

**Original Paper**

# IL-27 Modulates Chemokine Production in TNF- $\alpha$ -Stimulated Human Oral Epithelial Cells

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**Key Words**IL-27 • Chemokine • TNF- $\alpha$  • Oral epithelial cells**Abstract**

**Background/Aims:** Interleukin-27 (IL-27) is a cytokine which belongs to the IL-12 family. However, the role of IL-27 in the pathogenesis of periodontal disease is uncertain. The aim of this study was to examine the effect of IL-27 on chemokine production in TNF- $\alpha$ -stimulated human oral epithelial cells (TR146). **Methods:** We measured chemokine production in TR146 by ELISA. We used western blot analysis to detect the phosphorylation levels of signal transduction molecules, including STAT1 and STAT3 in TR146. We used inhibitors to examine the role of STAT1 and STAT3 activation. **Results:** IL-27 increased CXCR3 ligands production in TNF- $\alpha$ -stimulated TR146. Meanwhile, IL-27 suppressed IL-8 and CCL20 production induced by TNF- $\alpha$ . STAT1 phosphorylation level in IL-27 and TNF- $\alpha$ -stimulated TR146 was enhanced in comparison to TNF- $\alpha$ -stimulated TR146. STAT3 phosphorylation level in IL-27-treated TR146 did not change by TNF- $\alpha$ . Both STAT1 inhibitor and STAT3 inhibitor decreased CXCR3 ligands production. STAT1 inhibitor overrode the inhibitory effect of IL-27 on IL-8 and CCL20 production in TNF- $\alpha$ -stimulated TR146. Meanwhile, STAT3 inhibitor did not modulate IL-8 and CCL20 production. **Conclusion:** IL-27 might control leukocyte migration in periodontal lesion by modulating chemokine production from epithelial cells.

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**Introduction**

Periodontal disease is a chronic inflammatory disease caused by periodontal disease cause bacteria. The characteristic is alveolar bone destruction and tooth loss. Excessive immunoreaction participates in the onset and progression of periodontal disease [1]. It is reported that the role of the immune cells, including neutrophil and T cells, in particular is important because mediators released from immune cells could destroy both soft tissues and alveolar bone [1-3].

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Chemokines play an essential role in the permeation or accumulation of immune cells in inflammatory lesion [4, 5]. We previously reported that TNF- $\alpha$ -stimulated periodontium constitution cells could produce chemokines which have already participated in neutrophils [6], Th1 cells [7], Th2 cells [8], and Th17 cells infiltration [9]. However, we do not understand the mechanism how chemokine production is controlled in periodontal lesion enough because new inflammatory mediator expressions are reported.

IL-27, which is an IL-12 family member, is a heterodimeric cytokine consisting of p28 and Epstein-Barr virus-induced gene 3 (EBI3) [10]. IL-27 is mainly produced in antigen-presenting cells stimulated with toll-like receptor ligands or inflammatory cytokines [11]. IL-27 is recognized by a receptor consisting of WSX-1 and gp130 [12]. IL-27 receptor is widely expressed in monocytes [12], keratinocytes [13], B cells [14], naïve T cells [10] and so on. It is reported that the IL-27 could influence on immune response including leukocyte biology. IL-27 could induce naïve T cells proliferation and IFN- $\gamma$  production. So, IL-27 could induce Th1 cells differentiation [15]. On the other hand, there are several reports that demonstrated that IL-27 inhibited Th2 and Th17 responses [16, 17]. It is known that IL-27 could suppress neutrophil function to inhibit neutrophil adhesion [18]. It was reported IL-27 is expressed in gingival crevicular fluid of human subjects [19]. However, the effect of IL-27 on chemokine production in periodontal lesion is still uncertain.

Gingival epithelial cells play an important role to disturb a bacterial invasion to periodontal tissues. Qian et al. reported that oral epithelial cells produce  $\beta$  defensin 1 and 3 which are antimicrobial peptides [20]. Moreover, it is certain gingival epithelial cells are involved in immune response in periodontal tissues. Imai et al. reported that *Aggregatibacter actinomycetemcomitans* could induce IL-6 and IL-8 mRNA expression in gingival epithelial cells. Therefore, we used oral epithelial cells in this experiment [21].

