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*ImmunoHorizons* 2020, 4 (11) 754-761
doi: https://doi.org/10.4049/immunohorizons.2000083
http://www.immunohorizons.org/content/4/11/754

This information is current as of May 31, 2022.

**Supplementary Material**
http://www.immunohorizons.org/content/suppl/2020/11/25/4.11.754.DCSupplemental

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IL-37 Expression Is Downregulated in Lesional Psoriasis Skin

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ABSTRACT

IL-37 broadly suppresses inflammation in various disease models. However, studies of the regulation and role of IL-37 in psoriasis are limited and contradictory. Using transcriptome analysis, PCR, protein determination, and immunofluorescence, we demonstrated marked downregulation of IL-37 in biopsies from human lesional psoriasis skin compared with paired samples of nonlesional skin. Immunofluorescence analysis showed that IL-37 was localized to stratum granulosum of the epidermis. TNF-α stimulation of normal human epidermal keratinocytes led to increased IL37 expression through a p38 MAPK-mediated mechanism, whereas IL-17A, IL-17C, IL-17F, and IL-22 acted suppressively. Intradermal injection with recombinant human IL-37 into imiquimod-induced psoriasis skin of C57BL/6J mice demonstrated a trend toward a protective effect, however NS. Altogether, these results demonstrate that IL-37 is downregulated in human lesional psoriasis skin. This may be a consequence of the loss of stratum granulosum, but key cytokines in the development of psoriasis also seem to contribute to this downregulation. ImmunoHorizons, 2020, 4: 754–761.

INTRODUCTION

Psoriasis is a common, chronic inflammatory skin disease characterized by skin inflammation, keratinocyte hyperproliferation, and differentiation abnormality. Tremendous progress in the treatment of moderate to severe psoriasis has been achieved with the introduction of biologics targeting disease-driving proinflammatory cytokines such as IL-23 and IL-17A (1, 2). However, patients with psoriasis differ with respect to genetic predisposition, trigger factors, phenotype, comorbidities, and response to treatments. Therefore, further modulators of psoriasis pathogenesis remain to be explored.

IL-37 was discovered in 2000. Five isoforms have so far been described, IL-37a-e, of which IL-37b is the longest canonical form and the most intensively studied (3, 4). IL-37 has been identified in various tissues such as colon, breast, testis, muscle, and lung (5). Recently, PCR and subsequent DNA sequencing showed that in human keratinocytes, IL-37b was the most pronounced expressed isoform followed by IL-37c (6).

Studies of IL-37 function have mainly focused on its role in various inflammatory conditions, including cardiovascular disease and cancer. Mice do not express IL37, and therefore experiments in mice have been performed using the human form of IL-37. Recombinant human IL-37 suppress inflammation in, for example, acute arthritis (rIL-37b aa 46–218), allergic airway inflammation, and atherosclerosis (7–9). Mice transgenic for the longest form of human IL37b (pro–IL37b, aa 1–218) are protected from inflammation in models of contact dermatitis, colitis, endotoxic shock, and obesity (10–13).

Received for publication October 29, 2020. Accepted for publication November 2, 2020.

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The array data presented in this article have been submitted to the Gene Expression Omnibus under accession number GSE161683.

This work was supported by grants from The Danish Society of Dermatology, Aage Bang’s Foundation, Augustinus Foundation, The Danish Research Council, and Dr. Sofus Carl Emil Friis and Olga Doris Friis’ Foundation. C.A.D. is supported by National Institutes of Health Grant AI-15614.

Abbreviations used in this article: 3D, three-dimensional; EGF, epidermal growth factor; hBD3, human β-defensin 3; IMQ, imiquimod; IQR, interquartile rage; logFC, logarithmic fold change; LP, lesional psoriasis; NHEK, normal human epidermal keratinocyte; NP, non-LP; PASI, Psoriasis Area Severity Index; Poly I.C, polyinosinic-polycytidylic acid; qPCR, quantitative PCR.

