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IL-37 Ameliorates the Inflammatory Process in Psoriasis by Suppressing Proinflammatory Cytokine Production

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IL-37 is a potent inhibitor of innate immunity by shifting the cytokine equilibrium away from excessive inflammation. Psoriasis is thought to be initiated by abnormal interactions between the cutaneous keratinocytes and systemic immune cells, triggering keratinocyte hyperproliferation. In the current study, we assessed IL-37 in two well-known psoriasis models: a human keratinocyte cell line (HaCat) and the keratin 14 VEGF-A–transgenic mouse model. First, we used the HaCaT cell line, which was transiently transfected with an overexpressing IL-37 vector, and tested the effect of IL-37 on these cells using a mixture of five proinflammatory cytokines. IL-37 was effective in suppressing the production of CXCL8, IL-6, and S100A7, which were highly upregulated by the mixture of five proinflammatory cytokines. Keratin 14 VEGF-A–transgenic mice were treated with plasmid coding human IL-37 sequence–formulated cationic liposomes, and we observed potent immunosuppressive effects over the 18-d period. In this model, we observed reduced systemic IL-10 levels, local IFN-γ gene transcripts, as well as mild mast cell infiltration into the psoriatic lesions of the mice. Immunohistochemical analysis indicated that IL-37 was expressed by effector memory T cells, as well as macrophages, in human psoriatic plaques. In conclusion, our studies strongly indicate that IL-37 plays a potent immunosuppressive role in the pathogenesis of both experimental psoriasis models in vitro and in vivo by downregulating proinflammatory cytokines. Importantly, our findings highlight new therapeutic strategies that can be designed to use this immunosuppressive anti-inflammatory cytokine in psoriasis and other inflammatory cutaneous diseases. The Journal of Immunology, 2014, 192: 1815–1823.

Interleukin-37 (formerly IL-1F7), earlier referred to as FIL-1β/IL-1H4/IL-1H/IL-1RP1, is a new member of the IL-1 family that encompasses 11 structurally related members sharing a β-barrel motif (1–3). However, quite distinct from most IL-1 family members, which have been characterized with proinflammatory functions, IL-37 has emerged as a fundamental inhibitor of the innate immune response. A total of five splice variants (IL-1F7a–e) exist in IL-1F7 transcripts. IL-1F7b (NM014439.3) is the largest cytokine member and is encoded by five of the six exons spanning the IL-37 gene, of which exon 1 encodes the putative caspase-1–processing site (4, 5). The IL-37b precursor can be processed by caspase-1 into the mature form, requiring the mature form to translocate actively into the cell nucleus (3, 5). IL-37b–specific mRNA has been found in diverse human tissues, including the lymph node, thymus, bone marrow, placenta, lung, and testis. IL-37 protein production in PBMCs and dendritic cells (DCs) was shown to be upregulated when stimulated by TLR ligands, as well as several proinflammatory cytokines (4, 6).

In vitro, the expression of IL-37 in macrophages or epithelial cells was shown to greatly dampen constitutive or induced production of several major proinflammatory cytokines, such as IL-1β, TNF, IL-6, and MIP-2 (3, 5). In vivo, IL-37 protects mice from LPS-induced shock, chemical-induced colitis, and hepatitis (3, 7–9). However, the inhibitory effects of IL-37 in the pathogenesis of inflammatory skin diseases, including psoriasis, have not been demonstrated.

Psoriasis is one of the most common immune-mediated chronic inflammatory cutaneous disorders and is characterized by epidermal hyperplasia, dilated blood vessels, and increased leukocyte infiltration (10, 11). Although the pathogenesis of this autoimmune disease has not been fully characterized, there is growing evidence that the formation and maintenance of psoriatic plaques are mediated by cell–cell interactions between resident skin cells (mainly keratinocytes) and elements of the immune system in the context of a combined Th1/Th17/Th22 immune response (12–14).
In the early stage of psoriasis, keratinocyte-derived chemokines and cytokines have major roles in continuing the recruitment of leukocytes invading the inflammatory sites. Secondly, intrinsic defects in cytokine and growth factor signaling in keratinocytes could be responsible for aberrant keratinocyte hyperproliferation and differentiation. Moreover, a series of recent reports (15–17) demonstrated that specific inhibition of the innate immune response may be one of the most promising therapeutic approaches in the treatment of psoriasis. Based on these reports, our laboratory is focused on IL-37b, the largest and most widely researched inflammatory cytokine and differentiation. Moreover, a series of recent reports (15–17) demonstrated that specific inhibition of the innate immune response may be one of the most promising therapeutic approaches in the treatment of psoriasis (18).

Our results support the idea that the presence of IL-37 attenuates the inflammatory process in psoriasis by downsizeing essential proinflammatory cytokine production and highlight new therapeutic strategies that can be designed using this immunosuppressive anti-inflammatory cytokine in psoriasis and other inflammatory cutaneous diseases.