The aim of this study was clarify the role of IL-27 on leucocytes accumulation in periodontal lesion. So, we examined the effect of IL-27 on chemokines production in oral epithelial cells. Especially, we checked whether IL-27 modulate chemokine production in TNF- $\alpha$ -stimulated oral epithelial cells because TNF- $\alpha$  could induce chemokine production in periodontal resident cells. We focused on IL-8, which is a neutrophil chemokine, CCL20, which is a Th17 chemokine, and CXCR3 ligands (CXCL9, CXCL10, and CXCL11), which are Th1 chemokines, in this experiment. Moreover, we investigated which signal transduction pathway participated in the control of the chemokines production.

## Materials and Methods

### Cell culture

TR146 is a human oral epithelial cell line and was kindly provided by Dr. Mark Herzberg (University of Minnesota, MN, USA). TR146 was grown in Ham's F12 medium (Nakarai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (JRH Biosciences, Lenexa, KS, USA), 1 mmol/L sodium pyruvate (Gibco, Grand Island, MI, USA), and antibiotics (penicillin G, 100 units/ml; streptomycin, 100  $\mu$ g/ml; Gibco) at 37°C in a humidified air with 5% CO<sub>2</sub>. When the cells reached subconfluence, they were harvested and subcultured.

### Chemokine production in TR146

TR146 was stimulated with recombinant human IL-27 (Peprotech, Rocky Hill, NJ, USA) with or without TNF- $\alpha$  (Peprotech) for 24 hours. The supernatants of TR146 were collected, and IL-8, CCL20, CXCL9, CXCL10, and CXCL11 concentration was measured in triplicate using enzyme-linked immunosorbent assays (ELISA). Duoset ELISA kit (R&D Systems, Minneapolis, MN, USA) was used to obtain these measurements. All assays were performed according to the manufacturer's instructions, and chemokine level was determined using the standard curve prepared for each assay. In selected experiments, the TR146 were cultured for 1 hour in the presence or absence of fludarabine (50  $\mu$ M; Cayman Chemical, Ann Arbor, MI, USA) or WP1066 (5  $\mu$ M; Santa Cruz Biotechnology) prior to the incubation with IL-27 and TNF- $\alpha$ .

#### Western blot analysis

Western blot analysis was performed to detect IL-27 receptor expression and the IL-27 with or without TNF- $\alpha$ -induced phosphorylation of signal transduction molecules. TR146 were stimulated with IL-27 (10 ng/ml) or TNF- $\alpha$  (10 ng/ml) for 15, 30, or 60 minutes, and washed once with cold phosphate-buffered saline and then incubated on ice for 10 min with cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA) supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). After the removal of debris by centrifugation, the protein concentrations of the lysates were quantified with the Bradford protein assay using IgG as a standard. Twenty- $\mu$ g protein samples were loaded onto 4-20% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel, before being electrotransferred to polyvinylidene difluoride membranes. The membranes were then blocked with 1% non-fat dried milk for 1 hour and then reacted with anti-human gp130 mouse monoclonal antibody (R&D Systems), anti-human WSX-1 mouse monoclonal antibody (R&D systems), anti-phospho-STAT1 rabbit monoclonal antibody (Cell Signaling Technology), anti-phospho-STAT3 rabbit monoclonal antibody (Cell Signaling Technology), anti-STAT1 rabbit monoclonal antibody (Cell Signaling Technology), anti-STAT3 mouse monoclonal antibody (Cell Signaling Technology), or anti-GAPDH rabbit monoclonal antibody (Cell Signaling Technology) overnight. After washing, the membranes were incubated with the horseradish peroxidase-conjugated secondary antibody (Sigma) for 1 hour at room temperature, and then immunodetection was performed using the ECL prime Western blotting detection system (GE Healthcare, Uppsala, Sweden).

#### Statistical analysis

Statistical significance was analyzed using the Student's *t* test. *P* values of <0.05 were considered to be significant.