The online version of this article contains supplemental material.

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IL-37 has only sparsely been investigated in psoriasis. To the best of our knowledge, only one study has previously investigated the anti-inflammatory property of IL-37 in psoriasis. In this study, mice transgenic for keratin 14 vascular endothelial growth factor A were injected with plasmid-coding IL37 sequence-formulated cationic liposomes. Protection by IL-37 against the development of skin inflammation was demonstrated on H&E-stained mice skin sections and by the assessment of VCAMs and mast cells. Using immunofluorescence analysis, a positive correlation was found between amounts of IL-37 and psoriasis severity. However, this is at odds with other studies that demonstrate downregulation of IL-37 in lesional psoriasis (LP) skin compared with non-LP (NP) skin (3, 15–17).

Most recently, Lachner et al. (6) described a positive correlation between IL-37 expression and the differentiation level of keratinocytes. IL-37 was upregulated more than 100-fold in differentiated three-dimensional (3D) epidermal cultures compared with subconfluent, monolayer keratinocyte cultures. Immunofluorescence analysis showed that IL-37 localized to the stratum granulosum of human epidermis (6).

In psoriasis, keratinocyte differentiation is altered, which results in loss of stratum granulosum. The process coincides with inflammation. We therefore hypothesized that IL-37 is downregulated in psoriasis, and psoriasis inflammation is aggravated as a consequence. Using in vitro experiments, we investigated the expression and regulation of IL-37 in normal human epidermal keratinocytes (NHEK), human epidermal 3D models, and in biopsies from psoriasis patients and healthy controls. Then, we examined the effect of IL-37 on inflammation in vivo in the imiquimod (IMQ)-induced psoriasis-like skin inflammation mouse model (IMQ model).

**MATERIALS AND METHODS**

**Patients**

Punch biopsies, excision biopsies, and keratome biopsies were collected from patients with psoriasis vulgaris in our outpatient clinic at the Department of Dermatology, Aarhus University Hospital, Denmark. Inclusion criteria comprised age >18 y, no topical treatment ≥2 wk, and no systemic treatment for ≥4 wk or 5× half-lives. The punch biopsies were collected from patients who had been treated with either adalimumab or ustekinumab (adalimumab [n = 6], 80-mg initial dose, then 40 mg every 2 wk, starting 1 wk after the initial dose or ustekinumab [n = 12], six responders and six nonresponders, 45 mg [≥100 kg] or 90 mg [≥100 kg] on week 0 and 4, then every 12 wk). Demographic data, Psoriasis Area Severity Index score (PASI), and biopsies (NP [defined as ≥5 cm from LP skin] and LP) were collected at baseline. Clinical assessment and biopsies from LP were obtained at follow-up (adalimumab on day 4, 14, 42, and 84; ustekinumab on day 4, 28, and 109). Healthy controls were included from our institution and were >18 y old. The study was approved by the Danish Ethical Committee and conducted in compliance with good clinical practice according to the principles of the Declaration of Helsinki.

**Mice**

C57BL/6J 6- to 10-wk-old female mice were purchased from Janvier Labs (Le Genest-Saint-Isle, France). Studies were approved by the Animal Experiments Inspectorate, Denmark, and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Mice were anesthetized with sevoflurane prior to handling. IMQ cream (Aldara 5%; Meda Pharma) or vehicle creme (16% isostearic acid, 4% glycerol monostearate 40–50, 5% ceteosteryl alcohol, 5% white soft paraffin, 0.5% satiavane CX 800, 5% glycerol 85%, 1% polysorbate 80, 1% benzyl alcohol, 1% paraxoxybenzoat-diluentum, 10% DAK 63, 61.5% purified water; Skanderborg Pharmacy, Skanderborg, Denmark) (45 mg) was applied to the shaved back skin once a day, from day −2 to day 1, while a single injection of IL-37 (recombinant human IL-37b [aa 46–218], 7585-IL-025/CF, 100 ng in 80 μl of PBS; R&D Systems) or PBS (80 μl) was given as an intradermal injection on day 0 in the same area. Mice were sacrificed on day 2 (control, n = 2; placebo, n = 7; IL-37, n = 7). Skin inflammation on the treated area was documented by digital photo. Biopsies from treated skin were either snap frozen for later RNA purification or fixed in formalin for H&E staining for measurement of epidermal thickness.