Materials and Methods

Cell culture, cytokines, and plasmids

The keratinocyte cell line HaCaT was obtained from the China Center for Type Culture Collection (Wuhan, China). The mixture of five proinflammatory cytokines (M5) included 10 ng/ml TNF-α, IL-17A, IL-22, IL-1α, and Oncostatin-M (ProSpec, East Brunswick, NJ). The model pCMV6-entry (pNull) vector tagged C-terminal Myc-DDK (Origene Technologies, Rockville, MD) and human IL-37 (NM 014439) cDNA open reading frame entry (pNull) vector tagged C-terminal Myc-DDK (Origene Technologies, Rockville, MD) and human IL-37 (NM 014439) cDNA open reading frame were cloned into pCMV6-entry vector (pIL-37; Origene Technologies) and were sequenced to exclude mutations. The pmax-EGFP plasmid (pEGFP; Lonza, Allendale, NJ) encoding EGFP was used as an indication of transfection. All plasmids were purified using Endo-Free plasmid purification kits (QIAGEN, Düsseldorf, Germany).

Transient transfections

HaCaT cells were transiently transfected with pCL or pNull using an Ammax Cell Line Nucleofector Kit V (Lonza), according to the manufacturer’s instructions. After overnight recovery, the transfected cell lysates were tested for IL-37 expression by Western blot analysis. The keratinocyte lysates were obtained at 24, 48, and 72 h posttransfection for the duration of IL-37 expression by keratinocytes transiently transfected with pCL-37.

Induction of psoriatic model in vitro

Keratinocytes were stimulated with M5 to induce inflammation that recapitulates numerous features of psoriasis (19). Total RNA was isolated from keratinocytes at 0, 6, 12, and 24 h poststimulation with M5. Culture supernatants were collected at 12; 24, 36, and 48 h poststimulation with M5 for quantification of the cytokine levels.

Preparation of cationic liposome–plasmid complexes

Cationic liposomes containing 1, 2-dioleoyl-3-trimethylammoniumpropane, 1, 2-dioleoyl-3-trimethylammoniumpropane (Avanti Polar Liposomes, Alabaster, AL), in a 1:1 molar ratio with cholesterol (Sigma-Aldrich, St. Louis, MO) was formulated to enhance the efficiency of gene transfection in vivo. To formulate the cationic liposome (CL)–plasmid complexes, pIL-37 or pNull was dispersed into prediluted CLs according to their mass ratios (CL to plasmid). We evaluated the size distribution and encapsulation efficiency of in vivo gene transfection in vivo. To formulate the cationic liposome (CL)–plasmid complexes, pIL-37 or pNull was dispersed into prediluted CLs according to their mass ratios (CL to plasmid). We evaluated the size distribution and encapsulation efficiency of in vivo gene transfection in vivo. To formulate the cationic liposome (CL)–plasmid complexes, pIL-37 or pNull was dispersed into prediluted CLs according to their mass ratios (CL to plasmid). We evaluated the size distribution and encapsulation efficiency of in vivo gene transfection in vivo. To formulate the cationic liposome (CL)–plasmid complexes, pIL-37 or pNull was dispersed into prediluted CLs according to their mass ratios (CL to plasmid). We evaluated the size distribution and encapsulation efficiency of in vivo gene transfection in vivo. To formulate the cationic liposome (CL)–plasmid complexes, pIL-37 or pNull was dispersed into prediluted CLs according to their mass ratios (CL to plasmid).

In vivo experiments

The psoriasis animal model in our research was the keratin 14 VEGF–transgenic (K14–VEGF–Tg) mouse model, in which VEGF is overexpressed in the epidermis, and the mouse spontaneously develops a chronic inflammatory skin disease with many features similar to human psoriasis (21). To study IL-37 expression kinetics in vivo, 8–10-week-old K14–VEGF–Tg homozygous mice (n = 21; The Jackson Laboratory, Bar Harbor, ME) were injected with CL–pIL-37 complex (5 μg pDNA/mouse) via the tail vein. Exogenous IL-37 gene expression in the spleen was assessed at 0, 6, 12, 18, 24, 48, and 72 h after transfection. In the second series of experiments, 8–10-week-old K14–VEGF–Tg homozygous mice were randomly assigned to three groups (n = 5/group): mice treated with 5% dexamethasum group; mice treated with CL–pNull complexes (5 μg pDNA/mouse); and mice treated with CL–pCL–37 complex (5 μg pDNA/mouse) six times (i.e. once every 3 d). Forty-eight hours after the previous treatment, the animals were anesthetized by i.p. injection of 10% glutaraldehyde for macroscopic photography. The animals were then sacrificed, and serum and psoriatic lesion tissues were collected. All studies involving mice were approved by the State Key Laboratory of Biotherapy Institutional Animal Care and Use Committee.

