**Decreased expression of IL-27 in moderate-to-severe psoriasis and its
anti-inflammation role in imiquimod-induced psoriasis-like mouse model**

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Abstract

Background: Psoriasis is a high-incident T-cell-mediated autoimmune disease mainly affecting the skin. Interleukin (IL)-27 is a novel member of the IL-6/IL-12 cytokine family, which plays a versatile role in the differentiation and function of distinct T cell subsets. Previous studies uncovered that IL-27 promoted the onset of psoriasis through enhancing the differentiation of T helper (Th) 1 cells. However, the role of IL-27 in other psoriasis-related Th lineages, especially Th17 cells, remains elusive.

Objects: The study aimed to investigate the role of IL-27 in the progression of psoriasis and its underlying mechanisms, particularly its influence on Th1 and Th17.

Methods: IL-27 and IL-27 receptor α (IL-27R α) expressions in normal and lesional

skin were determined by immunohistochemistry and western blot analysis. Serum levels of IL-27 and IL-10 were measured by ELISA. Expression levels of IL-27 and IL-27 receptor (IL-27R) mRNA in the skin tissue and peripheral blood mononuclear cells (PBMC) were assessed by quantitative polymerase chain reaction (PCR) analysis. To explore the function of IL-27 *in vivo*, we used imiquimod (IMQ)-induced psoriasis mouse model. We treated mice with IL-27 or its antagonist, evaluated disease severity and detected the cytokine secretion from splenic CD4⁺ T cells by flow cytometric analysis and the expression levels of IL-17 and IFN- γ in serum and skin lesion.

Results: The expression levels of IL-27 and IL-27R α were significantly reduced in the moderate-to-severe psoriatic lesions, along with a consistent decrease in serum IL-27 levels, compared with those of healthy control subjects. Moreover, subcutaneous administration of IL-27 recombinant protein lessened severity of IMQ-induced psoriasis-like cutaneous lesions, whereas IL-27p28 antagonist exaggerated the disease severity. Further analysis revealed that IL-27 significantly repressed IL-17 secretion from CD4⁺ T lymphocytes. Also administration of IL-27 decreased IL-17A level while IL-27p28 antagonist increased IL-17A level in serum and psoriasis-like lesion in the IMQ-treated mice.

Conclusion: Our results suggest that IL-27 might predominantly play a protective role in the pathogenesis of psoriasis through abrogating Th17 differentiation. The potential therapeutic benefit of harnessing IL-27 in treating psoriasis awaits future investigation.

Keywords: Psoriasis; IL-27; Th17; Imiquimod

Introduction:

Psoriasis is a chronic autoimmune disease characterized by epidermal hyperplasia [1], inflammatory infiltration and blood vessel dilation [2, 3]. Accumulating evidence suggests that the pathogenesis of psoriasis is principally mediated by T lymphocytes [2]. Originally, T helper (Th) 1 cells were identified as a predominant regulator in psoriasis [4]. Later research uncovered that Th17 was

essential in the exacerbation of psoriasis [5]. Furthermore, innate immune cells, including antigen-presenting cells (APC) and natural killer (NK) cells, also participate in the pathogenesis of psoriasis by secreting immunoregulatory cytokines, such as interleukin (IL)-12 and IL-23 [6]. These cytokines profoundly modulate the differentiation and function of multiple T cell lineages, thereby contributing to the progression of psoriasis [7, 8].

As a novel member of the IL-6/IL-12 cytokine family, IL-27 is composed of Epstein–Barr virus-induced gene 3 protein (EBI3) and p28 subunit, which signals through a receptor complex constituted by IL-27 receptor α (IL-27R α) and glycoprotein 130 (gp130) [9]. IL-27 is primarily secreted by activated APCs, including dendritic cells (DC), macrophages (M ϕ) and monocytes [10]. It has been reported that IL-27 can drive the rapid clonal expansion of naïve but not memory CD4⁺ T cells, and further synergize with IL-12 to trigger their subsequent IFN- γ production [11]. Moreover, Owaki *et al* [12] demonstrated that IL-27, which is produced from activated APCs earlier than IL-12, can efficiently induce Th1 differentiation in the absence of IL-12, suggesting a pivotal role of IL-27 in the initial triggering of Th1 differentiation. In addition, Murugaiyan *et al* [13] discovered that IL-27 promotes IL-10-secreting regulatory T cells (Tregs) while potently inhibiting Th17 cells generation and functions. Taken together, these results imply a pleiotropic role of IL- 27 in the differentiation and function of distinct T-cell subsets. In accordance, previous studies showed a predominantly protective role of IL-27 in various inflammatory disorders partly via regulating the activities of Th1, Th17 and Treg [15, 16, 18-20].