**NHEK**

NHEK were isolated by trypsinization of skin from patients undergoing plastic surgery as previously described (18). Second-passage keratinocytes were grown in a serum-free keratinocyte medium (Life Technologies, Invitrogen) supplemented with recombinant epidermal growth factor (EGF), bovine pituitary extract, and gentamicin in six-well plates at 37°C and 5% CO2. The medium was changed to keratinocyte medium without growth factors 24 h prior to stimulation. Cytokines used for stimulation experiments included the following: TNF-α (210- TA-010; R&D Systems), IFN-γ (285-IF; R&D Systems), IL-22 (782-IL; R&D Systems), IL-17A (34-8180; eBioscience), IL-17C (34-8477; eBioscience), IL-17F (14-8479; eBioscience), EGF (236-EG-200; R&D Systems), polyinosinic-polycytidylic acid (Poly I:C) (P1530; Sigma-Aldrich), the antimicrobial peptide LL-37 (5213; Tocris, Bristol, U.K.), and human β-defensin 3 (hBD3) (4435-BD-050; R&D Systems). Each stimulation was performed in duplicate.

**In vitro caspase-1 cleavage of recombinant pro–IL-37b**

rIL-37b aa 1–218 (AG-40A-0174Y; Adipogen/vWR), 0.1 ng in 100 μl of medium (50 nM HEPES pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 10% glycerol, 10 nM DTT) was added to a 96-well plate. Caspase-1 (CC126; Merck Millipore) was added in increasing amounts, and the plate was incubated at 37°C for 2 h. The medium was harvested, and Laemmli buffer ×2 was added 1:1 before it was heated and transferred to a gel for Western blotting. Specificity was validated by blocking with recombinant pro–IL-37 1-218 (AG-40A-0174Y; Adipogen/vWR) in the relation 1:5 (anti–IL-37 Ab: recombinant pro–IL-37 1–218).

https://doi.org/10.4049/immunohorizons.2000083
**Gene array**

Human Gene 1.0 Array analysis (Affymetrix) was performed on paired excision biopsies from nine psoriasis patients. A total of 100 ng of RNA was labeled with the Ambion WT Expression Kit (Ambion) according to the manufacturer's instructions. Samples were hybridized overnight to the GeneChip and scanned using an Affymetrix GCS 3000 7G scanner. Paired biopsies were analyzed by gene array, and 16,736 probes were annotated. A threshold for up- or downregulation was set at 1.5-fold change. Array data can be found at Geo Expression Omnibus database.

**Quantitative PCR**

NHEK were harvested for RNA isolation using the SV96 Total RNA Isolation System (Promega), and RNA concentration was measured with a Nanodrop 2000 spectrometer (Thermo Fischer Scientific). Skin biopsies in cryotubes were treated with −80°C RNA Later-ICE (Ambion) for 20 min, transferred to −20°C, and left overnight before RNA isolation. Transcription reagents, including TaqMan Gene Expression Master Mix (4369016), and TaqMan Gene Expression Assays were purchased from Thermo Fisher Scientific. Human primer/probes used are as follows: **IL37** (Hs00367201_m1) (also used for mice experiments), **IL18R1** (Hs00175381_m1), **SIGIRR** (Hs01546500_g1), and **RPLP0** (Hs99999902_m1) for normalization of target genes. Mice primer/probes used are as follows: **Il17a** (Mm00439619_m1), **Il1b** (Mm00434228_m1), **Il1c** (Mm00521397_m1), **Tgf** (Mm00443258_m1), **Il22** (Mm01226722_g1), **Defb4** (defensin β 4, Mm00731768_m1), **Lcn2** (lipocalin 2, Mm00656925_m1), **Si0039** (Mm00656925_m1), **Si0037a** (Mm01218201_m1), and **GAPDH** (Mm99999915_g1) or **Ubiquitin C** (Mm02525934_g1), as appropriate for target gene normalization. Gene expression was analyzed in triplicate on Applied Biosystems StepOneTM Real-Time PCR System in accordance with the protocol applied from the supplier (Thermo Fisher Scientific). Results were processed in the PlusOne Software v2.3. A standard curve was generated from each gene with a 4-fold serial dilution, and the amount of gene in each sample was determined from the standard curve.