## Results

#### IL-27 receptor expression in TR146

It is reported gp130 and WSX-1 are necessary to recognize IL-27 [12]. Therefore, we firstly examined IL-27 receptor expression in non-stimulated TR146. Western blot analysis revealed that both gp130 and WSX-1 were expressed in TR146 (Fig.1).

#### The effect of IL-27 on IL-8 and CCL20 production in TNF- $\alpha$ -stimulated TR146

It is certain that chemokines are important to control the migration of leucocyte in inflammatory lesion [4, 5]. Neutrophil and Th17 cells are involved in the pathogenesis of periodontal disease [6, 9]. So, we examined the effect of IL-27 on IL-8, which could induce neutrophil accumulation, and CCL20, which is a Th17 chemokine, production in TR146. It was impossible to induce IL-8 or CCL20 production in TR146 only by IL-27 stimulation (data not shown). Meanwhile, IL-27 was able to reduce IL-8 and CCL20 production in TNF- $\alpha$ -stimulated TR146 (Fig.2).

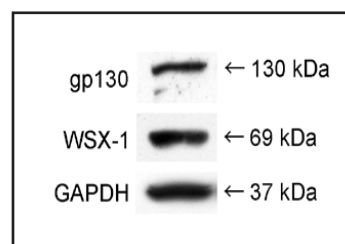
#### The effect of IL-27 on CXCR3 ligand production in TNF- $\alpha$ -stimulated TR146.

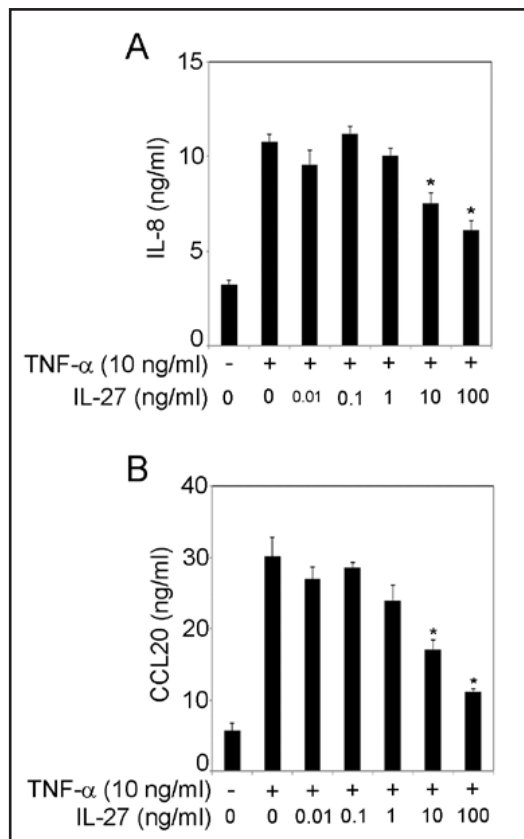
Th1 cells in periodontal lesion are essential in pathological process. It is known that CXCR3 ligands, including CXCL9, CXCL10, and CXCL11, are important in explaining Th1 cells migration [22]. Fig.3 shows that IL-27 could induce CXCL9, CXCL10, and CXCL11 production in TR146 in a concentration dependent manner. Moreover, IL-27 significantly increased CXCR3 ligands production in TNF- $\alpha$ -stimulated TR146 (Fig.3).