Shibata *et al* [14] recently reported an enhanced lesional infiltration of IL-27-secreting cells along with an elevated serum IL-27 level in psoriatic patients, which correlated with disease severity and serum IFN- γ level, indicating that IL-27 probably promotes the onset of psoriasis. Subsequently, they uncovered that IL-27 injection exacerbated imiquimod (IMQ)-induced psoriasis-like skin lesions through specifically facilitating Th1 differentiation [15]. However, our study showed that the

expressions of IL-27 and IL-27R α were significantly reduced in the moderate-to-severe psoriatic lesions compared with those of healthy controls. Also, the serum IL-27 level was consistently downregulated in psoriasis patients. Furthermore, the treatment of IL-27 recombinant protein alleviated the disease severity of IMQ-induced psoriasis-like skin lesions, principally by suppressing IL-17 production by CD4⁺ T cells.

Material and methods

Patients

This study was performed at Shanghai Tenth People's Hospital, Tongji University School of Medicine between July 2012 and December 2014. It was approved by the Ethics Committee of Shanghai Tenth People's Hospital. Moderate-to-severe psoriasis patients were all diagnosed by dermatologists. Exclusion criteria were current or recent treatment (within 1 month) with disease-modifying systemic drugs. 25 psoriasis patients and 25 healthy controls were enrolled after obtaining informed, written consent in accordance with the Declaration of Helsinki principles for research involving human subjects. Clinical information, blood samples and skin biopsies were collected.

Mice

Female BALB/c mice were purchased from the Jackson Laboratory. Experiments were conducted at 6 to 8 weeks of age, unless otherwise indicated. Mice were maintained in a specific pathogen-free barrier facility. All studies and procedures were performed in accordance with requirements of the Institutional Animal Care and Use Committee.

Imiquimod-induced psoriasis mouse model

Psoriasis-like skin inflammation was induced on the shaved back skin of BALB/c mice by topical application of 62.5 mg cream containing 5% IMQ (Mingxin

Pharmaceuticals, Sichuan, China). The application was repeated daily for 6 days until a psoriasis-like skin inflammation was apparent. For comparison, vaseline was applied onto the back skin of control mice. Blood samples, dorsal skin and spleens were collected 24 hours after the last topical treatment. Disease severity was evaluated regarding erythema, scaling and thickening on a scale from 0 (no alteration) to 4 (very distinct alteration). These three individual assessments were added in a cumulative score from 0 to 12.

Injection of IL-27 and anti-IL-27p28

On the day before IMQ treatment (day 0), mice were subcutaneously injected with PBS or 200ng/day recombinant mouse IL-27 (eBioscience, San Diego, CA, USA) for 1 week. Some mice were intraperitoneally injected with 50µg of IL-27p28 antibody (eBioscience, San Diego, CA, USA) on days 0, 2, 4, and 6.

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using lymphocyte separation medium (MP Bio, Santa Ana, CA, USA). PBMCs were washed and counted using Trypan Blue exclusion on a hemocytometer and light microscope. Aliquots were TRIzol (Invitrogen, Carlsbad, CA, USA) for gene expression analysis.

***In vitro* PMA and ionomycin activation assay**

Mouse spleens were excised and minced via cell strainer (200 strainer) (BD) to obtain single cell suspensions. After erythrocyte lysis, cells were cultured at a density of 2×10^6 cells per ml in R10 medium (RPMI1640 with 10 % FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM glutamine) in the presence of phorbol myristate acetate (Sigma-Aldrich) (PMA, 50 ng/mL) and ionomycin (1µmol/mL) (Sigma) for 1 hour; then 0.67 µl/mL Golgistop (eBioscience, San Diego, CA, USA) was added and the cells were incubated for an additional 3 hours. IL-17A and IFN-γ were detected by intracellular staining and flow cytometry.

Enzyme-Linked Immunosorbent Assay

IL-27 and IL-10 levels in the serum samples collected from psoriasis patients and healthy controls were measured using human IL-27p28 ELISA kit (eBioscience, San Diego, CA, USA) and human IL-10 ELISA kit (Multisciences). IL-17A and IFN- γ levels in the serum of mice in different groups were detected using mouse IL-17 ELISA kit (Leide Biosciences) and mouse IFN- γ ELISA Kit (Leide Biosciences) according to the manufacturers' instructions.