**Immunofluorescence analysis**

Paired LP and NP (n = 4) skin biopsies and biopsies from healthy controls (n = 4) were fixed in formalin and paraffin embedded. The sections were deparaffinized for 3 × 5 min in xylene substitute buffer followed by a wash in ethanol-distilled water. Unmasking was performed by heating the sections in citrate buffer for 36 min in a microwave oven (650 W). Cryo-fixed 3D epidermal models were treated with acetone before blocking. Blocking was performed for 30 min with an Image iT Fx signal enhancer before incubation with an anti–IL-37 Ab (HPA054371, 1:100 in blocking buffer; Sigma-Aldrich) overnight. After washing, samples were incubated with a secondary Ab, goat anti-rabbit (A11008; Invitrogen). Specificity was validated by blocking with recombinant pro–IL-37 1-218 (AG-40A-0174Y; Adipogen/vWR) in the relation 1:5 (anti–IL-37 Ab: recombinant pro–IL-37 1-218). Nuclear staining was performed with ProLong Gold antifade reagent with DAPI, and sections were analyzed by epifluorescence microscopy.

**ELISA**

Protein from skin biopsies was isolated as previously described (19). ELISA was performed on purified protein from keratome biopsies from psoriasis patients. For detection of IL-37, DuoSet ELISA Development System, Human IL-37/IL-1F7 (DY9175; R&D Systems) was used as described by the manufacturer. Briefly, the ELISA plate was coated with the capture Ab overnight. After blocking, samples were added in duplicate, followed by the detection Ab, streptavidin HRP, substrate, and stop solution. OD was determined using a microplate reader (Laboratory systems iEMS Reader MF, Copenhagen, Denmark) set to 450 nm. Wavelength correction was set to 540 nm.

**Western blotting**

Isolation of protein from NHEK was performed as previously described (20). Proteins were separated according to size by SDS-PAGE and transferred on to nitrocellulose membranes by electroblotting. The membrane was probed with primary anti–IL-37 Ab (Ab116282 i:1000; Abcam, Cambridge, U.K.) and detected by secondary anti-rabbit IgG, HRP-linked Ab (7074; Cell Signaling Technology). The Ab used for immunofluorescence analysis was also tested in Western blotting (HPA054371 [1:200]; Sigma-Aldrich), and specificity was validated by blocking with recombinant pro–IL-37 1-218 (AG-40A-0174Y; Adipogen/vWR) in the relation 1:5 (anti–IL-37 Ab: recombinant pro–IL-37 1-218). The ECL detection system was used as described by the manufacturer (ClarityTM Western ECL Substrate, 1705061; Bio-Rad) for band imaging.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism 7.04. For statistical comparison of two groups, Mann–Whitney (nonpaired) or Wilcoxon matched-pairs signed rank test (paired data) for continuous data and Fishers exact test for category data were used, as groups were too small or did not meet assumptions of normality for these analyses. For comparison of more than two groups, one-way ANOVA with Tukey post hoc test (nonpaired data) was used as a parametric test, whereas for nonparametric data, Kruskall Wallis test with Dunn post hoc test (for nonpaired data) and Friedman test with Dunn post hoc test (for paired data) were performed. For gene array data, background correction and normalization of raw data and robust multiasay analysis were conducted with Affy package in R language. Next, Limma package in R language was applied to analyze the changes in gene expression values, LP versus NP. Multiple testing was performed with the Benjamini-Hochberg method to obtain the corresponding logarithmic fold change (logFC) and p value of changed gene expression. A p value <0.05 was considered statistically significant.

