Trends in Immunotherapy (2022) Volume 6 Issue 1
doi:10.24294/ti.v6.i1.1436

ORIGINAL RESEARCH ARTICLE

IL-27 regulates cytokine production as a double-edged sword in keratinocytes

Akihiro Aioi[1*], Tomozumi Imamichi[2]

[1] *Septem-Soken, Dojima, Kita-ku, Osaka 530-0003, Japan. E-mail: a-aioi@septem-so.com*

[2] *Laboratory of Human Retrovirology and Immunoinformatics, Applied and Developmental Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA.*

ABSTRACT

Inflammaging is a subject of considerable attention, because aging is characterized by low-grade, chronic, and asymptomatic inflammation, concomitant with increased blood levels of senescence-associated secretory phenotype (SASP) factors, including IL-1, IL-6, IL-8, IL-18, and tumor necrosis factor-α (TNF-α). However, IL-27 is currently not categorized as a SASP factor, although it is known to play pleiotropic roles in inflammation. In this study, we evaluated the interaction between TNF-α and IL-27 in the context of low-grade inflammation using HaCaT cells. TNF-α induced significant upregulation of the mRNA levels of IL-6 and IL-8 at the experimental concentration (~10 ng/ml), while the mRNA levels of IL-1RA, IL-10, and IL-18BP were unchanged. After confirming the expression of functional IL-27 receptors in HaCaT cells, we examined the effects of IL-27 alone on cytokine expression. IL-27 alone significantly upregulated the mRNA levels of IL-10, IL-18BP and IL-6 by 1.61-fold, 1.46-fold, and 2.32-fold, respectively. In the presence of 100 ng/ml of IL-27, the mRNA levels of the anti-inflammatory cytokines IL-1RA, IL-10, and IL-18BP, were significantly upregulated upon treatment with TNF-α at the physiological concentration (1 ng/ml). Taken together, this study indicates that a high concentration of IL-27 exhibits anti-inflammatory effects in the presence of a low concentration of TNF-α in keratinocytes, suggesting that the anti-inflammatory role of IL-27 in inflammaging may be regulated by TNF-α concentration.

Keywords: IL-27; Keratinocyte; Pleiotropic Function; Inflammaging

ARTICLE INFO

Received 6 March 2022
Accepted 16 March 2022
Available online 12 May 2022

1. Introduction

Interleukin (IL)-27, a member of the IL-12 family, is a heterodimeric cytokine composed of IL-27 p28 and Epstein–Barr virus-induced gene protein 3 subunits. The functional IL-27 receptor consists of IL-27Rα (WSX-1) and glycoprotein 130 (gp130) and is a commonly used cytokine receptor of the IL-6 family[1]. Binding of IL-27 to its receptor activates JAK 1 and 2, TYK2, and STAT-1, 2, 3, 4, and 5[2–5], and is responsible for the diverse regulatory functions of IL-27. Previous studies have reported that IL-27 exerts pro-inflammatory and anti-inflammatory effects[6–9] as well as anti-viral effects[10], and therefore plays a dual role in inflammation. The potential role of IL-27 in the skin has been previously studied. Yang *et al.* demonstrated that IL-27 produced by $CD301b^+$ cells infiltrating wound lesions stimulated keratinocyte proliferation and re-epithelialization during the wound-healing process[11]. Another study reported that IL-27 is expressed in chronic lesional allergic eczematous[12]. In addition, a previous study showed that serum IL-27 level in

psoriatic patients were significantly higher than those in healthy controls, correlating with disease severity[13]. Aging becomes a serious issue in an aging society. Previous studies have reported that aging is strongly associated with inflammation[14–16], which is the basis of the concept of inflammaging[17]. Clinically, inflammaging consists of low-grade, chronic, and asymptomatic inflammation, characterized by increased blood levels of several inflammatory biomarkers, including IL-1, IL-6, IL-8, IL-18, and tumor necrosis factor-α (TNF-α), which are together called senescence-associated secretory phenotype (SASP) factors[18–20]. The SASP factors secreted from senescent cells, which are considered passive bystanders, play pivotal roles in aging. Skin aging can be divided into two distinct types, intrinsic aging and extrinsic aging, based on the fact that the skin is the outermost organ and is therefore exposed to the external environment. However, despite their different histological features and functional triggers, intrinsic and extrinsic aging share common biochemical mechanisms, involving SASP factors[21]. Here, we explored the effect of IL-27 on the inflammatory environment of HaCaT cells, a human-derived keratinocyte cell line.

2. Materials and methods

2.1 Cytokine

IL-27 and TNF-α were purchased from Proteintech (Rosemont, IL, USA). Both cytokines were dissolved in sterilized PBS at a concentration of 100 μg/ml for use in the experiments.

2.2 Cell culture

To maintain the distinct differentiation stage of HaCaT cells, the cells were cultured according to the method previously reported[22,23]. Calcium in fetal bovine serum (FBS) was depleted by incubation with Chelex 100 resin (Bio-Rad, Hercules, CA, USA) for 1 h at 4 ℃. HaCaT cells were maintained in Ca^{2+}-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% Ca^{2+}-depleted FBS, 4 mM glutamine, 1 mM sodium pyruvate, and 2 mM $CaCl_2$, at 37 ℃ in a 5% CO_2-humidified atmosphere.