Western blotting

Sample tissues from psoriasis patients (10 samples) and healthy controls (10 samples) were lysed with strong radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Institute of Biotechnology, Beijing, China). Western blot analysis was performed with standard protocol with anti- β -actin antibody, anti-IL-27 antibody (Genetex, USA), anti-IL-27 α (Abcam, USA). Secondary antibodies conjugated with HRP were used at 1:1000 dilutions (Jackson ImmunoResearch Laboratories, Inc). Protein bands were detected with the Odyssey Infrared Imaging System (LI-COR, USA).

To further identify the role of IL-27 in the expression of IL-17 and IFN- γ in skin lesion of psoriatic-like mouse, western blot was supplied to detect the level of IL-17 (Abcam, USA) and IFN- γ (PeproTech, USA) in different group mice.

RNA isolation and quantitative PCR

Total RNAs were extracted from frozen tissues (10 samples from psoriasis patients and 10 samples from healthy controls) and PBMCs (25 samples from psoriasis patients and 25 samples from healthy controls) using TRIzol solution (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed with PrimeScript RT Reagent Kit (TaKaRa Biotechnology, Japan). The expression levels of IL-27p28, EBI3, IL-27 α and gp130 were detected with SYBR GREEN quantitative PCR analysis (KAPA).

Primer sequences were as follows: β -actin (FW 5'-TGGCACCCAGCACAATGAA-3', RV 5'-CTAAGTCATAGTCCGCCTAGAA GCA-3'), IL-27p28 (FW 5'-AGGTCAGGGAAACATCAGGGA-3', RV 5'-AGGTCA GGGAAACATCAGGG A-3') , EBI3 (FW 5'-TCATTGCCACGTACAGGCTC-3', RV 5'-GGGTCTGGGCTTGATGATGTG-3') , IL-27R α (FW 5'-TCTGGGAGTGCTG TTCTGCTT-3', RV 5'-TGTGCCTTGGAGGAGTGTGA-3') , gp130 (FW 5'-GTGAGTGGGATGGTGGGAAGG-3', RV 5'-CAAACCTTGTGTGTTGCCCAT TC-3'). Samples were normalized to β -actin expressions.

Histological analyses and immunohistochemical staining

Human tissue specimens were embedded in paraffin wax and the expressions of IL-27 and IL-27R α were detected in skin tissues (10 samples from psoriasis patients and 10 samples from healthy controls) using a standard immunohistochemical staining method. Staining quantification criteria were as follows: epidermal staining intensity was graded as follows: (1) none; (2) weak; (3) moderate; and (4) strong. Staining extent was graded as follows: (1) 0%-25%; (2) 25%-50%; (3) 50%-75%; (4) 75%-100%. For each specimen, the staining index was assessed by multiplying the staining intensity score by the extent of staining score as previously reported [16, 17]. Based on the formula, the index ranged from 1 to 16.

Mouse skin samples were processed with standard methods, and stained with hematoxylin and eosin. A researcher blind to the group allocations performed the analyses. The severity of acanthosis was evaluated by epidermal cell layers. For dermal infiltrating cell count, nine areas in three tissue sections of each sample were randomly taken.

Flow cytometry and antibodies

The single-cell suspensions treated by PMA and ionomycin according to the previously stated protocol, were stained for surface and intracellular markers with the conjugated monoclonal antibodies listed as follows: Anti-CD4 (RM4-5) and anti-IL-17A

(eBio17B7) were purchased from eBioscience (San Diego, CA, USA), anti-IFN- γ (REA638) was purchased from Miltenyibiotec (USA). Data were analyzed using FlowJo software (Tree Star Inc, Ashland, OR, USA).

Statistical analysis

Data were analyzed with GraphPad Prism 5 (GraphPad Software) and SPSS 20.0. The two-tailed Student's t-test and nonparametric Mann-Whitney test were used between the two groups. Differences were considered statistically significant with values of $P < 0.05$.