Skin samples

The use of skin biopsies for this study was approved by the Ethical Committee of the Sichuan Provincial People’s Hospital and Health Care Hospital of Sichuan University. After obtaining informed consent, psoriatic plaque biopsies were obtained from patients with moderate-to-severe psoriasis vulgaris (n = 15, mean age = 28 y, skin involvement, 10–70% of body surface area) who did not receive any therapy for 4 wk. Skin biopsies obtained from healthy volunteers undergoing circumcision (n = 15, mean age = 8 y) were used as control.

Histological studies and immunohistochemistry

Immunohistochemical single staining of 4-μm vertical sections of formalin-fixed, paraffin-embedded (FFPE) human psoriatic plaques was performed using the avidin-biotin technique (ZSGB-Bio, Beijing, China). After deparaffination, rehydration, and blocking of endogenous peroxidase activity, heat-induced Ag retrieval was performed using Tris/EDTA (pH 9) in a pressure cooker for 3 min. After cooling, the specific binding was blocked with goat serum reagent, followed by incubation with rabbit polyclonal anti-human IL-37 Ab (Abcam, Cambridge, MA) overnight at 4°C in a humidified chamber. To detect IL-37+ cells, the sections were sequentially washed and incubated with biotinylated secondary Ab (15 min; Vector Laboratories, Burlingame, CA). The sections were visualized by indigo blue. The sections were blocked with serum and incubated with anti-human CD4 (Maixin, Fuzhou, China), which was designed based on alkaline phosphatase visualized by 5-bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium (indigo blue) and HRP and visualized by 3-aminopropyl-ethyl carbazole (red color) system, was utilized to identify IL-37+ cells. In FFPE human severe psoriatic plaques. Similar to single staining, the sections were incubated with primary anti-human IL-37 Ab (Abcam), biotinylated secondary Ab, and alkaline phosphatase–labeled streptavidin complex and visualized by indigo blue. The sections were blocked with serum and incubated with anti-human CD4 (Maixin), anti-cytotoxic lymphocyte Ag (CLA; e Bioscience, San Diego, CA), anti-Foxp3 (Abcam), anti-CD68 (Gene Tech, Shanghai, China) Abs (as recommended by the manufacturer, Maixin), biotinylated secondary Ab, and HRP-labeled streptavidin complex (15 min) and visualized using diaminobenzidine. The specific staining of IL-37 was confirmed by another anti-human IL-37 polyclonal Ab (pAb; R&D Systems, Minneapolis, MN; Supplemental Fig. 3). Sequential double-staining immunohistochemistry kit (Maixin, Fuzhou, China), which was designed based on alkaline phosphatase visualized by 5-bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium (indigo blue) and HRP and visualized by 3-aminopropyl-ethyl carbazole (red color) system, was utilized to identify IL-37+ cells. In FFPE human severe psoriatic plaques. Similar to single staining, the sections were incubated with primary anti-human IL-37 Ab (Abcam), biotinylated secondary Ab, and alkaline phosphatase–labeled streptavidin complex and visualized by indigo blue. The sections were blocked with serum and incubated with anti-human CD4 (Maixin), anti-cytotoxic lymphocyte Ag (CLA; e Bioscience, San Diego, CA), anti-Foxp3 (Abcam), anti-CD68 (Gene Tech, Shanghai, China) Abs (as recommended by the manufacturer, Maixin), biotinylated secondary Ab, and HRP-labeled streptavidin complex; and visualized by red color. Similarly, FFPE mice psoriatic plaques were stained with H&E and stained with anti-mouse VCAM pAb (Boster, Wuhan, China). Additionally, to detect mast cells, mice psoriatic plaque sections were stained with toluidine blue (AMRESCO, Solon, OH), as previously described (22). Images of the stained slides of animal biopsies and human psoriatic plaques were obtained with an Olympus BX60 or Olympus DP72 microscope (both from Olympus Optical, Tokyo, Japan). Five random fields of view (400×), of each mouse psoriatic plaque section stained with H&E, were assessed using the Baker Score system. Similarly, mast cells were quantified by counting five randomly selected fields in toluidine blue–stained slides.

