the signal transducer STAT6 (35). To see whether effects of IL-4 stimulation on PP also involved this pathway, we measured STAT6 mRNA levels. In the presence of IL-4, STAT6 mRNA expression was significantly upregulated in PP epidermis and whole PP biopsies (Supplemental Fig. 4). Immunofluorescence staining confirms that IL-4 stimulation of psoriatic biopsies leads to an increase in the expression of phospho-STAT6 (Fig. 5).

IL-4 upregulates expression of GATA3 in psoriatic skin and human skin KC

Epidermal GATA3 expression is downregulated under regenerative, inflammatory, and hyperproliferative skin conditions and is highly expressed in normal steady-state conditions. Stimulation of PP with IL-4 strongly upregulated GATA3 mRNA expression in the epidermis and in whole biopsies from PP (Fig. 6A). As shown previously (2), IL-4 also enhanced epidermal GATA3 mRNA expression in cultured healthy skin (Supplemental Fig. 4). Immunofluorescent staining of cryosections from PP biopsies cultured with IL-4 led to a bright and upregulated GATA3 signal in the majority of the cells in the epidermis, whereas minimal GATA3 staining was visible in biopsies cultured in medium alone (Fig. 6B). However, the psoriatic epidermis is composed
of different cell types, including lymphocytes and LC, which could respond to IL-4 and modify GATA3 expression in KC via paracrine signaling. To specifically assess the response of KC and to exclude the contribution of other resident epidermal or dermal cells, the effect of IL-4 on HaCaT cells was investigated using in situ immunofluorescent staining. After 24 h of stimulation, IL-4 induced a strong upregulation of GATA3 expression and GATA3 was also expressed in the nucleus, whereas minimal staining of GATA3 was observed in HaCaT cultured in medium alone (Fig. 6B).

**Discussion**

This study shows that IL-4 has a strong anti-inflammatory effect on the psoriatic epidermis ex vivo. Hence, the therapeutic effects of IL-4 in the treatment of psoriasis may not be solely explained by its effects on the dermal infiltrate but also by effects on the epidermal compartment. By the inhibition of IL-1β and IL-6 in psoriatic epidermal cells, IL-4 acts early in the immunological cascade in psoriasis. In addition, IL-4 inhibits IL-1β– and IL-17A–induced antimicrobial peptide expression in normal skin and hBD2 protein in psoriatic skin ex vivo, a hallmark of psoriatic lesional epidermal skin. Psoriatic EC produce increased levels of several members of the IL-1 family of cytokines including IL-1β (36), which is capable of inducing the regenerative epidermal phenotype in normal human skin (9, 37–39), and upregulates IL-6, IL-8, TNF-α, and hBD2 expression (36, 37). More importantly, IL-1β together with IL-23 is crucial in inducing Th17 and Th22 differentiation and IL-17 and IL-22 production (6, 40). Our results indicate that IL-4 is a powerful inhibitor of IL-1β mRNA expression and protein secretion, and via that pathway, IL-4 may be able to reverse the psoriatic phenotype toward a healthy skin phenotype at the beginning of the inflammatory cascade. IL-23–mediated psoriatic epidermal hyperplasia is dependent on IL-6, which is also known to hamper regulatory T cell function in PP skin (41, 42). Hence, we expected IL-4 to inhibit IL-6 mRNA expression in PP skin. We did not observe an effect on IL-6 mRNA expression in our experiments; however, in psoriatic EC, the secretion of IL-6 protein was inhibited by IL-4. Thus, the expression of IL-1β and IL-6 is reduced in psoriatic EC but likely via different mechanisms; IL-1β possibly via inhibition of gene transcription and IL-6 via inhibition of translation or secretion. These differences are likely explained by the conclusion that IL-6 is regulated

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** IL-4 inhibits IL-1β– and IL-17A–induced hBD2 expression in normal skin. RT-PCR using mRNA from normal skin (NN) stimulated with or without IL-4 (100 ng/ml), IL-1β (10 ng/ml), or IL-17A (100 ng/ml) for 24 h (A). Dots connected by a line represent cells from the same individual under different conditions. The hBD2 mRNA expression/ABL is shown. One-way ANOVA with Bonferroni’s posttest, and results are displayed with median; *p < 0.05, **p < 0.01, ***p < 0.001. Immunohistochemical staining of hBD2 in PP biopsies cultured with 100 ng/ml IL-4 for 24 h or without (control). Note the absence of epidermis derived hBD2 staining in the dermis when cultured with IL-4. Original magnification ×2.5 (B).