Results

Decreased IL-27 and IL-27R α expressions in the lesions of moderate-to-severe psoriasis patients

To explore a possible role of IL-27 signaling in the pathogenesis of psoriasis, the expression levels of IL-27 and IL-27R α in psoriatic lesions from moderate-to-severe patients and normal skin tissues from healthy controls were detected by immunohistochemical staining. The demographic description and clinical characteristics of psoriasis patients and healthy controls included in our study are summarized in Table 1. As depicted in Fig 1a, a proportion of epidermal and dermal cells within normal skin expressed IL-27, Conversely, this ratio of IL-27-positive skin cells was considerably downregulated in the psoriatic lesions. Similarly, whereas IL-27R α was widely expressed throughout both epidermis and dermis under normal conditions (Fig 1a), moderate-to-severe psoriasis patients exhibited a significantly lower level of IL-27R α expression in their cutaneous lesions (Fig.1a). The staining score of IL-27 (epidermal: $P < 0.01$, dermis: $P < 0.05$) and IL-27R α (epidermal: $P < 0.05$, dermis: $P < 0.01$) was significantly higher in the skin from healthy controls than from psoriasis patients (Fig.1b). Western blot analyses also confirmed the downregulation of IL-27 and IL-27R α at the protein level (Fig 1c). Further evaluation by real-time PCR showed that the expression levels of IL-27p28, IL-27R α and Gp130 mRNA were decreased in skin tissues of psoriasis patients compared with healthy

controls. However, EBI3 mRNA was comparable in skin tissues between psoriatic patients and health controls (Fig. 1d). Taken together, these results demonstrated that the expressions of IL-27 and IL-27R α were significantly downregulated in psoriatic conditions.

Circulating IL-27 level was downregulated in moderate-to-severe psoriasis patients

To investigate a potential role of IL-27 signaling in the systemic inflammation of psoriasis, we first examined the expression levels of IL-27 and IL-27R in the peripheral blood mononuclear cells (PBMC) of psoriasis patients and healthy controls. As depicted in Fig. 2a, whereas the EBI3 mRNA level in PBMCs was comparable between psoriasis patients and healthy controls, the mRNA level of IL-27p28 was significantly downregulated in psoriasis patients compared with healthy controls, indicating that the expression of IL-27 was probably diminished in PBMCs of psoriasis patients. However, we were unable to determine the expression level of IL-27R, because the IL-27R α mRNA level was increased and the gp130 level was considerably decreased in PBMCs of psoriasis patients (Fig. 2a). Next, we assessed the serum IL-27 levels in psoriasis patients and healthy controls by ELISA. Consistent with the alternation of IL-27 mRNA level in PBMCs, we observed a significant reduction in the serum level of IL-27 among moderate-to-severe psoriasis patients compared with healthy controls (Fig. 2b). Previous studies demonstrated that IL-27 could promote CD4⁺ T cells to secrete IL-10, which predominantly mediated anti-inflammatory responses [18, 19]. As expected, the serum IL-10 level was also drastically downregulated in psoriasis patients (Fig. 2c), although there was no significant correlation between the serum levels of IL-27 and IL-10 (Fig. 2d). In brief, the circulating IL-27 level was downregulated in moderate-to-severe psoriasis patients, suggesting that IL-27 is also involved in the systemic inflammatory responses of psoriasis.

IL-27 alleviated the severity of IMQ-induced psoriasis

To further explore the anti-inflammatory role of IL-27 in the pathogenesis of psoriasis, we used the IMQ-induced psoriasis mouse model. In accordance with previous reports, topical application of IMQ on the back skin of BALB/c mice for 6 consecutive days elicited psoriasis-like lesions featuring redness, scales and infiltration (Fig. 3b) [20, 21]. We treated BALB/c mice with either PBS, IL-27 or anti-IL-27 antibody as shown in Fig. 3a. Whereas injection of IL-27 lessened the severity of IMQ-induced psoriasis as measured by total disease score, anti-IL-27p28 antibody exacerbated the skin inflammation significantly (Fig. 3b). In line with the disease scoring, IL-27 alleviated the acanthosis and dermal infiltrate of immune cells induced by IMQ, whereas IL-27p28 antagonism aggravated the characteristic changes of psoriasis-like lesions significantly (Fig. 3d&e). In summary, our results here further confirmed an inhibitory role of IL-27 during the development of psoriasis.