#### The effect of IL-27 on STAT1 and STAT3 phosphorylation in TNF- $\alpha$ -stimulated TR146.

It is reported that IL-27 could induce STAT1 and STAT3

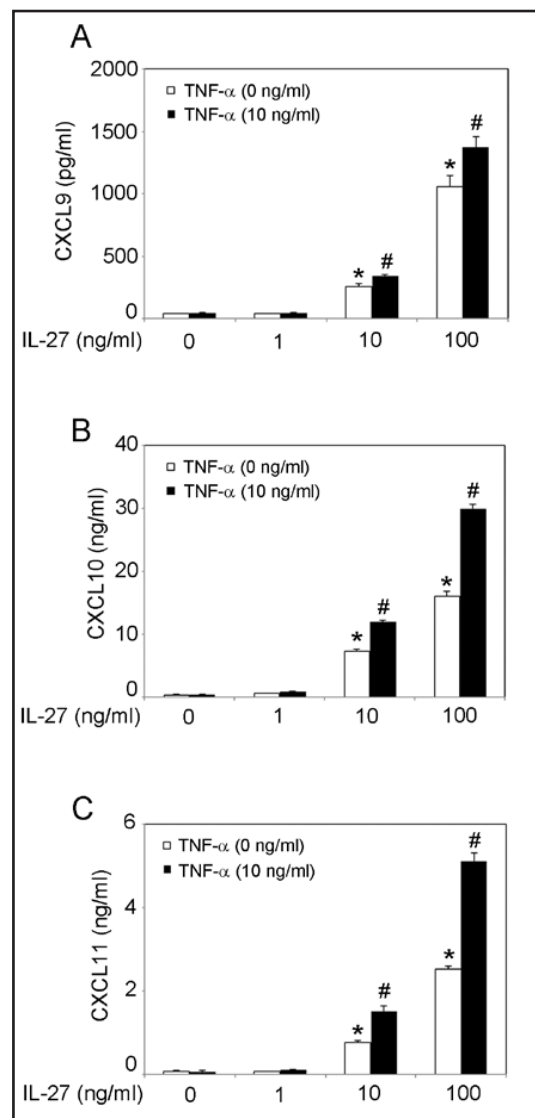
**Fig. 1.** gp130 and WSX-1 expressions in TR146. The extracts of non-stimulated TR146 were subjected to SDS-PAGE. Western blot analysis with antibodies against gp130, WSX-1, and GAPDH were performed.



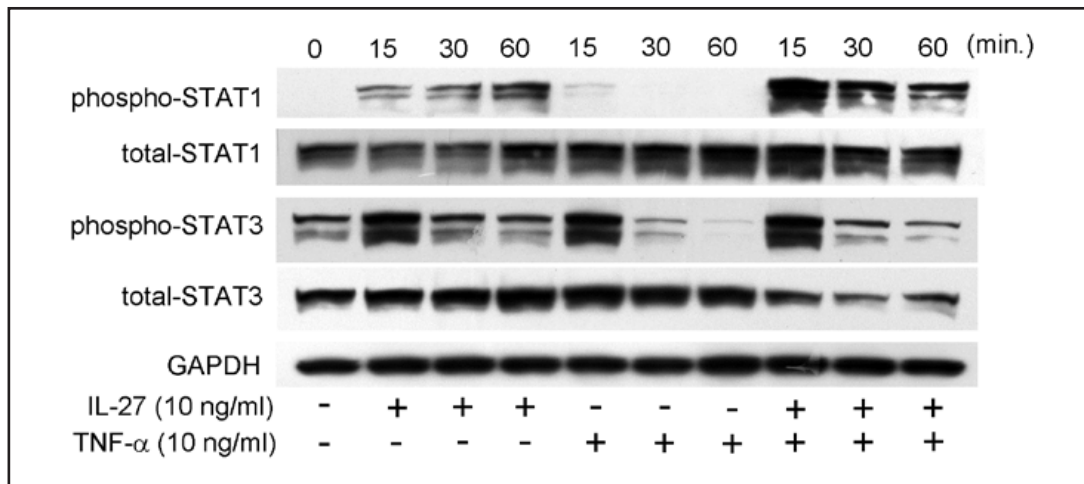


**Fig. 2.** The effect of IL-27 on IL-8 and CCL20 production in TNF- $\alpha$ -TR146. TR146 was incubated with human recombinant TNF- $\alpha$ (10 ng/ml) with or without human recombinant IL-27 (0.01, 0.1, 1, 10, or 100 ng/ml) with for 24 hours, and then the supernatant was collected. The concentration of IL-8 and CCL20 in the supernatant was measured using ELISA. The result is shown as the mean and SD values of a representative experiment performed in triplicate. The error bars represent the SD. \* = P<0.05, significantly different from the non-stimulated TR146.

phosphorylation in some types of cells [12, 23]. Therefore, we examined whether IL-27 could activate STAT1 and STAT3 pathways in TR146. Fig.4 shows that IL-27 could induce STAT1 and STAT3 phosphorylation in TR146. TNF- $\alpha$  did not induce STAT1 phosphorylation though activate STAT3 pathway in TR146. STAT1 phosphorylation in IL-27 and TNF- $\alpha$ -stimulated TR146 was enhanced in comparison with IL-27-stimulated TR146. The deepness of the band of STAT3 phosphorylation in IL-27/TNF- $\alpha$ -stimulated TR146 did not change relative to IL-27 or TNF- $\alpha$ -stimulated TR146.