Quantitative real-time PCR analysis

Total cellular RNA, mice splenic RNA, and RNA in psoriatic lesions were isolated using TRIzol reagent (Ambion, Carlsbad, CA). Quantitative real-time PCR (QRT-PCR) was carried out using the One Step SYBR PrimeScript PLUS RT-PCR Kit (TaKaRa, Dalian, China) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The following
primers (Takara) were used for QRT-PCR analysis: human β-actin: forward 5'-CCACGAAACACTACACCTCA-3' and reverse 5'-GTTGAGGTGCCCATGCTAC-3'; human IL-6: forward 5'-AAATGCCTGCTGTCGAGAAGC-3' and reverse 5'-GACCCACAAGGACTTGTC-3'; mouse β-actin: forward 5'-CCTCTATGGCCAACAAGTGTC-3' and reverse 5'-GACCTGTCTGGGAAGAGGAC-3'; mouse IL-4: forward 5'-ACAGGAAAGGCGCACTA-3' and reverse 5'-GAACCCCTTACAGCGAGAAC-3'; mouse IL-23a: forward 5'-GACCCCAAAGGACTCAAAGGC-3' and reverse 5'-ATGCGGCTTACGAGGATGAG-3'; human CXCL8: forward 5'-GGCTTCCACATGTCCTCACAA-3' and reverse 5'-TGTCCTTTTCTCAAAACAT-3'; mouse S100A7: forward 5'-CGCTGCTCAGGATTTTCATG-3' and reverse 5'-TACCTGCTCTGAAGGAGGC-3'; mouse IFN-γ: forward 5'-TCAAGTGGCATAGATGTGGAAGAA-3' and reverse 5'-TGCTGCTCAGGATTTTCATG-3'; mouse IL-4: forward 5'-ACAGGAAAGGCGCACTA-3' and reverse 5'-GAACCCCTTACAGCGAGAAC-3'; mouse IL-23a: forward 5'-GACCCCAAAGGACTCAAAGGC-3' and reverse 5'-ATGCGGCTTACGAGGATGAG-3'; mouse IL-12p70: forward 5'-TCGCTTTTTCTCAAAACAT-3' and reverse 5'-GTCCTTGAGATGGAAGAGG-3'; mouse IL-17A: forward 5'-TCGCTTTTTCTCAAAACAT-3' and reverse 5'-GTCCTTGAGATGGAAGAGG-3'; IL-10: forward 5'-ACAGGAAAGGCGCACTA-3' and reverse 5'-GAACCCCTTACAGCGAGAAC-3'; mouse IL-10 mAb (E Bioscience, San Diego, CA). The quantitative analysis of IL-12p70, IL-17A, and IL-10 was performed using the human CXCL8 ELISA Ready-SET-Go kit (eBioscience, San Diego, CA). The levels of IL-12p70, IL-17A, and IL-10 in mouse serum were detected using specific mouse ELISA kits (Neobioscience, Shenzhen, China). IL-12p70, IL-17A, and IL-10 in mouse serum were detected using specific mouse ELISA kits (Neobioscience, Shenzhen, China).

Western blot analysis

The expression of IL-37 and S100A7 in keratinocytes was detected with mouse anti-DDK mAb (Stratagen, Santa Clara, CA) (23) and anti-human S100A7 mAb (Imgenex, San Diego, CA). The quantitative analysis of integral OD of the bands in the Western blot was performed using the Gel-Pro analyzer 4.0 (Media Cybernetics, Bethesda, MD).

Statistical analysis

Levels of statistical significance were calculated using SPSS 17.0 (IBM, Chicago, IL), one-way ANOVA by ranks with a Tukey test. The p values < 0.05 were considered statistically significant, and all data are mean ± SD.

Results

IL-37 inhibited the production of CXCL8, IL-6, and S100A7 in stimulated keratinocytes

After overnight recovery from nucleofection, overexpression of IL-37 in keratinocytes was identified by Western blotting (Fig. 1A, left panel). The kinetics study demonstrated that, at 24 h post-transfection, IL-37 was highly expressed by keratinocytes transfected with pIL-37; this extended to the 48-h time point. At 72 h, IL-37 expression had decreased compared with that observed at 24 and 48 h (Fig. 1A, right panel, Supplemental Fig. 1A), offering a relative stable condition to study the roles of IL-37 in vitro. In keratinocytes that had not been transfected with pIL-37, there was a strong increase in CXCL8, IL-6, IL-13, and S100A7 gene transcripts within 12 h poststimulation with M5 (Fig. 1B–D, left panels, Supplemental Fig. 1B), followed by a rapid decrease after an additional 12 or 36 h. Therefore, we treated control and transfected keratinocytes with M5 to investigate the effects of IL-37 on the expression of the above-mentioned molecules. First, IL-37–overexpressing keratinocytes expressed lower CXCL8 gene transcription at 6, 12, and 24 h poststimulation with M5 (at 6 h, *p < 0.05 and #p < 0.01, at 12 h: *p < 0.05 and $p < 0.05, Fig. 1C, left panel). IL-6 quantification by ELISA in culture supernatants confirmed the reduction led by IL-37 (e.g., at 12 h, 79.02 pg/ml for pIL-37 versus 105.87 pg/ml for control culture and 118.91 pg/ml for pNull keratinocyte culture, Fig. 1C, right panel). Also, IL-13 gene transcription was lower compared with that observed in the control and pNull keratinocytes (Supplemental Fig. 1B). However, we were not able to evaluate the effect of IL-37 overexpression on the level of IL-13 in supernatants because of its low level using ELISA.