IL-27 modulated IL-17 secretion by CD4⁺ T cells

Previous research showed that IL-27 prohibited IL-17 secretion by Th17 cells, but enhanced IFN- γ production by Th1 cell [22, 23]. To study the underlying mechanisms of IL-27 in alleviating psoriasis, spleen cells from IMQ-induced psoriasis mice were stimulated *in vitro* with PMA and ionomycin for 5 hours to assess the role of IL-27 in CD4⁺ T cell function. As depicted in Fig.4a and 4b, administration of IL-27 significantly repressed IL-17 secretion by CD4⁺ T cells, whereas anti-IL-27p28 antibody led to an increase in IL-17 production by CD4⁺ T cells. Whereas the secretion of IFN- γ by splenic CD4⁺ T cells remained unaltered after IL-27 injection, its production increased in response to IL-27p28 antagonism. Hence, IL-27 not only suppressed IL-17 production by CD4⁺ T cells, but also influenced their IFN- γ secretion to some extent. As both Th1 and Th17 greatly contribute to the pathogenesis of psoriasis, these data indicate a complicated role of IL-27 in the mechanisms of psoriasis.

To further explore the function of IL-27 in the systemic psoriatic inflammation, we assessed the mouse serum levels of IL-17A and IFN- γ . As expected, IMQ-treated mice exhibited higher levels of IL-17A and IFN- γ (Fig. 4c). And, administration of

IL-27 downregulated while IL-27p28 antagonist enhanced the mouse serum IL-17A level, which was consistent with the findings in CD4⁺ lymphocytes. Intriguingly, IL-27 increased the serum level of IFN- γ in the IMQ-treated mice, whereas blockage of IL-27 signaling drastically reduced IFN- γ level (Fig. 4c). The divergence of IL-27 effect on IFN- γ production by splenic CD4⁺ cells and mouse serum IFN- γ level indicated that IL-27 might target other IFN- γ -secreting immune cells. All in all, IL-27 primarily control IL-17A generation by CD4⁺ T lymphocytes in the pathogenesis of psoriasis.

IL-27 altered the expression of IL-17A and IFN- γ in skin lesion of psoriasis-like mouse model

We also determined the protein levels of IL-17 and IFN- γ in IL-27, IL-27p28 antagonist and PBS injection groups in the IMQ-treated mice. We found IL-27 injection decreased IL-17 expression levels while IL-27p28 antagonist increased IL-17A expression levels, and IL-27 injection alone could induce the expression of IFN- γ compared with PBS injection in IMQ-treated mice, while IL-27p28 antagonist down-regulate its expression significantly.

Discussion

In general, psoriasis has been considered as a stubborn T-cell mediated autoimmune disease. Previous patient and animal studies demonstrated that both IFN- γ -secreting Th1 and IL-17A-producing Th17 cells played indispensable roles in the pathogenesis of psoriasis [20, 24]. Collecting data supported that skin-infiltrating Th17 cells might also promote the disease onset by interacting with other cell components, such as keratinocytes and endothelial cells [25]. Notably, IL-17, which is a vital inflammatory cytokine factor secreted by Th17 cells, could be produced by expanded innate lymphoid cells, $\gamma\delta$ T cells and resident-memory T cells within psoriatic lesions as well [26]. Apart from T lymphocytes, distinct subpopulations of cutaneous DCs also contributed to the onset of psoriasis by supplying inflammatory cytokines, which would further modulate T-cell immunity [27] [28]. Taken together, the pathogenesis of psoriasis involves complicated immune disturbance with prominent Th17

malfunction.

Despite that IL-27 was initially regarded as a pro-inflammatory cytokine by promoting Th1 differentiation, accumulating evidence suggested that IL-27 played an anti-inflammatory role by suppressing Th17-cell differentiation and their IL-17 production [8, 13]. IL-27 also suppressed the reinforcement of IL-17 on TNF- α induced secretion of psoriasis-related chemokines by keratinocytes [29]. IL-27R α deficient mice produced more Th17 cells and were considerably vulnerable to experimental autoimmune encephalomyelitis (EAE), the underpinning mechanism of which was postulated that IL-27 abrogated IL-6- and TGF- β 1-mediated Th17 differentiation in a STAT1-dependent manner [23]. Diveu *et al* further illuminated that IL-27 prevented mouse and human Th17 development partly through STAT1-mediated suppression of ROR γ t, which was the house-keeping transcription factor of Th17 lineage, that IL-27p28 null mice were hypersusceptible to EAE [30]. Consistently, IL-27 treatment alleviated the severity of EAE and reduced the proportion of Th17 cells [31]. Similar findings that IL-27 inhibited the generation and function of pro-inflammatory Th17 cells were also observed in patients studies of Bechet's disease and Vogt-Koyanagi-Harada disease [32] [33]. In accordance, we showed here an unusually low level of IL-27 expression in the condition of psoriasis together with its anti-inflammatory role by negatively modulating Th17 cells, which further suggested that IL-27 might predominantly impede, instead of promote, the occurrence of autoimmunity.