**RESULTS**

**IL37 is downregulated in human psoriasis skin**

To examine the relative gene expression of **IL37** among a large number of genes, transcriptome analysis was performed on paired
TABLE I. Array results on the top 10 up- and downregulated genes in LP skin compared with NP

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>LogFC</th>
<th>Adj. p</th>
<th>Gene Symbol</th>
<th>LogFC</th>
<th>Adj. p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCN1</td>
<td>5.94</td>
<td>$1.32 \times 10^{-13}$</td>
<td>DGAT2L6</td>
<td>$-2.77$</td>
<td>$2.33 \times 10^{-4}$</td>
</tr>
<tr>
<td>S100A12</td>
<td>5.71</td>
<td>$2.52 \times 10^{-11}$</td>
<td>PM20D1</td>
<td>$-2.64$</td>
<td>$4.57 \times 10^{-4}$</td>
</tr>
<tr>
<td>SERPINA4</td>
<td>5.50</td>
<td>$4.67 \times 10^{-7}$</td>
<td>IL37</td>
<td>$-2.57$</td>
<td>$4.81 \times 10^{-6}$</td>
</tr>
<tr>
<td>S100A7A</td>
<td>5.16</td>
<td>$1.44 \times 10^{-8}$</td>
<td>WIFI</td>
<td>$-2.56$</td>
<td>$1.60 \times 10^{-5}$</td>
</tr>
<tr>
<td>TMPRSS11D</td>
<td>4.78</td>
<td>$3.89 \times 10^{-11}$</td>
<td>AWAT2</td>
<td>$-2.46$</td>
<td>$4.48 \times 10^{-4}$</td>
</tr>
<tr>
<td>RHCG</td>
<td>4.69</td>
<td>$2.13 \times 10^{-9}$</td>
<td>CLEC2A</td>
<td>$-2.24$</td>
<td>$1.27 \times 10^{-6}$</td>
</tr>
<tr>
<td>HEPHL1</td>
<td>4.62</td>
<td>$4.24 \times 10^{-8}$</td>
<td>THRS</td>
<td>$-2.24$</td>
<td>$1.64 \times 10^{-4}$</td>
</tr>
<tr>
<td>PI3</td>
<td>4.39</td>
<td>$3.65 \times 10^{-8}$</td>
<td>UGT3A2</td>
<td>$-2.21$</td>
<td>$1.47 \times 10^{-7}$</td>
</tr>
<tr>
<td>S100A9</td>
<td>4.36</td>
<td>$9.11 \times 10^{-7}$</td>
<td>PAMR1</td>
<td>$-2.18$</td>
<td>$7.30 \times 10^{-9}$</td>
</tr>
<tr>
<td>SPRR2A</td>
<td>4.22</td>
<td>$3.47 \times 10^{-6}$</td>
<td>MSMB</td>
<td>$-2.14$</td>
<td>$2.74 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

Adj. adjusted.

LP and NP excision biopsies from nine psoriasis patients, all men with a median age of 43 y (interquartile range [IQR]: 35–57 y), median body mass index of 28.7 (IQR: 25.9–33.7), and median PASI score of 15.6 (IQR = 6.0–20.1). Overall, array results showed a significant upregulation of 876 genes and downregulation of 889 genes in LP skin compared with NP skin. Not only was the gene expression of IL37 downregulated in LP compared with NP skin, but it was also the third most downregulated gene throughout the array (logFC = $-2.57$, $p = 4.81 \times 10^{-6}$, Table I).