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** IL-4 stimulation leads to an increase in the protein expression of phospho-STAT6 in psoriatic skin biopsies. Immunofluorescence staining of phospho-STAT6 in PP biopsies stimulated with 100 ng/ml IL-4 for 24 h and without (control). Phospho-STAT6 was stained in green, and nuclei are stained in blue with DAPI. Scale bar, 100 μm.
hBD2 and S100A7 are typical markers of alterations in epidermal activation and skin barrier function in psoriasis and in atopic dermatitis. In fact, serum hBD2 has been proposed as a biomarker of psoriatic disease activity (34). Because IL-1β stimulation leads to upregulated expression of hBD2 and IL-1β secretion can be inhibited by IL-4, we expected IL-4 to reduce hBD2 expression in psoriatic skin. We confirmed that hBD2 protein was reduced in psoriatic skin after exposure to IL-4. Addition of IL-4 to a Th1 cytokine mixture led to inhibition of hBD2 in keratinocytes (33). This is in line with our findings and that of other investigators using IL-4 in KC (45, 46).

Other markers of the regenerative psoriatic phenotype include NGF, IL-23p19, K17, and GATA3. NGF plays a role in the pathogenesis of psoriasis and can modulate inflammation by regulating neuropeptides, angiogenesis, cell trafficking molecules, and T cell activation (47). NGF is not only produced by nerves but also by several immune cells, endothelial cells, fibroblasts, and KC. In psoriasis patients, NGF expression is increased in both lesional and nonlesional KC (48). NGF is strongly induced by IL-1, and its importance is demonstrated by the fact that NGF is a strong inducer of TNF-α (47–49). In our experiments, we observed a trend toward reduction of the epidermal NGF expression. However, IL-4 significantly reduced NGF in whole PP biopsies ex vivo, probably via inhibition of IL-1β. This suggests additional inhibition by IL-4 of NGF producing dermal cells including DC, macrophages, and fibroblasts (50).

IL-23p19 is increased in PP and can be produced by KC, but DC are the main source (40, 51, 52). Reports showed that IL-23 production by DC is inhibited by IL-4 and that downmodulation of DC and IL-23p19 is an early effect during psoriasis treatment (20, 26). We did not observe a reduction in IL-23p19 mRNA expression in epidermal and whole PP skin after IL-4 stimulation. However, this corresponds with a previous study stating that psoriatic KC lack intrinsic aberrant expression of IL-23, and therefore, IL-23 may not be further reduced by IL-4 in culture conditions (52). In addition, IL-1β and IL-6 are necessary for Th17 induction by DC (6, 10). Because of the early inhibition of IL-1β and IL-6 by IL-4, likely DC are not activated and new IL-12 and IL-23 production and the consequent Th17 differentiation are inhibited.

GATA3 is a crucial transcription factor in KC homeostasis (53), activation and proliferation (54), and epidermal GATA3 is downregulated in PP and during wound healing (2). We show that the expression of GATA3 is strongly upregulated by IL-4 in KC and also in psoriatic epidermis. The prominent increased protein expression of GATA3 in nuclei of the KC indicates nuclear translocation and an activated state. GATA3 mRNA and protein in psoriatic skin were significantly upregulated by IL-4, comparable to its expression in healthy skin. Our results are in line with previous reports that collectively underscore the importance of KC in the pathogenesis of psoriasis (55). This is further illustrated by the observation that, during etanercept (anti–TNF-α) treatment of psoriasis, epidermal improvement precedes dermal improvement (56). The effects of IL-4 on the epidermis are likely mediated through the upregulated IL-4R on psoriatic KC (19). This IL-4R upregulation could be the result of a negative feedback loop in an attempt to restore the epidermis from its inflammatory state. The relative low levels of IL-4 in psoriatic skin can be replenished by the addition of exogenous IL-4, hence, driving the cytokine balance in inflamed psoriatic skin away from the Th1/Th17-dominated pathologic state via the upregulated IL-4R.

In conclusion, our results indicate that IL-4 not only improves psoriasis via modification of dermal Th2 cells and induction of type II DC function but also has anti-inflammatory and antiregenerative effects on the psoriatic epidermal compartment and is thereby able to shift a psoriatic skin phenotype toward a healthy skin phenotype.

**FIGURE 6.** IL-4 upregulates GATA3 mRNA expression in psoriatic epidermal sheets and whole psoriatic skin and upregulates the protein expression in cultured psoriatic epidermis and in cultured human skin keratinocytes. RT-PCR using mRNA from epidermal sheets (PP epidermis) and whole biopsies from psoriatic skin (PP whole skin) stimulated with or without IL-4 for 24 h (A). Wilcoxon signed-rank test, and results are displayed with median; *p < 0.05. Biopsies from PP skin and HaCaT were cultured for 24 h without IL-4 and with IL-4 and analyzed by immunofluorescent microscopy (results were repeatable). Note the main induction of GATA3 in the epidermis (bright fluorescent staining) by IL-4 and the preferential nuclear localization in HaCaT. Scale bar, 100 µm for PP biopsies; 20 µm for HaCaT (B).