**Fig. 3.** Effect of IL-27 on CXCL9, CXCL10, and CXCL11 production in TNF- $\alpha$ -stimulated TR146. TR146 were stimulated with IL-27 (1, 10, or 100 ng/ml) with or without TNF- $\alpha$  (10 ng/ml). Their supernatants were collected after 24 hours. The concentrations of CXCL9, CXCL10, or CXCL11 in the supernatants were measured using ELISA. The results are shown as the mean and SD of a representative experiment performed in triplicate. The error bars indicate the SD. \* = P<0.05, significantly different from the non-stimulated TR146 that were not treated with IL-27, # = P<0.05, significantly different from the TNF- $\alpha$ -stimulated TR146 that were not treated with IL-27.



**Fig. 4.** STAT1 and STAT3 phosphorylation in IL-27 with or without TNF- $\alpha$  in TR146. The cultured cells were treated with IL-27 (10 ng/ml) alone or IL-27 (10 ng/ml) with TNF- $\alpha$  (10 ng/ml) for 15, 30, or 60 min. The cells were lysed in lysis buffer containing protease inhibitors, and the phosphorylation of STAT1 and STAT3 was assessed using Western blot analysis. A representative western blot data that indicates the phospho-STAT1, total STAT1, phospho-STAT3, total STAT3, and GAPDH levels detected in the TR146 during three independent experiments is shown.

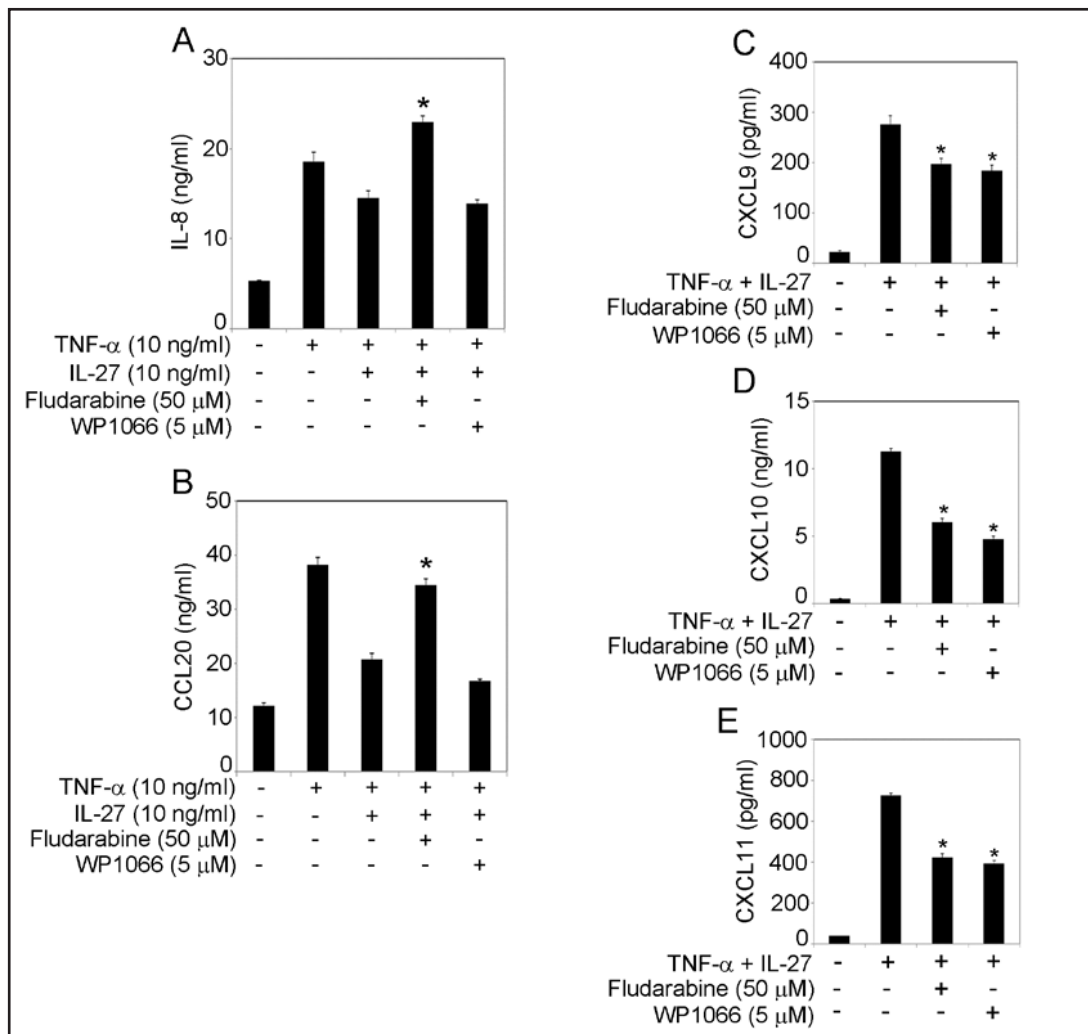
*Effects of signal transduction inhibitors on chemokine production in IL-27/TNF- $\alpha$ -stimulated TR146.*