However, Shibata *et al* [14] reported an opposite role of IL-27 in the pathogenesis of psoriasis that the expression of IL-27 was enhanced in psoriatic patients and the injection of IL-27 led to the exacerbation of psoriasis in mouse models [15]. The huge discrepancy between their results and our data might be due to: (1) the disease stages of the psoriatic patients recruited might differ between the two studies. As the study showed, IL-27 treatment starting at the peak of Influenza virus load resulted in the amelioration of systemic infection, whereas the application of IL-27 at an earlier stage

impaired virus clearance, indicating that the function of IL-27 might dynamically change at different stages of disease progression [34]. (2) IL-27 might exert distinct effects in psoriasis patients of different ethnic groups [35, 36]. (3) The two studies used different drug-delivery methods, Shibata *et al* treated the mice with a continuous pumping of IL-27, we administrated IL-27 by intermittent subcutaneous injections. The time-dependent changes in drug concentration might lead to different therapeutic outcomes. Future investigations applying other mouse models of psoriasis might provide better understanding of the role of IL-27 in the disease progression of psoriasis.

In summary, we demonstrated here for the first time that both lesional and circulating levels of IL-27 expression are considerably reduced in Chinese patients with moderate-to-severe psoriasis. Moreover, administration of IL-27 can alleviate the severity of IMQ-induced psoriasis-like skin inflammation, mainly by inhibiting IL-17 secretions by CD4⁺ T cells. Our results indicated a potent protective role of IL-27 in the pathogenesis of psoriasis, and the therapeutic effect of IL-27 awaits future investigation.

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References

- [1] J.E. Gudjonsson, A. Johnston, H. Sigmundsdottir, H. Valdimarsson, Immunopathogenic mechanisms in psoriasis, *Clinical and experimental immunology* 135 (2004) 1-8.
- [2] Y. Deng, C. Chang, Q. Lu, The Inflammatory Response in Psoriasis: a Comprehensive Review, *Clin Rev Allergy Immunol* 50 (2016) 377-89.
- [3] P. Quaglino, M. Bergallo, R. Ponti, E. Barberio, S. Cicchelli, E. Buffa, et al., Th1, Th2, Th17 and regulatory T cell pattern in psoriatic patients: modulation of cytokines and gene targets induced by etanercept treatment and correlation with clinical response, *Dermatology* 22 (2011) 57-67.
- [4] L.M. Austin, M. Ozawa, T. Kikuchi, I.B. Walters, J.G. Krueger, The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients, *J Invest Dermatol* 113 (1999) 752-9.
- [5] M.B. Teunissen, C.W. Koomen, R. de Waal Malefyt, E.A. Wierenga, J.D. Bos, Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes, *J Invest Dermatol* 111 (1998) 645-9.
- [6] A.A. Gaspari, Innate and adaptive immunity and the pathophysiology of psoriasis, *Journal of the American Academy of Dermatology* 54(3 Suppl 2) (2006) S67-80.
- [7] J.S. Stumhofer, E.D. Tait, W.J. Quinn, 3rd, N. Hosken, B. Spudy, R. Goenka, et al., A role for IL-27p28 as an antagonist of gp130-mediated signaling, *Nat Immunol* 11 (2010) 1119-26.
- [8] C. Heinemann, S. Heink, F. Petermann, A. Vasanthakumar, V. Rothhammer, E. Doorduyn, et al., IL-27 and IL-12 oppose pro-inflammatory IL-23 in CD4⁺ T cells by inducing Blimp1, *Nature communications* 5 (2014) 3770.