2.3 Immunoblotting

HaCaT cells were treated with the indicated concentration of IL-27 for 20 min at 37 ℃, and then subjected to immunoblotting. HaCaT cells were collected in radioimmunoprecipitation assay buffer supplemented with protease and phosphatase inhibitors. Equal amounts of protein (10 μg) were loaded, resolved via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and transferred to polyvinylidene difluoride (PVDF) membranes, followed by immunoblotting with phosphorylated-STAT3 (p-STAT3) rabbit monoclonal antibody (Cell Signaling Technology; Danvers, MA, USA). Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Millipore; Billerica, MA, USA).

2.4 Treatment with IL-27 or (and) TNF-α

To study the effects of IL-27 on the mRNA levels of the cytokines, HaCaT cells were treated with 10, 30, and 100 ng/ml of IL-27 for 24 h at 37 ℃. To study the effects of TNF-α on the mRNA levels of cytokines, HaCaT cells were treated with 1, 5, and 10 ng/ml of TNF-α for 24 h at 37 ℃. To study the effects of the interaction of TNF-α and IL-27 on the mRNA levels of cytokines, HaCaT cells were treated with 1 or 10 ng/ml of TNF-α for 24 h at 37 ℃, in the presence of 10 or 100 ng/ml of IL-27.

2.5 Real-time qPCR

Real-time qPCR was conducted as the following, according to a previous study[24]. After the treatments, cells were harvested and total RNA was extracted using the SV RNA isolation kit (Promega; Madison, WI, USA), according to the manufacturer's instructions, followed by reverse transcription using ReverTra Ace® qPCR RT Master Mix (TOYOBO; Osaka, Japan). PCR amplification and detection were conducted using a CFX96 real-time PCR system (Bio-Rad; Hercules, CA, USA) with KAPA SYBR FAST qPCR master mix (KAPA Biosystems; Woburn, MA, USA). The sequences of primer pairs were shown in **Table 1**. Target mRNA expression was quantified using the comparative threshold cycle (Ct) method for relative quantifica-

tion ($2^{-\Delta\Delta}$Ct), and normalized to the geometric mean of the reference gene (β-actin) expression.

Table 1. Primer pairs

β-actin	sense	5′-GATGAGATTGGCATGGCTTT-3′
	antisense	5′-CACCTTCACCGTTCCAGTTT-3′
IL-27Rα	sense	5′-AGGCCACCTCACCCACTACA-3′
	antisense	5′-ATGCTGTCACCCACAGCTCA-3′
gp130	sense	5′-ATCCTGTGGATCTGGGCAAA-3′
	antisense	5′-GCCTCCATGCCAACTGTTTC-3′
IL-1RA	sense	5′-TGGAGGGAAGATGTGCCTGT-3′
	antisense	5′-GCGCTTGTCCTGCTTTCTGT-3′
IL-6	sense	5′-ATGCAATAACCACCCCTGAC-3′
	antisense	5′-AAAGCTGCGCAGAATGAGAT-3′
IL-8	sense	5′-ACCACCGGAAGGAACCATCT-3′
	antisense	5′-TTGGCAAAACTGCACCTTCA-3′
IL-10	sense	5′-CAAGCCTGACCACGCTTTCT-3′
	antisense	5′-AAAGGGGCTCCCTGGTTTCT-3′
IL-18BP	sense	5′-TGGGTTCACACGCAGCTAGA-3′
	antisense	5′-GGCATCTGCTTCCTCCTTCA-3′

2.6 Statistical analysis

Data are expressed as the mean ± SEM. Statistical comparisons between experimental groups and controls were performed using unpaired student's *t*-test. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Functional IL-27 receptor expression in HaCaT cells

Real-time qPCR was conducted to estimate the mRNA levels of IL-27 Rα and gp130. The mRNA level of IL-27Rα was 0.21 ± 0.05-fold that of involucrin, which is a differentiation marker of HaCaT cells, while the mRNA level of gp130 was 9.24 ± 1.06-fold that of involucrin (**Figure 1a**). Next, immunoblotting for p-STAT3 was performed to confirm the function of the IL-27 receptor expressed in HaCaT cells. p-STAT3 was detected in HaCaT cells treated with IL-27 at concentrations ranging from 3 to 100 ng/ml (**Figure 1b**).

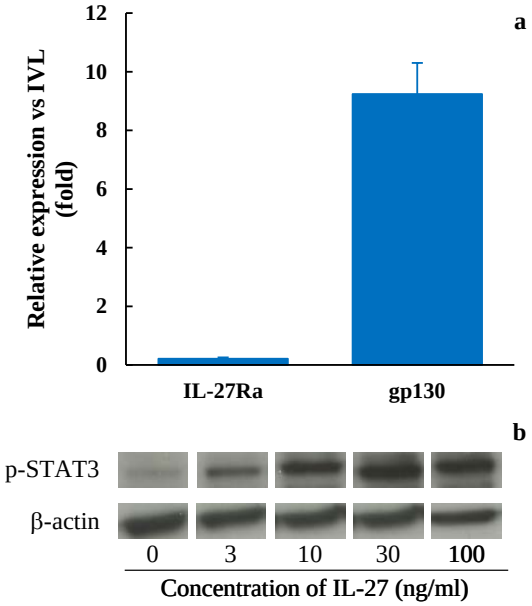

Figure 1. The functional IL-27 receptor is expressed in HaCaT cells.
To evaluate the functional IL-27 receptor expression in HaCaT cells, qPCR (**a**), and immunoblotting of p-STAT3 (**b**) were performed. Significant mRNA levels of IL-27Ra and gp130 against IVL were confirmed in HaCaT cells (**a**). The spots of p-STAT3 in the immunoblot of cells treated with IL-27 were detected within a concentration range of 3 to 100 ng/ml of IL-27 (**b**).