In epidermal skin cells at the posttranscriptional level via enhancing the stability of IL-6 mRNA. Indeed, it has been previously shown that IL-6 mRNA levels do not correspond to IL-6 protein levels (31, 43).

In the activated PBMC, representing the dermal infiltrate, we see an inhibition of LPS-induced IL-1β as well as IL-6 cytokine release by IL-4. In addition, stimulation of cultured keratinocytes and purified LC was done to specify the different cell types involved. IL-4 reduced IL-1β mRNA expression in keratinocytes and purified LC and IL-6 protein in all keratinocyte cell lines. In vitro, the effects of IL-4 were more subtle than observed in the psoriatic ECS. This can be explained by 1) synergistic interactions between KC and LC in contiguous intact skin ex vivo compared with a single-cell in vitro milieu, 2) the proinflammatory environment in psoriatic EC differs from our artificial in vitro proinflammatory cytokine environment, 3) time of culture, 4) the genetically predisposed psoriatic EC respond to IL-4 than the healthy skin KC and LC used in our experiments, 5) the upregulation of the IL-4R on psoriatic epidermal cells may lead to a larger effect of IL-4 in psoriatic EC, and 6) we cannot rule out that melanocytes and sporadic Merkel cells are also a target for IL-4 (44). The observed upregulation of phospho-STAT6 indicates that the anti-inflammatory effects of IL-4 are mediated via a pathway involving at least STAT6.
Supplemental Fig 1. IL-4 significantly reduces IL-1β mRNA expression induced by IL-1β+TNF-α or IL-17A in normal human keratinocytes. RT-PCR using mRNA from normal human keratinocytes (KC) cultured for 2 h with IL-1β (100U/ml) + TNF-α (5 ng/ml) or IL-17A (100 ng/ml), with or without IL-4 (100 ng/ml) (A). The Y axis shows the mRNA expression of IL-1β and IL-6 relative to the expression of the housekeeping gene ABL. Supernatants of keratinocytes were collected after 16 h and tested by ELISA (B). Dots connected by a line represent cells from the same individual in different conditions. Results are displayed with median, paired t-test, p < 0.05 (*) and p < 0.01 (**).
Supplemental Fig 2. IL-4 significantly downregulates IL-1β mRNA expression in Langerhans cells.

RT-PCR using mRNA from Langerhans cells (LC) cultured for 16 h with IL-1β (100U/ml) + TNF-α (1 ng/ml) or IL-17A (100 ng/ml), with or without IL-4 (100 ng/ml). The Y axis shows the mRNA expression of IL-1β and IL-6 relative to the expression of the housekeeping gene ABL. Wilcoxon signed rank test, results are displayed with median, p < 0.05 (*).
Supplemental Fig 3. IL-4 inhibits S100A7 and hBD2 mRNA expression and IL-1β induced S100A7 expression in normal skin. RT-PCR using mRNA from psoriatic epidermal sheets (PP epidermis), normal skin (NN) and whole psoriatic skin (PP) stimulated with or without IL-4 (100 ng/ml). There was a considerable inter-donor variation in the mRNA expression levels, to illustrate the effect in the
individual patients more clearly we used a ratio (control was set as ‘1’). The Y axis shows the mRNA expression relative to the expression of ABL (A, B). RT-PCR using mRNA from NN stimulated and with or without IL-4 (100 ng/ml) and IL-1β (10 ng/ml) or IL-17A (100 ng/ml) for 24 h. Dots connected by a line represent cells from the same individual in different conditions (C). Wilcoxon signed rank test and one way ANOVA with Bonferroni’s post-test, results are displayed with median, p < 0.05 (*) and p < 0.01 (**).
Supplemental Fig 4. IL-4 downregulates NGF expression in normal skin and psoriatic skin, upregulates STAT-6 gene expression in psoriatic epidermal sheets as well as in whole psoriatic skin, and upregulates GATA-3 expression in whole normal skin and whole psoriatic skin. RT-PCR using mRNA from epidermal sheets (left column) and whole biopsies from psoriatic skin (PP) and whole
biopsies from normal skin (NN) (right column) stimulated with or without IL-4 in a skin explant culture system for 24 h. There was a considerable inter-donor variation in the mRNA expression levels, to illustrate the effect in the individual patients more clearly we used a ratio (control was set as ‘1’). The Y axis shows the mRNA expression relative to the expression of ABL. Wilcoxon signed rank test, results are displayed with median, p < 0.05 (*) and p < 0.01 (**).