- [9] M. Bosmann, P.A. Ward, Modulation of inflammation by interleukin-27, *Journal of leukocyte biology* 94 (2013) 1159-65.
- [10] A.V. Villarino, E. Huang, C.A. Hunter, Understanding the pro- and anti-inflammatory properties of IL-27, *Journal of immunology* 173 (2004) 715-20.
- [11] S. Pflanz, J.C. Timans, J. Cheung, R. Rosales, H. Kanzler, J. Gilbert, et al., IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4⁺ T cells, *Immunity* 16 (2002) 779-90.
- [12] T. Owaki, M. Asakawa, N. Morishima, K. Hata, F. Fukai, M. Matsui, et al., A role for IL-27 in early regulation of Th1 differentiation, *Journal of immunology* 175(2005) 2191-200.
- [13] G. Murugaiyan, A. Mittal, R. Lopez-Diego, L.M. Maier, D.E. Anderson, H.L. Weiner, IL-27 is a key regulator of IL-10 and IL-17 production by human CD4⁺ T cells, *Journal of immunology* 183 (2009) 2435-43.
- [14] S. Shibata, Y. Tada, N. Kanda, K. Nashiro, M. Kamata, M. Karakawa, et al., Possible roles of IL-27 in the pathogenesis of psoriasis, *The Journal of investigative dermatology* 130 (2010) 1034-9.
- [15] S. Shibata, Y. Tada, Y. Asano, K. Yanaba, M. Sugaya, T. Kadono, et al., IL-27 activates Th1-mediated responses in imiquimod-induced psoriasis-like skin lesions, *The Journal of investigative dermatology* 133 (2013) 479-88.
- [16] M. Terao, S. Itoi, H. Murota, I. Katayama, Expression profiles of cortisol-inactivating enzyme, 11beta-hydroxysteroid dehydrogenase-2, in human epidermal tumors and its role in keratinocyte proliferation, *Exp Dermatol* 22 (2013) 98-101.
- [17] M.C. Meulener, E.E. Ayli, R. Elenitsas, J.T. Seykora, Decreased Srcasm expression in hyperproliferative cutaneous lesions, *J Cutan Pathol* 36 (2009) 291-5.
- [18] Y. Iwasaki, K. Fujio, T. Okamura, A. Yanai, S. Sumitomo, H. Shoda, et al., Egr-2 transcription factor is required for Blimp-1-mediated IL-10 production in IL-27-stimulated CD4⁺ T cells, *Eur J Immunol* 43 (2013) 1063-73.
- [19] A. Visperas, J.S. Do, K. Bulek, X. Li, B. Min, IL-27, targeting antigen-presenting cells, promotes Th17 differentiation and colitis in mice, *Mucosal Immunol* 7 (2014)

625-33.

[20] L. van der Fits, S. Mourits, J.S. Voerman, M. Kant, L. Boon, J.D. Laman, et al., Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, *Journal of immunology* 182 (2009) 5836-45.

[21] C.S. Hau, N. Kanda, Y. Tada, S. Shibata, S. Sato, S. Watanabe, Prolactin induces the production of Th17 and Th1 cytokines/chemokines in murine Imiquimod-induced psoriasiform skin, *J Eur Acad Dermatol Venereol* 28(10) (2014) 1370-9.

[22] M. Batten, N.M. Kljavin, J. Li, M.J. Walter, F.J. de Sauvage, N. Ghilardi, Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells, *Journal of immunology* 180 (2008) 2752-6.

[23] M. Batten, J. Li, S. Yi, N.M. Kljavin, D.M. Danilenko, S. Lucas, et al., Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells, *Nat Immunol* 7 (2006) 929-36.

[24] M. Mortezaei, C. Ritchlin, Immunologic advances reveal new targets in psoriasis and psoriatic arthritis, *Discov Med* 20 (2015) 169-75.

[25] F.C. Eberle, J. Bruck, J. Holstein, K. Hirahara, K. Ghoreschi, Recent advances in understanding psoriasis, *F1000Res* 5 (2016). pii: F1000 Faculty Rev-770.

[26] S. Cheuk, M. Wiken, L. Blomqvist, S. Nylen, T. Talme, M. Stahle, et al., Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis, *Journal of immunology* 192 (2014) 3111-20.

[27] F.O. Nestle, C. Conrad, A. Tun-Kyi, B. Homey, M. Gombert, O. Boyman, et al., Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production, *J Exp Med* 202 (2005) 135-43.

[28] C. Gunther, K. Blau, U. Forster, A. Viehweg, G. Wozel, K. Schakel, Reduction of inflammatory slan (6-sulfo LacNAc) dendritic cells in psoriatic skin of patients treated with etanercept, *Exp Dermatol* 22 (2013) 535-40.

[29] S. Fujiwara, H. Nagai, S. Oniki, T. Yoshimoto, C. Nishigori, Interleukin (IL)-17 versus IL-27: opposite effects on tumor necrosis factor-alpha-mediated chemokine production in human keratinocytes, *Exp Dermatol* 21 (2012) 70-2.

[30] C. Diveu, M.J. McGeachy, K. Boniface, J.S. Stumhofer, M. Sathe, B.

Joyce-Shaikh, et al., IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells, *Journal of immunology* 182 (2009) 5748-56.