To verify array results, quantitative PCR (qPCR) analysis was performed on 4-mm punch biopsies from 18 psoriasis patients and six healthy controls (patient characteristics in Supplemental Table I). In line with array results, IL37 expression was downregulated in LP compared with NP skin ($p < 0.0001$) and compared with healthy control skin ($p = 0.049$) (Fig. 1A). The same patients were followed during treatment with adalimumab or ustekinumab with consecutive biopsies that were analyzed with qPCR for IL37 expression during treatment (adalimumab, all responders $n = 6$; ustekinumab, responders $n = 6$; nonresponders $n = 6$). Interestingly, IL37 expression increased over time in all responders, reaching the expression level seen in NP skin at baseline. At the last visit, IL37 expression was significantly higher in LP skin than at baseline for patients responding to treatment (adalimumab on day 109 versus day 0, $p < 0.05$ and ustekinumab responders on day 109 versus day 0, $p < 0.01$). By contrast, patients not responding to ustekinumab showed no significant increase in IL37 expression in LP skin during treatment (Fig. 1B).

To verify correspondence between IL-37 mRNA and protein, five patients had paired LP and NP keratome biopsies taken for ELISA (all men, median age = 43 y [IQR: 36–53] and median PASI score = 17.3 [IQR = 12.0–18.6]). Similar to IL37 mRNA findings, a lower IL-37 protein level was also seen in LP compared with NP skin, although this difference did not reach significance ($p = 0.063$) (Fig. 1C).

Altogether, these results demonstrate that IL-37 is downregulated in LP skin and that IL37 expression is normalized in association with treatment response.

FIGURE 1. IL-37 gene and protein in patients with psoriasis. (A) IL37 expression was analyzed by qPCR in skin biopsies from healthy controls (HC), $n = 6$; NP, $n = 16$; and LP, $n = 18$. (B) IL37 mRNA expression in NP and LP biopsies from patient responders and nonresponders to treatment. (C) IL-37 protein was measured by ELISA in paired NP and LP keratome biopsies, $n = 5$. Horizontal bars indicate mean values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. 

https://doi.org/10.4049/immunohorizons.2000083
IL-37 is localized to stratum granulosum of epidermis

In the study by Lachner et al. (6), IL-37 was localized to stratum granulosum, and its expression level was positively correlated with a high level of keratinocyte differentiation. We therefore took advantage of a 3D epidermal model to study IL37 expression in keratinocytes during differentiation. In this model, IL37 expression, standardized to RPLP0, increased proportionally with the degree of differentiation in three unstimulated 3D cultures (Fig. 2A). The median cycle threshold value of three unstimulated, subconfluent NHEK cultures was 31.3 (IQR: 30.7–31.4), whereas the cycle threshold value for the fully differentiated 3D epidermal model was 26.5. Immunofluorescence analysis detected IL-37 in the upper epidermal layer of the 3D epidermal models (Fig. 2B). In biopsies from healthy controls and NP skin, IL-37 was most detectable in stratum granulosum of the epidermis (Fig. 2C). The staining was mainly cellular within the flattened nuclei and in the cytoplasm of the keratinocytes. In biopsies from LP skin, IL-37 was not detectable. After preincubation with rIL-37, the IL-37 staining in NP skin was not detected (Supplemental Fig. 1).

Cytokines in psoriasis regulate IL-37 expression in NHEK cultures

The exact time for the loss of stratum granulosum and thereby probably also the IL-37 cytokine during development of psoriasis is not known, but changes in the cytokine milieu are thought to induce the structural changes. Therefore, it is possible that important cytokines in psoriasis regulate IL37 expression. We stimulated NHEK cultures with cytokines known to play a role in psoriasis pathogenesis (TNF-α, IFN-γ, IL-17A, and IL-22), molecules previously demonstrated to induce IL37 (EGF, Poly I:C, and hBD3 (21)), and the antimicrobial peptide LL37, involved in psoriasis pathogenesis. Significant upregulation of IL37 expression was seen with TNF-α stimulation for 18 h (NHEK, n = 12, 2.7-fold, p < 0.01) (Fig. 3A). The combination of TNF-α and IFN-γ for 18 h increased IL37 expression at the same magnitude as TNF-α alone (Fig. 3B), whereas increased expression of the receptors for IL-37 required the combination of TNF-α and IFN-γ (Fig. 3C). Similarly, single stimulations with EGF, Poly I:C, or LL-37 did not alter IL37 expression (Supplemental Fig. 2).