3.2 The treatment with TNF-α upregulated the mRNA expression of pro-inflammatory cytokines in HaCaT cells

The mRNA levels of IL-6, IL-8, IL-1RA, IL-10, and IL-18BP were examined using real-time qPCR. The mRNA levels of IL-6 were significantly upregulated to 1.56 ± 0.21-fold and 1.37 ± 0.12-fold by treatment of 5 and 10 ng/ml TNF-α respectively. The mRNA levels of IL-8 were significantly upregulated up to 8.43 ± 1.07-fold following treatment with TNF-α at experimental concentrations. The mRNA levels of IL-1RA, IL-10, and IL-18BP were unaffected by TNF-α treatment (**Figure 2**).

3.3 The treatment with IL-27 affected the mRNA expression of IL-6, IL-10 and IL-18BP

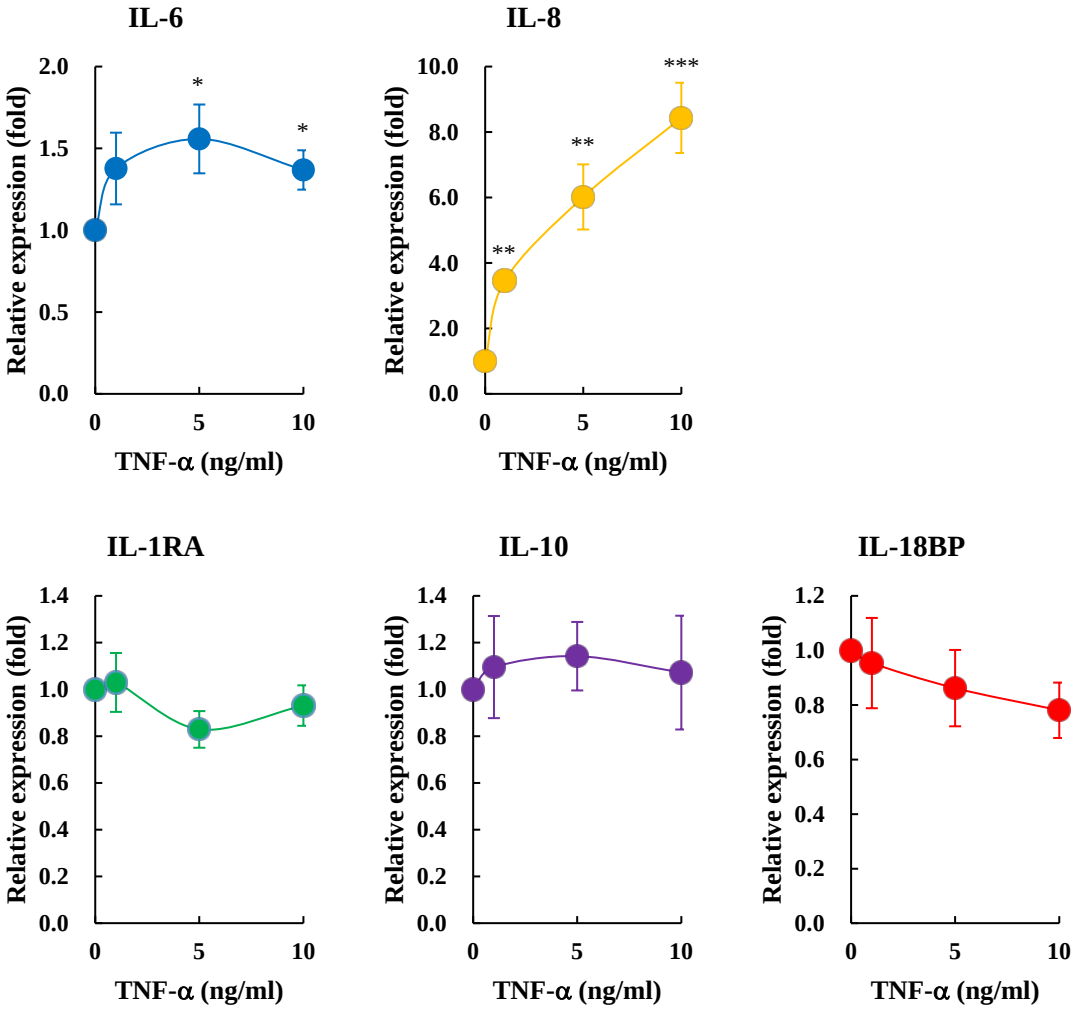

Figure 2. TNF-α-induced upregulation of IL-6 and IL-8 mRNA expression.
To evaluate the effect of TNF-α, the mRNA levels of IL-6, IL-8, IL-1RA, IL-10, and IL-18BP were measured in HaCaT cells. TNF-α stimulation upregulated the mRNA levels of IL-6 and IL-8, while those of IL-1RA, IL-10, and IL-18BP remained unchanged at experimental concentrations. Each value is represented as the mean ± SEM of at least three independent experiments (*$p < 0.05$, ***$p < 0.001$ compared with the control).

The mRNA levels of IL-6, IL-8, IL-1RA, IL-10 and IL-18BP were examined by real-time qPCR. The mRNA levels of IL-6 were significantly upregulated up to 2.32 ± 0.10-fold in HaCaT cells treated with IL-27 at the experimental concentrations. The mRNA levels of IL-10 were significantly upregulated to 1.61 ± 0.23-fold after treatment with 100 ng/ml IL-27. The mRNA levels of IL-18BP were significantly upregulated to 1.46 ± 0.16-fold after treatment with 10 ng/ml IL-27. The mRNA levels of IL-8 and IL-1RA were not affected by the treatment with IL-27 (**Figure 3**).