Third, expression of the S100A7 gene was significantly decreased in IL-37–overexpressing keratinocytes compared with control and pNull keratinocytes over 12 h poststimulation with M5 (e.g., at 12 h, 736.64-fold increase for pIL-37 keratinocytes versus 120.64-fold decrease for pNull keratinocytes and 1224.79-fold decrease for control keratinocytes, respectively, *p < 0.05 and $p < 0.05, Fig. 1D, left panel). This was confirmed by Western blotting at the protein level over 24 and 48 h poststimulation with M5 (at 24 h, *p < 0.05 and **p < 0.01; at 48 h, ***p < 0.001 and ****p < 0.001, Fig. 1D, right panel, Supplemental Fig. 1C). To summarize, the results obtained in vitro clearly demonstrated that IL-37 suppresses the expression of CXCL8, IL-6, and S100A7 in stimulated keratinocytes, recapturing critical features of psoriasis.

IL-37 gene delivery in vivo using K14-VEGF–Tg mice

IL-37 transgene expression in K14-VEGF–Tg mice that were treated with a single injection lasted for ~72 h (Fig. 2A). Relatively high transgene expression was obtained as early as 6 h after injection (~23% of the peak level) and increased over time, reaching a maximum at 18 h. At later time points, the level of IL-37 expression dropped sharply; at 24 h the expression was ~15% of its maximum value, followed by a slow decline in the next 24 h; it became nearly undetectable at 72 h.

Psoriatic symptoms of K14-VEGF–Tg mice were assessed through macroscopic observation and pathological examination (Fig. 2B). The mice treated with medium or pNull gene delivery exhibited typical features of psoriasis, such as scabby, erythematous, and scaly skin lesions (Fig. 2B, top panels). Mice injected with pIL-37 showed mild symptoms, including scattered calluses and barely visible diabrosis and scales (Fig. 2B). Histological examination of psoriatic plaques stained with H&E revealed that mice treated with either medium or pNull gene delivery demonstrated psoriatic disease that was characterized by epithelial parakeratosis and keratoplasia, severe lymphocyte infiltration into the dermis and epidermal layers, and apparent angiectasis (Fig. 2B, middle panels). Importantly, reduced epithelial hyperplasia and less severe hemangiectasis were observed in the pIL-37–injected animals (Fig. 2B, middle panels). We also assessed the expression of VCAM in psoriatic plaques using immunochemical analysis; it was found perivascularly in mice treated with medium or the pNull gene (Fig. 2B, bottom panels). However, localization of VCAM in the pIL-37 treatment group was negligible. Moreover, we also thoroughly evaluated the pathological characteristics and assessed the psoriatic symptoms of mice lesions according to the Baker Score system: scores in the IL-37 gene delivery group were significantly lower compared with the other two groups (*p < 0.05 and #p < 0.05, Fig. 2C). Together, these observations demonstrate that IL-37 gene delivery was highly effective in relieving the psoriatic symptoms of K14-VEGF–Tg mice.

IL-37 gene delivery ameliorated systemic and local immune pathology

Because our study using pIL-37 gene delivery was highly promising, we next investigated the molecular mechanism of IL-37. We measured various cytokines in mice serum using ELISA. The levels
of IL-12p70 and IL-17A were below the lower detection limits, so these differences could not be compared (data not shown). Systemic IL-10 in the pIL-37 delivery group of mice was lower than in the other groups, indicating that systemic immune conditions were restored to homeostatic status (Fig. 3A). We also detected IFN-γ, IL-4, and IL-23a gene levels in psoriatic plaques using QRT-PCR. We observed that the expression level of IFN-γ in the pIL-37 delivery group declined nearly 3-fold compared with the control group at 0 h. Level of CXCL8 and IL-6 in lysates (right panel). At the indicated time points, levels of CXCL8 and IL-6 were measured by ELISA, and S100A7 was detected by Western blot (M₀ of S100A7 was ~11 kDa). The data are representative of experiments that were carried out in triplicate. *p < 0.05, **p < 0.01, ***p < 0.001, versus control keratinocytes at the same time point; #p < 0.05, ##p < 0.01, ###p < 0.001, versus pNull group at the same time point. ns, not significant compared with the control or pNull group.

Abundant IL-37 expression in human psoriatic lesions

IL-37 was highly expressed in human psoriatic lesions compared with the skin of healthy individuals, as assessed by immunohistochemical analysis. We did not observe specific staining in the epithelial keratinocytes in healthy human skin or psoriatic plaques (Fig. 4, top panels). In psoriatic plaques, we detected a strong staining in the papillary areas and sparse localization in cells of the epidermis (Fig. 4, middle panels). IL-37 was scarce in the dermal part of healthy skin, but it was weakly localized in the middle dermis region of the psoriatic plaques (Fig. 4, bottom panels). Furthermore, IL-37 was found at high densities in severe plaques compared with mild plaques. The distribution pattern of IL-37 in psoriatic plaques was characterized as “More severe pathogenesis, higher expression of IL-37.”
IL-37 was expressed primarily by effector memory T cells and macrophages in human psoriatic lesions