In line with qPCR data, Western blotting of purified protein from NHEK lysate showed that TNF-α stimulation induced the expression level of IL-37 protein. As TNF-α is a known trigger of the p38 MAPK signaling pathway, NHEK cultures were preincubated with a p38 MAPK inhibitor (SP202190) before stimulation with TNF-α. This abolished the TNF-α-mediated induction of IL-37 protein (Fig. 4). Interestingly, preincubation with IL-17A, IL-17C, IL-17F, and IL-22 before TNF-α stimulation prevented IL-37 induction (Fig. 5A).

As most commercially available Abs for Western blot against IL-37 are polyclonal, lack of specificity may influence the results.
For further analysis of the Abs used for Western blotting (ab16282; Abcam) and immunofluorescence analysis (HPA054371; Sigma-Aldrich) in the current study, a Western blotting experiment was also performed with the HPA054371, Sigma-Aldrich, anti–IL-37 Ab. This Ab recognized a similar TNF-α–regulated band in NHEK cultures at the same size (around 30 kDa) as Abcam ab16282 anti–IL-37 Ab. Recombinant pro–IL-37 (aa 1–218) was also detected at 30 kDa, whereas caspase-1–cleaved IL-37 subunits were detected below the band for the pro–IL-37 form. Furthermore, a strong band was detected in normal human skin (Fig. 5B, SC). When recombinant pro–IL-37 was added for blocking, the band at 30 kDa was not detected (Supplemental Fig. 3), whereas the nonspecific bands persisted. In normal human skin, a smaller band also disappeared with blocking, indicating that the IL-37 Ab also detects a posttranslational processed form of IL-37.

In summary, these results demonstrate that psoriasis-specific cytokines and the degree of keratinocyte differentiation regulate IL-37 expression.

**Intradermal treatment with human recombinant IL-37 does not significantly reduce inflammation in the IMQ model**
IMQ or vehicle was applied to the back skin from day –2 to 1, whereas the injection of rIL-37b was given on day 0 and mice were sacrificed on day 2. Both at day 1 and day 2, there was clinically less thickening of the back skin in IL-37–treated mice than in mice given placebo. At day 2, there was at trend toward lower epidermal thickness of back skin on H&E-stained sections of IL-37–treated mice, although this was NS. Along this line, the expression of Il17b, Il17c, Defb4, Lnc2, S100a9, and S100a7a in biopsies of treated skin showed a trend toward downregulation in mice treated with IL-37, whereas this was not seen for Il17a. However, there were no significant changes in cytokine expression in any of the groups, and expression levels were below the detection limit for Il17a and Il22 (Fig. 6).