3.4 TNF-α affected IL-27-induced mRNA expression of cytokines

To estimate the effect of the interaction of TNF-α and IL-27 on the mRNA levels of cytokines, IL-27-regulated mRNA expression of cytokines was determined in the presence of 1 or 10 ng/ml TNF-α. In the presence of a low dose of IL-27 (10 ng/ml), the mRNA levels of IL10 and IL-18BP were significantly upregulated following treatment with TNF-α, while the expression levels of IL-6 and IL-8 were significantly enhanced as well (**Figure 4**). In contrast, in the presence of a high dose of IL-27 (100 ng/ml), the mRNA levels of anti-inflammatory cytokines, IL-1RA, IL-10, and IL-18BP, were significantly upregulated to 1.70 ± 0.0.16-fold, 3.30 ± 0.28-fold and 2.87 ± 0.49-fold, respectively, following treatment with 1 ng/ml TNF-α, while the expression levels of proinflammatory cytokines also upregulated (**Figure 5**).

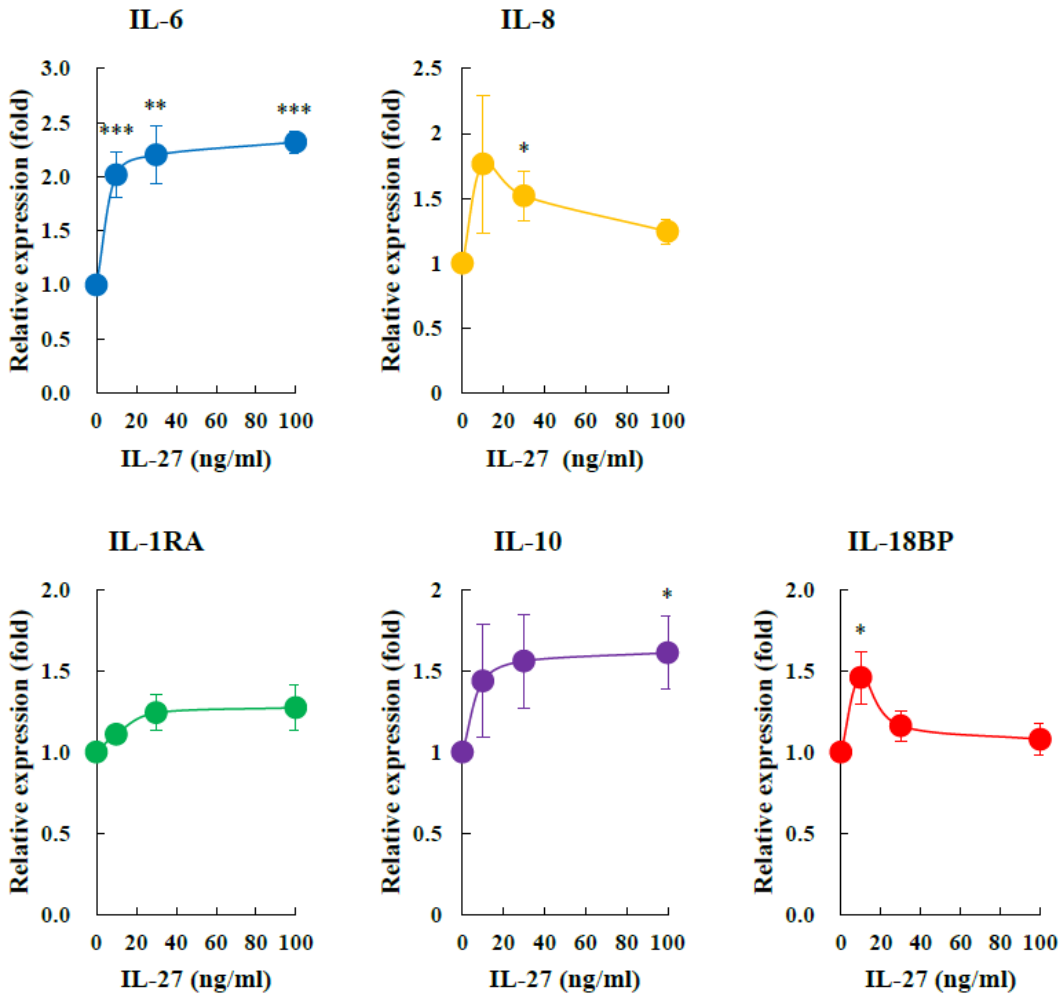

Figure 3. IL-27 induced the upregulation of mRNA expression of IL-6, IL-10 and IL-18BP.
To evaluate the effect of IL-27, the mRNA levels of IL-6, IL-8, IL-1RA, IL-10 and IL-18BP were measured in HaCaT cells. The mRNA expression of IL-6 was significantly upregulated in a dose-dependent manner. The mRNA expression of IL-10 and IL-18BP was also upregulated at concentrations of 30 ng/ml and 10ng/ml respectively. Each value is represented as the mean ± SEM from at least three independent experiments (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the production from the control).