[31] D.C. Fitzgerald, B. Ciric, T. Touil, H. Harle, J. Grammatikopoulou, J. Das Sarma, et al., Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector phase of experimental autoimmune encephalomyelitis, *Journal of immunology* 179 (2007) 3268-75.

[32] C. Wang, Y. Tian, Z. Ye, A. Kijlstra, Y. Zhou, P. Yang, Decreased interleukin 27 expression is associated with active uveitis in Behcet's disease, *Arthritis research & therapy* 16 (2014) R117.

[33] C. Wang, Y. Tian, B. Lei, X. Xiao, Z. Ye, F. Li, et al., Decreased IL-27 expression in association with an increased Th17 response in Vogt-Koyanagi-Harada disease, *Investigative ophthalmology & visual science* 53 (2012) 4668-75.

[34] F.D. Liu, E.E. Kenngott, M.F. Schroter, A. Kuhl, S. Jennrich, R. Watzlawick, et al., Timed action of IL-27 protects from immunopathology while preserving defense in influenza, *PLoS pathogens* 10 (2014) e1004110.

[35] L. Jiang, L. Liu, Y. Cheng, Y. Lin, C. Shen, C. Zhu, et al., More heritability probably captured by psoriasis genome-wide association study in Han Chinese, *Gene* 573 (2015) 46-9.

[36] S. Munir, S. ber Rahman, S. Rehman, N. Saba, W. Ahmad, S. Nilsson, et al., Association analysis of GWAS and candidate gene loci in a Pakistani population with psoriasis, *Mol Immunol* 64 (2015) 190-4.

Figure Legends

Figure1. IL-27 and IL-27R expressions in human psoriatic skin. (a) Representative hematoxylin and eosin staining (bar=50 μ m), and IL-27 and IL-27R α immunostaining (bar =100 μ m) of skin biopsies from moderate-to-severe psoriasis patients and health controls. (b) Staining score analysis of IL-27 and IL-27R α immunostaining in psoriasis patients and health controls. (c) Representative western blotting analysis of IL-27 and IL-27R α expressions in skin tissues from moderate-to-severe psoriasis patients and health controls. (d) Relative expression levels of IL-27p28, EBI3, IL-27R α and gp130 by qRT-PCR in the skin biopsies from moderate-to-severe psoriasis patients and health controls. The level of expression is normalized to β -actin. *: P<0.05, **: P<0.01. All these studies were conducted with 10 psoriasis patients and 10 health controls.

Figure2. IL-27 and IL-27R expressions in the peripheral blood mononuclear cells (PBMC) of psoriasis patients. (a) Relative expression levels of IL-27p28, EBI3, IL-27R α and gp130 by qRT-PCR in the PBMCs of moderate-to-severe psoriasis patients and health controls. The level of expression is normalized to β -actin. (b) Quantitative evaluation of serum IL-27 level in psoriasis patients and health controls by ELISA. (c) Quantitative evaluation of serum IL-10 level in psoriasis patients and health controls by ELISA. (d) The correlation between the serum level of IL-27 and IL-10 was analyzed by spearman correlation regression test. *: P<0.05, **: P<0.01. All these studies were conducted with 25 psoriasis patients and 25 health controls.

Figure3. The impact of IL-27 on imiquimod (IMQ)-induced psoriasis-like skin inflammation. (a) Schematic representation of the experimental plan with the application of IL-27 or IL-27p28 antagonist on IMQ-treated mice. (b) Representative pictures of IMQ-induced psoriasis-like cutaneous lesions from each group. (c)

Disease severity scoring of IMQ-induced psoriasis-like inflammation based on erythema, scaling and skin thickness with a scale from 0 to 4 on the indicated days. (d) Representative hematoxylin and eosin staining of skin sections from each group on day 7 (bar =100 μ m). (e) Epidermal thickness and inflammatory cell infiltrate analysis of different group mice. All experiments were repeated twice and each group included three mice. *: P<0.05, **: P<0.01.

Figure4. IL-27 alters the polarization of Th17. Experiments were conducted as described in Fig 3a, and splenic cells were harvested from each group on day 7. (a, b) Representative dot plots and the percentages of IL-17A-positive and IFN- γ -positive splenic CD4⁺ T cells. (c) Mice serum IL-17A and IFN- γ levels on day 7. (d) Western blotting analysis of IL-17A and IFN- γ expressions in skin tissues. *: P<0.05, **: P<0.01. (All experiments were repeated twice and each group included three mice)

Fig. 1

Fig. 2

Fig. 3

Fig. 4