The highly distributed pattern of IL-37 in human psoriatic lesions prompted us to identify the IL-37\(^+\) cells to reveal the role of IL-37 in psoriasis. Based on the pathological observations, immunocytes are most likely the IL-37\(^+\) cells and are not structural cells of the skin, such as keratinocytes and fibroblasts. We identified IL-37\(^+\) positive cells by sequential immunohistochemical double staining with specific markers. We identified that most of the IL-37\(^+\) cells localized in the papillae and epidermis were CD4\(^+\) cells (Fig. 5A). These IL-37-producing CD4\(^+\) T cells were identified as effector memory T (T\(_{EM}\)) cells with the hallmark CLA (Fig. 5B), rather than as regulatory T cells (Tregs) with marked Foxp3 staining (Fig. 5C). In the middle dermis, IL-37-expressing cells appeared to be macrophages, with the colocalization of IL-37 and CD68 (Fig. 5D).

Discussion

In this article, we describe an essential immunosuppressive role of IL-37 in the pathogenesis of psoriasis in vitro and in vivo. We show that overexpression of IL-37 in human keratinocytes inhibits the production of CXCL8, IL-6, and S100A7 in vitro. We also demonstrate that IL-37 gene delivery relieves the psoriatic symptoms and ameliorates systemic and local immune pathology in K14-VEGF-Tg mice. By immunohistochemical analysis, we demonstrate that IL-37 is strongly expressed by T\(_{EM}\) cells, as well as macrophages, in human psoriatic plaques, appearing to reflect the capacity of IL-37 to curb the overreacted pathogenesis of psoriasis.

To study the possible immunomodulatory role of IL-37 in psoriasis in vitro, the keratinocyte cell line HaCaT was stimulated with proinflammatory cytokines (M5) to mimic the Th1/Th17/Th22 inflammatory response between keratinocytes and immune cells in the pathogenesis of psoriasis (19). M5 activated the STAT3-, MAPK-, and NF-κB–signaling pathways (reinforced by C/EBP transcription factor activation induced by IL-17A), resulting in a synergistic effect on CXCL8, IL-6, and S100A7 production by human keratinocytes, which is effectively inhibited by IL-37, suggesting the harnessing effect of IL-37 in the autoimmune response. The anti-inflammatory property of IL-37 could be facilitated by interaction with Smad3, as revealed by mass spectrometry and biological function researches (3). Smad3, the downstream transcriptional effector of TGF-β signaling, translocates into the nucleus upon TGF-β-mediated phosphorylation and binds to cellular DNA to affect gene transcription, modulating the proinflammatory signal pathways. Foremost, Smad3 directly binds to and represses the proximal promoter of IFN-γ and inhibits the expression of its positive regulator T-BET, thus regulating the expression of chemokines and chemokine receptors (24). In other studies, Smad3 antagonized C/EBP-STAT3 transcriptional activation and, thereby, suppressed IL-6 production and blocked the IL-17A–signaling pathways (25, 26). Smad3 also is involved in the inhibition of the activation of transcription factor AP-1 induced by IL-1β or TNF-α (27). We conclude that the expression of IL-37 contributes to counter against M5-mediated proinflammatory effects through transcriptional factors affected by the interaction between IL-37 and Smad3.

Overall, psoriasis is triggered by an imbalance among proinflammatory cytokines, chemokines, and growth factors, which orchestrate the cross-talk among immune cells, epidermal keratinocytes, and blood vessels. Indeed, the gene expression levels of CXCL8 and S100A7 are upregulated up to 17- and 18-fold, respectively, in psoriatic plaques compared with normal skin. In the innate immune–response stage of psoriasis, activated epithelial keratinocytes are a rich source of CXCL8, which are mainly chemotactic for the JAK3-mediated migration of neutrophils into the epidermis, contributing to microabscesses and pustules in the case of psoriasis (28). Serum IL-6 levels are significantly higher in patients with active psoriasis than in controls, and the levels correlate with disease severity of psoriatic arthritis (29, 30). Upon stimulation of DCs in disease initiation, keratinocytes are activated to produce autologous IL-6 to occur the epidermal hyperproliferation and impaired differentiation, as demonstrated by the hyperkeratosis exhibited by another psoriasis model: keratin 14–IL-6 transgenic mice (11). In contrast, keratinocyte-derived IL-6 activates myeloid DCs to initiate communication between the DCs and T cells in adaptive immunity (10). IL-6 enhances IL-1β–induced Th17 polarization or IL-2–induced Th22 differentiation from naive CD4\(^+\) T cells, facilitating the Th17/Th22 context in...
versus medium group, the total RNA isolated from mice lesions. The gene transcript of IFN- 