4. Discussion

The aging of organs starts at the time of birth and continues throughout life. How to cope with aging, which causes systemic as well as topical frailty, is a growing subject of interest because of the aging society. Previous studies have reported strong association between inflammation and aging[14–16], which is the basis of the concept of inflammaging[17]. In inflammaging, which is clinically characterized by low-grade, chronic, and asymptomatic inflammation[18,19], SASP factors such as IL-1, IL-6, IL-8, and TNF-α play a pivotal role in the progression of aging. Among these SASP factors, TNF-α, an immunological biomarker of frailty and aging[15], is known to play a vital role in inflammatory diseases such as psoriasis[25]. In addition, a previous study has reported that TNF-α induces the production of IL-27 from human macrophages[26]. Moreover, other previous studies have demonstrated that IL-27, whose serum levels in patients with psoriasis were elevated, suppressed the TNF-α-induced production of IL-1α and CCL20[13]. Moreover, as per another study, IL-27 suppressed the TNF-α-induced production of CXCL1, CXCL2, CXCL8, and CCL20 from human keratinocytes[27]. These previous studies suggest that the interaction between TNF-α and IL-27 plays a vital role in psoriasis. Here, by using HaCaT cells, a human-derived keratinocyte cell line, we explored how IL-27 affects the TNF-α-induced inflammatory environment, although IL-27 is not categorized as a SASP factor. We evaluated the expression and function of IL-27 receptor in HaCaT cells, as to the best

of our knowledge, it has not been evaluated before. The results of qPCR and immunoblotting demonstrated that both subunits of the IL-27 receptor, IL-27Rα and gp130, were expressed in HaCaT cells, and that p-STAT3 was present in IL-27-treated HaCaT cells. These results suggest that a functional IL-27 receptor is expressed in HaCaT cells. We also examined the effects of TNF-α on cytokine production in HaCaT cells. As shown in **Figure 2**, the treatment with TNF-α upregulated the mRNA levels of IL-6 and IL-8, while those of IL-1RA, IL-10 and IL-18BP were not affected. In agreement with previous studies, this study demonstrated the pro-inflammatory effects of TNF-α. Next, we evaluated the effects of IL-27 alone on the mRNA levels of IL-6, IL-8, IL-1RA, IL-10, and IL-18BP (**Figure 3**). Surprisingly, IL-27 upregulated IL-6 mRNA expression in a dose-dependent manner. Although Kalliolias *et al.* demonstrated that the priming of human macrophages with IL-27 enhanced IL-6 production by stimulation with toll-like receptor ligands, while IL-8 production induced by TNF-α was suppressed in the presence of IL-27[28]. To the best of our knowledge, the present study is the first to demonstrate that IL-27 alone can induce IL-6 production. Regarding the effects of IL-27 as an anti-inflammatory cytokine, IL-27 significantly upregulated the mRNA expression of IL-10 and IL-18BP at concentrations of 30 ng/ml and 10 ng/ml, respectively, which is in agreement with the findings of previous studies[29,30]. These results suggest that IL-27 exerts both pro- and anti- inflammatory effects on keratinocytes. Next, we evaluated the effects of IL-27 and TNF-α on cytokine production. To the best of our knowledge, although 10 ng/ml of TNF-α has been used in vitro to mimic symptomatic skin inflammation of the type observed in psoriasis, there has been no previous study that used 1ng/ml of TNF-α. We speculate that the reason why a low concentration of TNF-α has not been used so far is that it does not produce clear results with respect to cytokine production. However, because the elevated plasma concentration of TNF-α (below 10 pg/ml) in the elderly is associated with frailty and mortality, it is likely that low-dose TNF-α plays a role in inflammaging[15,31]. Thus, we used concentrations of 1 and 10 ng/ml of TNF-α in this study. We observed differential expression patterns of cytokines depending on the concentration of IL-27. In the presence of high doses of IL-27 (100 ng/ml), the mRNA levels of anti-inflammatory cytokines were significantly upregulated when the cells were treated with 1 ng/ml TNF-α (**Figure 5**), whereas the expression levels of IL-1RA and IL-10 were significantly upregulated with the treatment of 10 ng/ml TNF-α in the presence of low dose of IL-27 (10 ng/ml), as shown in **Figure 4**. Collectively, the findings of this study indicate that at high concentrations, IL-27 exhibits anti-inflammatory effects in the presence of low concentrations of TNF-α in keratinocytes, indicating its anti-inflammatory role in inflammaging. However, because it has been reported in a previous study that TNF-α signaling via IL-27R promotes the aging of hematopoietic stem cells[32], and it remains unclear whether a sufficient amount of IL-27 is supplied by dendritic cells and macrophages, which are major sources of IL-27 whose functions decline with aging[33,34],and further experiments are required.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

Acknowledgements

The authors thank Dr. Ryuta Muromoto and Dr. Jun-ichi Kashiwakura for their valuable suggestions regarding this study. We would like to thank Editage (www.editage.com) for the English language editing. Finally, the authors appreciate Professor Tadashi Matsuda for helping with this study.