**DISCUSSION**

IL-37 has only sparsely been investigated in psoriasis and with contradicting results. In 2014, using only immunofluorescence analysis, Teng et al. (14) found that IL-37 was upregulated in human LP skin compared with healthy control skin, correlated positively with increasing psoriasis severity, and localized to memory CD4+ T cells in the epidermis and macrophages in the dermis. This contrasts with the results of the current study. Using transcriptome analysis, qPCR, ELISA, and immunofluorescence analysis, we demonstrated a marked downregulation of IL-37 in LP compared with NP skin. Furthermore, IL37 mRNA was lower in LP skin than in healthy controls, and IL37 expression in LP skin increased to the level in NP skin in patients with psoriasis responding to treatment with adalimumab or ustekinumab. Immunofluorescence analysis using an Ab validated for specificity in the current study and by others (6) localized IL-37 to stratum granulosum of the epidermis. Our results from transcriptome analysis, immunofluorescence analysis, and qPCR have also been supported by others (15–17). Also, in situ hybridization demonstrated IL37 expression in the nuclei of keratinocytes in their final stages of differentiation in stratum granulosum of human skin (3).
Loss of stratum granulosum in psoriasis is an obvious mechanism for loss of IL-37 in LP skin. However, cytokines such as TNF-α, IFN-γ, IL-1β, and TGF-β1, which are important for the development of psoriasis (22), have been shown to regulate IL-37 in PBMCs and keratinocytes (13, 21). We demonstrated that TNF-α upregulated IL-37 gene and protein expression in NHEK through the p38 MAPK. This was surprising because TNF-α is highly expressed in psoriasis. However, IL-17A, IL-17C, IL-17F, and IL-22 inhibited TNF-α–induced IL-37 protein upregulation. Smithrithee et al. (21) found similar upregulation of IL37 expression in NHEK by stimulation with TNF-α but not in IL-37 protein with Western blotting. This may be due to the use of a different anti–IL-37 Ab. They also found upregulation of IL37 by hBD3, EGF, and Poly I:C alone, and the combination of hBD3 with TNF-α, EGF, or Poly I:C increased IL37 expression 17–24-fold. Differences in results may be due to the use of NHEK from neonatal foreskin in the study by Smithrithee et al. (21). Overall, in the current study, the magnitude of IL37 mRNA induction to any stimulus was small. As IL-37 was found in the stratum granulosum and with the most pronounced expression in the highest differentiated 3D epidermal model, NHEK cultures that consist of low differentiated keratinocytes may have little potential for IL37 expression. Also, as previously described, instability region within the coding region of IL37 mRNA may only leave a narrow window for detecting gene regulation (23).

IL-37 has protective effects in various models of inflammation (7–13). Treating mice transgenic for keratin 14 vascular endothelial growth factor A with injections of plasmid-coding human IL37 sequence-formulated cationic liposomes, Teng et al. (14) demonstrated protective effects of IL-37. IL-37 protection was demonstrated by assessment of psoriasis lesions by calculation of Baker score, by histological H&E staining (and toluidine-blue staining for mast cells), and immunofluorescence analysis against VCAM. Only IFN-γ was found reduced in local psoriasis lesions of IL-37–treated mice with qPCR, whereas IL-4 and IL-23a were unaltered. ELISA on serum showed reduced IL-10 in IL-37–treated mice, and IL-12p70 and IL-17A were below the detection level (14).

In contrast to the experiment by Teng et al. (14) in which repeated injections with plasmid coding IL37 were performed, only one injection was given locally in the epidermis during development of IMQ-induced psoriasis-like skin inflammation in this study. Treatment with recombinant human IL-37 did not inhibit inflammation in the IMQ model, although a trend for a protective effect was seen. This may be due to small group size, but timing of injection and dose may play a role. It is possible that treatment with a lower dose would have increased the anti-inflammatory effect because formation of homodimers at higher concentrations blocks the IL-18Rα (24). Also, in future studies,
treatment with a stable monomer rIL-37 may improve anti-inflammatory effects (24). It should also be considered that IL-37 may target proinflammatory pathways in psoriasis that are not the driving mechanisms in the IMQ model.

In this article, we present results that strongly support that IL-37 is downregulated in LP psoriasis skin. IL-37 was localized to the stratum granulosum, and it is therefore possible that the cytokine is almost abolished because of the loss of this part of the epidermis in psoriasis. However, cytokine signaling may also play a role, as TNF-α upregulation of IL-37 in NHEKs was inhibited by Th17-pathway cytokines. Future in vitro studies on IL-37 should, however, be considered performed in 3D epidermal models, as IL-37 is highly expressed in differentiated keratinocytes.

DISCLOSURES

The authors have no financial conflicts of interest.

ACKNOWLEDGMENTS

We thank the technicians at the laboratory at the Department of Dermatology, Aarhus University Hospital, Aarhus, Denmark.

REFERENCES