B

FIGURE 3. Serum and local proinflammatory cytokine production of K14-VEGF–Tg mice after CL–pIL-37 gene delivery. (A) The level of IL-10 in serum was measured by ELISA; the production of IL-10 from the CL–pIL-37 group was statistically significantly lower than for the control groups. (B) QRT-PCR was carried out for IFN-γ, IL-4, and IL-23a using the total RNA isolated from mice lesions. The gene transcript of IFN-γ of the CL–pIL-37 group was statistically significantly lower than that of the control group. There was no significant difference in the gene expression of IL-4 and IL-23a in the CL–pIL-37 group compared with the medium group or the CL–pNull group. Gene transcript levels were normalized using the housekeeping gene and expressed as the fold change in the expression compared with the medium group. (C) Toluidine blue–stained mast cells in the lesion sections from K14-VEGF–Tg mice (n = 15, original magnification ×400). Red arrows indicate the positive staining layers. (D) The number of toluidine blue+ cells was quantified by counting positive stained cells in five randomly selected fields of view/tissue section. Data are mean ± SD and are representative of three independent experiments. **p < 0.01, versus medium group, #p < 0.05, versus CL–pNull group. ns, no significance.

psoriasis (31, 32). IL-6 impairs Treg regulation and enables cutaneous T cells to escape from Treg suppression (33), thereby contributing to the poor suppression of Treg cells by Tregs, tipping the balance in favor of pathogenic Th1 and Th17 cells over Tregs. IL-13, a Th2 cytokine, which was shown to confer a modest risk for psoriasis, was also inhibited in our research (34). S100A7, also called psoriasin, belongs to the pleiotropic S100 family of calcium-binding proteins and was identified as a protein upregulated in abnormally differentiated psoriatic keratinocytes (35). In addition to an antimicrobial property for Escherichia coli, S100A7 functions as a chemotactic protein for neutrophils, monocytes, and CD4+ T lymphocytes, appearing to play prominent inflammatory roles in the genesis of psoriatic lesions (36, 37).

Based on the suppression of essential proinflammatory mediators in vitro, IL-37 regulated inflammatory cell infiltration and activation of the Th1/Th17 immune response. Additionally, it was reported that IL-37–overexpressing macrophages showed striking morphological differences compared with mock-transfected cells (3). Therefore, we evaluated the effect of IL-37 on keratinocyte proliferation and differentiation through air–liquid surface culture to determine the regulatory role of IL-37 in a psoriasis model in vitro and to explore the possible effect of IL-37 from the perspective of cellular growth or differentiation.

We examined the effect of IL-37 in vivo using the psoriasis model of K14-VEGF–Tg mice, which exhibit cellular hallmarks of and physical features that resemble human psoriasis through a CL–formulated plasmid gene-delivery system. The kinetics study of IL-37 expression in vivo showed that IL-37 was expressed successfully and could last for 72 h after a single treatment. K14-VEGF–Tg mice received a shot every 3 d to evaluate the effect of IL-37 in a psoriasis model in vivo. IL-37 relieved the psoriatic symptoms revealed by both macroscopic observation and immunological pathology, implying that it reduced the inflammatory response in this psoriatic model.

Psoriasis is a systemic immune disorder whose main manifestation is in the skin, and abnormal production of inflammatory mediators is believed to play an important role in the pathogenesis of the disease. Hence, the immunomodulatory role of IL-37 was assessed by systemic and local immune parameters using this psoriatic model. Among the cytokines measured in mice serum, only IL-10 in the pIL-37 treatment group was significantly lower than in the other two groups. Although IL-10 has been considered an anti-inflammatory cytokine, several reports (38–40) suggested the proinflammatory properties of IL-10 in many human infections or autoimmune diseases, including endotoxemia and Crohn’s disease. Multicenter clinical trials demonstrated that IL-10 application does not seem to be a therapeutic approach for psoriasis because of its ineffectiveness in reversing psoriatic symptoms (15). Systemic IL-10 levels were upregulated in K14-VEGF–Tg mice during the entire clinical course of psoriasis compared with wild-type mice (41). Upon IL-37 treatment, the IL-10 levels decreased, benefiting from the outstanding “cooling the fires of inflammation” characteristics of IL-37, with the possible blocking of the positive-feedback loop of IL-10–STAT3 signaling, which needs to be tested further, as mentioned earlier in discussing the role of Smad3 (38). Given the dilemma that the levels of cytokines, including systemic Th1-type cytokine IL-12p70 and Th17-type cytokine IL-17A, were below the detection limits of the as-

FIGURE 4. Immunohistochemical analysis of IL-37 expression in human healthy skin and psoriatic lesion biopsies. Four-micrometer vertical sections of FFPE biopsies were stained with anti-human IL-37 mAb and then photographed under a microscope (original magnification ×100 [top panels], ×200 [middle panels], and ×400 [bottom panels]). Red arrows indicate the positively stained layers. The images are representative of three independent experiments.
say, we determined the levels of Th1-, Th2-, and Th17-type cytokines at the gene-expression level.