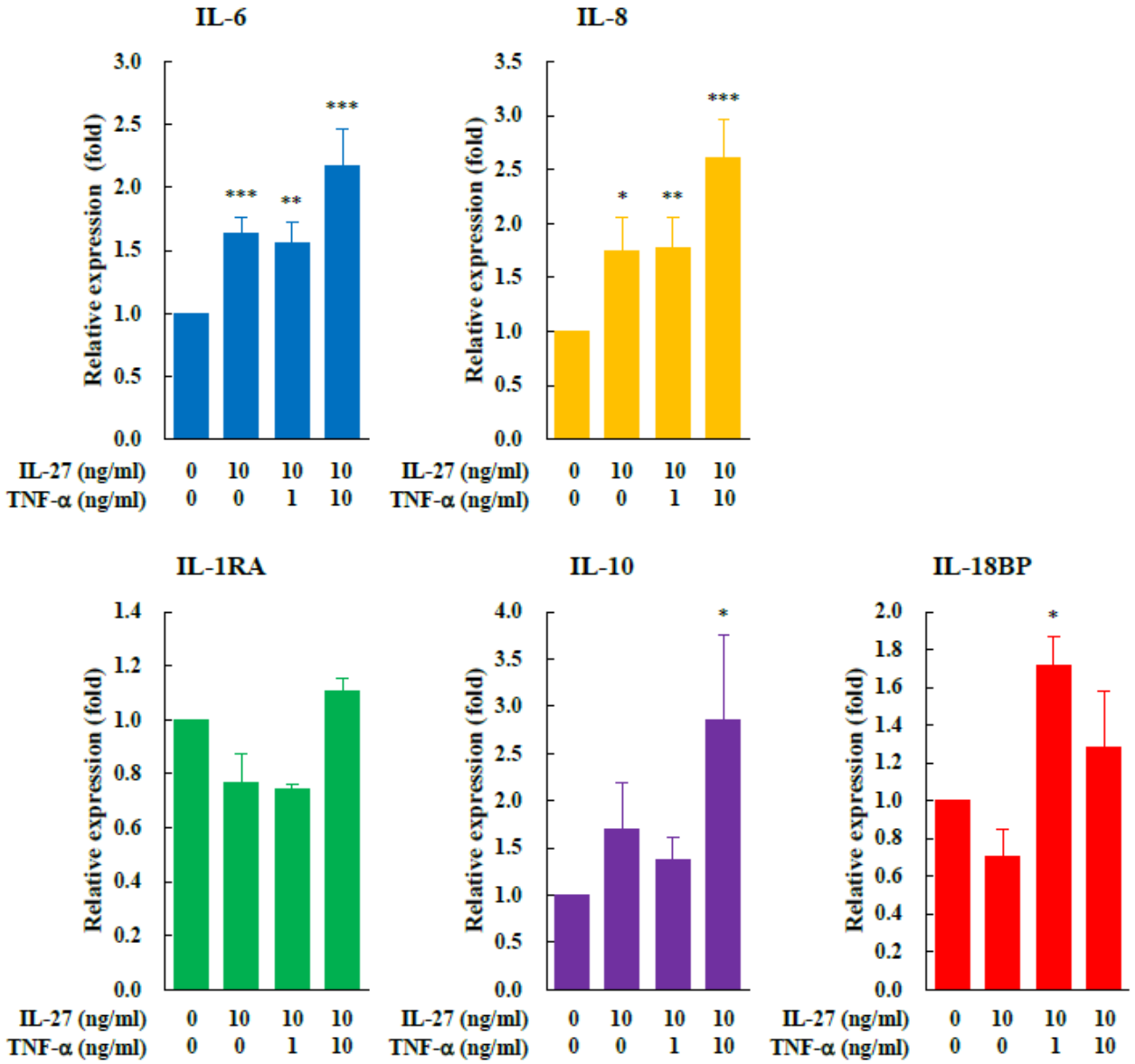

Figure 4. The interaction between IL-27 and TNF-α in the presence of 10 ng/ml IL-27.
To examine the effect of interaction between TNF-α and IL-27 on the mRNA levels of cytokines, IL-27-regulated mRNA levels were determined in the presence of 1 or 10 ng/ml TNF-α. In the presence of 10 ng/ml IL-27, the mRNA levels of IL1RA and IL-18BP were significantly upregulated by TNF-α, while the those of other cytokines were only slightly upregulated. Each value is represented as the mean ± SEM from at least three independent experiments (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the production from the control).

References

1. Pflanz S, Hibbert L, Mattson J, *et al.* WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. The Journal of Immunology 2004; 172(4): 2225–2231. doi: 10.4049/jimmunol.172.4.2225
2. Takeda A, Hamano S, Yamanaka A, *et al.* Cutting edge: Role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. The Journal of Immunology 2003; 170(10): 4886–4890. doi: 10.4049/jimmunol.170.10.4886
3. Hibbert L, Pflanz S, Malefyt RDM, *et al.* IL-27 and IFN-γ signal via Stat1 and Stat3 and induce T-Bet and IL-12Rα2 in naive T cells. Journal of Interferon & Cytokine Research 2003; 23: 513–522. doi: 10.1089/10799900360708632
4. Kamiya S, Owaki T, Morishima N, *et al.* An indispensable role for STAT1 in IL-27-induced T-bet expression but not proliferation of naive $CD4^+$ T cells. The Journal of Immunology 2004; 173(6): 3871–3877. doi: 10.4049/jimmunol.173.6.3871

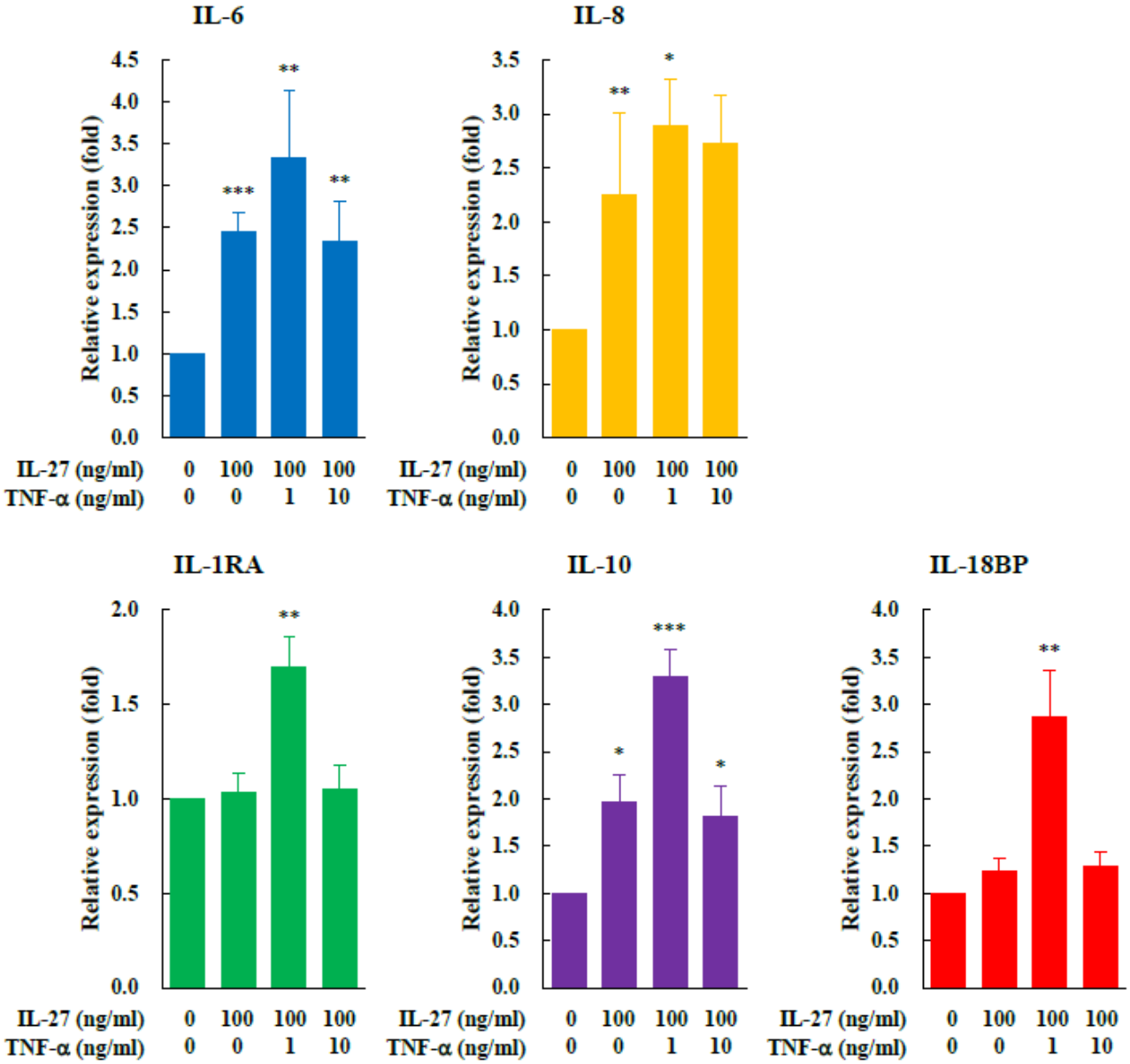

Figure 5. The interaction between IL-27 and TNF-α in the presence of 100 ng/ml IL-27.
To examine the effect of interaction between TNF-α and IL-27 on the mRNA levels of cytokines, IL-27-regulated mRNA levels were determined in the presence of 1 or 10 ng/ml TNF-α. In the presence of 100 ng/ml IL-27, the mRNA levels of IL1RA, IL-10 and IL-18BP were significantly upregulated in response to treatment with 1ng/ml TNF-α, while those of pro-inflammatory cytokines were not changed. Each value is represented as the mean ± SEM from at least three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001 compared with the production from the control).

5. Owaki T, Asakawa M, Morishima N, *et al.* STAT3 is indispensable to IL-27-mediated cell proliferation but not to IL-27-induced Th1 differentiation and suppression of pro-inflammatory cytokine production. The Journal of Immunology 2008; 180(5): 2903–2911. doi: 10.4049/jimmunol.180.5.2903
6. Pflanz S, Timans JC, Cheung J, *et al.* IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4$^+$ T cells. Immunity 2002; 16(6): 779–790. doi: 10.1016/s1074-7613(02)00324-2
7. Mei Y, Lv Z, Xiong L, *et al.* The dual role of IL-27 in CD4+T cells. Molecular Immunology 2021; 138: 172–180. doi: 10.1016/j.molimm.2021.08.001
8. Stumhofer JS, Hunter CA. Advances in understanding the anti-inflammatory properties of IL-27. Immunology Letters 2008; 117(2): 123–130. doi: 10.1016/j.imlet.2008.01.011
9. Yoshimura T, Takeda A, Hamano S, *et al.* Two-sided roles of IL-27: induction of Th1 differentiation on naive CD4$^+$ T cells versus suppression of pro-inflammatory cytokine production including IL-23-induced IL-17 on activated CD4$^+$ T cells partially through STAT3-dependent mechanism. The Journal Immunology 2006; 177(8): 5377–5385. doi: 10.4049/jimmunol.177.8.5377
10. Swaminathan S, Dai L, Lane HC, *et al.* Evaluating the potential of IL-27 as a novel therapeutic agent in

HIV-1 infection. Cytokine Growth Factor Reviews 2013; 24(6): 571–577. doi: 10.1016/j.cytogfr.2013.07.001