Insights into the immunopathology of psoriasis unraveled a central role for proinflammatory IFN-γ and IL-23–producing cells in psoriasis. IFN-γ or IL-23, alone or in combination, contributes to Th1- and Th17-lineage differentiation, DC maturation, and aberrant proliferation and impaired differentiation of epidermal keratinocytes in the initiation and maintenance of psoriasis lesions (42, 43). The downregulation of IFN-γ in psoriatic lesions by IL-37 agrees well with a previous report (44) that IL-37 enhanced the inhibitory capacity of IL-18BP on IFN-γ in vitro. Additionally, the effect of IL-37 on IFN-γ might be due to the suppression of IFN-γ gene promoter and its positive regulator, T-BET, by Smad3 (24). There have been some reports about the regulation of IL-23a by IL-37. In our study, IL-37 did not reduce local IL-23a mRNA expression, although the possible reduction in IL-23 gene expression could be inferred from the antagonism toward IL-17A–signaling pathways, as discussed above. An effective therapy against psoriasis is the deviation of either Th1 or Th17 responses to Th2 responses, for which IL-4 is the key cytokine (45, 46). However, such a deviation did not occur with IL-37 treatment. K14-VEGF–Tg mice showed more intensive localization of mast cells in the psoriatic lesions than that observed in wild-type mice. IL-37 successfully reduced the density of mast cells in lesions. Collective reports demonstrated that mast cells that are recruited into the upper dermis by keratinocyte-derived CXCL8 can create an environment required for the recruitment of neutrophils and lymphocytes by expressing a range of chemokines (CXCL8, MIP-1α), cytokines (IFN-γ, TNF), and cell surface proteins (costimulation molecules) during the development of human psoriasis (47, 48). First, decreased chemoattraction of CXCL8 and IL-6 resulting from IL-37 expression could account for the reduced density and activation of mast cells in the lesions (49, 50). Importantly, the scenario that IL-17–producing mast cells are found at greater densities than are IL-17–producing T cells in human psoriasis lesions is similar to the Th17-like response seen in K14-VEGF–Tg mice treated with 12-O-tetradecanoyl phorbol 13-acetate (51, 52), hinting that IL-37 could affect the Th17 response, albeit indirectly, via the negligible effect on IL-23a production.

Rather than being expressed by healthy skin biopsies, IL-37 was found at high densities in human psoriatic plaques, with strong expression in the papillary dermis, sparse localization in cells of the epidermis, and weak expression in the middle part of the dermis. Along with the recent study (53) that IL-37 is highly expressed by the human colon in inflammatory bowel diseases, our finding demonstrates that IL-37 appears to be highly expressed by inflammatory tissues with chronic pathology.

The IL-37–producing cells present in the papillary dermis and epidermis and middle dermis were TEM cells and macrophages, respectively. There is a conspicuous accumulation of lymphocytes, DCs, and macrophages in human psoriasis lesions. First, the presence of IL-37 in CD4+CLA+ T cells implied that IL-37 could be involved in the adaptive immune response in psoriasis. CD45RO TEM cells, highly expressing CLA, are Th1-polarized effector memory cells in psoriatic lesions and initiate epidermal inflammation (54, 55). The presence of IL-37 in TEM cells may illustrate the possible regulation of IL-37 in TEM cells through the inhibition of Th1 activity, as revealed by our in vivo study. Considerable numbers of Tregs, characterized by high expression of Foxp3 staining, are present in the skin lesions of psoriasis and are supposed to serve as a brake for cutaneous inflammation (56). A recent study (57) demonstrated that the capacity of Tregs to enforce tolerance to harmless and self-Ags is severely ablated by their less intrinsic activity and the proinflammatory cytokine milieu in psoriasis. Because IL-37 was not expressed in Tregs, it could imply that the absence of intrinsic IL-37 expression contributed to Treg deficiency. However, more studies need to be carried out to validate this. There is accumulating evidence that macrophages are key constituents of the psoriatic inflammatory process, and the macrophage-derived cytokines, including TNF-α, are essential for the development and maintenance of psoriasis (10, 58). It was reported that IL-37 expression significantly reduced the production of several proinflammatory cytokines, including CXCL8 and TNF, by human macrophages in vitro and prevented the activation of macrophages in mice (3). The presence of IL-37 in human macrophages suggests that IL-37 may affect the interplay between macrophages and effector T cells in adaptive immunity. The presence of IL-37 in activated proinflammatory immunocytes, rather than in immunosuppressive cells, appears to reflect the capacity of IL-37 to curb overreacted pathogenesis of psoriasis. Further studies are needed to understand this link between IL-37 and its anti-inflammatory role.
Taken together, our results indicate the important immune downregulating role of IL-37 in the pathogenesis of psoriasis by decreasing essential proinflammatory cytokine production, indicating a hopeful therapeutic approach for immune-mediated disorders. Additional studies will focus on understanding the effects of IL-37 on keratinocyte proliferation and differentiation, as well as understanding its precise role in the pathology of human psoriasis lesions.

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Disclosures

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