11. Yang B, Suwanpradid J, Sanchez-Lagunes R, *et al.* IL-27 facilitates skin wound healing through induction of epidermal proliferation and host defense. Journal of Investigative Dermatology 2017; 137(5): 1166–1175. doi: 10.1016/j.jid.2017.01.010
12. Wittmann M, Zeitvogel J, Wang D, *et al.* IL-27 is expressed in chronic human eczematous skin lesions and stimulates human keratinocytes. Journal of Allergy Clinical Immunology 2009; 124(1): 81–89. doi: 10.1016/j.jaci.2009.04.026
13. Shibata S, Tada Y, Kanda N, *et al.* Possible roles of IL-27 in the pathogenesis of psoriasis. Journal of Investigative Dermatology 2010; 130(4): 1034–1039. doi: 10.1038/jid.2009.349
14. Jergović M, Thompson HL, Bradshaw CM, *et al.* IL-6 can singlehandedly drive many features of frailty in mice. GeroScience 2021; 43: 539–549. doi: 10.1007/s11357-021-00343-z
15. Marcos-Pérez D, Sánchez-Flores M, Maseda A, *et al.* Frailty in older adults is associated with plasma concentrations of inflammatory mediators but not with lymphocyte subpopulations. Frontiers in Immunology 2018; 9: 1056. doi: 10.3389/fimmu.2018.01056
16. Van Epps PV, Oswald D, Higgins PA, *et al.* Frailty has a stronger association with inflammation than age in older veterans. Immunity and Ageing 2016; 13: 27. doi: 10.1186/s12979-016-0082-z
17. Xia S, Zhang X, Zheng S, *et al.* An update on inflamm-aging: mechanisms, prevention, and treatment. Journal of Immunology Research 2016; 2016: 8426874. doi: 10.1155/2016/8426874
18. Bektas A, Schurman SH, Sen R, *et al.* Aging, inflammation and the environment. Experimental Gerontology 2018; 105: 10–18. doi: 10.1016/j.exger.2017.12.015
19. Minciullo PL, Catalano A, Mandraffino G, *et al.* Inflammaging and anti-inflammaging: The role of cytokines in extreme longevity. Archivum Immunologiae et Therapiae Experimentalis 2016; 64: 111–126. doi: 10.1007/s00005-015-0377-3
20. Freund A, Orjalo AV, Desprez PY, *et al.* Inflammatory networks during cellular senescence: causes and consequences. Trends in Molecular Medicine 2010; 16(5): 238–246. doi: 10.1016/j.molmed.2010.03.003
21. Aioi A. Inflammaging in skin and intrinsic underlying factors. Trends in Immunotherapy 2021; 5(2): 44–53. doi: 10.24294/ti.v5.i2.1342
22. Deyrieux AF, Wilson VG. In vitro culture conditions to study keratinocyte differentiation using the HaCaT cell line. Cytotechnology 2007; 54: 77–83. doi: 10.1007/s10616-007-9076-1
23. Yamada T, Aioi A. Eucalyptus citriodora extract regulates cutaneous homeostasis including immune dysregulation and skin barrier dysfunction via the modulation of peroxisome proliferator-activated receptor-β/δ (PPAR-β/δ) pathway. Trends in Immunotherapy 2020; 4(2): 69–80. doi: 10.24294/ti.v4.i2.1130
24. Aioi A, Muromoto R, Mogami S, *et al.* Porcine placenta extract reduced wrinkle formation by potentiating epidermal hydration. Journal of Cosmetics, Dermatological Sciences and Applications 2021; 11(2): 101–109. doi: 10.4236/jcdsa.2021.112011
25. Zanni F, Vescovini R, Biasini C, *et al.* Marked increase with age of type 1 cytokines within memory and effector/cytotoxic $CD8^+$ T cells in humans: A contribution to understand the relationship between inflammation and immunosenescence. Experimental Gerontology 2003; 38(9): 981–987. doi: 10.1016/s0531-5565(03)00160-8
26. Kalliolias GD, Gordon RA, Ivashkiv LB. Suppression of TNF-α and IL-1 signaling identifies a mechanism of homeostatic regulation of macrophages by IL-27. The Journal of Immunology 2010; 185: 7047–7056. doi: 10.4049/jimmunol.1001290
27. Fujiwara S, Nagai H, Oniki S, *et al.* Interleukin (IL)-17 versus IL-27: Opposite effects on tumor necrosis factor-α-mediated chemokine production in human keratinocytes. Experimental Dermatology 2012; 21(1): 70–72. doi: 10.1111/j.1600-0625.2011.01384.x
28. De Groot M, Teunissen MBM, Picavet DI, *et al.* Reduction of different inflammatory cell types of the innate immune system in psoriatic skin during etanercept treatment. Experimental Dermatology 2010; 19: 754–756. doi: 10.1111/j.1600-0625.2010.01089.x
29. Batten M, Kljavin NM, Li J, *et al.* Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. The Journal of Immunology 2008; 180(5): 2752–2756. doi: 10.4049/jimmunol.180.5.2752
30. Wittmann M, Doble R, Bachmann M, *et al.* IL-27 regulates IL-18 binding protein in skin resident cells. PLoS ONE 2012; 7: e38751. doi: 10.1371/journal.pone.0038751
31. Bruunsgaard H, Andersen-Ranberg K, Hjelmborg Jv, *et al.* Elevated levels of tumor necrosis factor alpha and mortality in centenarians. The American Journal of Medicine 2003; 115(4): 278–283. doi: 10.1016/s0002-9343(03)00329-2
32. He H, Xu P, Zhang X, *et al.* Aging-induced IL27Ra signaling impairs hematopoietic stem cells. Blood 2020; 136(2): 183–198. doi: 10.1182/blood.2019003910
33. Boehmer ED, Meehan MJ, Cutro BT, *et al.* Aging negatively skews macrophage TLR2- and TLR4-mediated pro-inflammatory responses without affecting the IL-2-stimulated pathway. Mechanisms of Ageing and Development 2005; 126(12): 1305–1313. doi: 10.1016/j.mad.2005.07.009
34. Frasca D, Blomberg BB. Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology 2016; 17: 7–19. doi: 10.1007/s10522-015-9578-8