Finally, we examined the role of STAT1 and STAT3 pathways activation on chemokine production in TR146 using signal inhibitors (Fig. 5). Fludarabine (a STAT1 inhibitor) rescued inhibitory effects of IL-27 on IL-8 and CCL20 production in TNF- $\alpha$ -stimulated TR146 though WP1066 (a STAT3 inhibitor) did not change IL-8 and CCL20 production in IL-27/TNF- $\alpha$ -stimulated TR146. Both fludarabine and WP1066 significantly inhibited CXCL9, CXCL10, and CXCL11 production in IL-27 and TNF- $\alpha$ -stimulated TR146.

**Discussion**

Han and the colleagues reported that IL-27 was detected in gingival crevicular fluid in human subjects in 2013 [19]. On the other hand, Mitani and the colleagues reported that IL-27 was not detected in gingival tissues with chronic periodontitis in 2015 [24]. They examined IL-27 p28 mRNA expression in gingival tissues, though they did not check whether IL-27 was included in gingival crevicular fluid. Moreover, IL-27 is detected in blood serum in normal subjects [25]. It is known that gingival crevicular fluid includes blood serum component. Therefore, we think IL-27 is present in gingival crevicular fluid at least. And, component of gingival crevicular fluid could stimulate epithelial cells. So, IL-27 in gingival crevicular fluid could modulate chemokine production in epithelial cells.

The role of IL-27 in periodontal lesion was uncertain. We hypothesized IL-27 might modulate immune reaction. So, we examined the effects of IL-27 on chemokine production in periodontal resident cells because chemokines are important molecules to control leukocytes migration in periodontal lesion [26]. Our results mean that IL-27 could decrease neutrophil and Th17 cells migration, and increase the number of Th1 cells in periodontal lesion. It is reported that high levels of lysosomal enzymes, superoxides, and reactive oxygen derivatives in neutrophils are related with the destruction of periodontal tissues [27, 28]. Moreover, Th17 cells and IL-17 have an important role on bone destruction in inflammatory lesion [29, 30]. Therefore, IL-27 might inhibit tissue destruction in periodontal tissue by decreasing



**Fig. 5.** Effects of STAT1 or STAT3 inhibitors on chemokine production in IL-27 and TNF- $\alpha$ -stimulated TR146. TR146 were incubated with fludarabine (STAT1 inhibitor: 50  $\mu$ M) or WP1066 (STAT3 inhibitor: 5  $\mu$ M), for 1 hour and then stimulated with human recombinant IL-27 (10 ng/ml) and TNF- $\alpha$  (10 ng/ml). Their supernatants were collected after 24 hours. The concentrations of IL-8, CCL20, CXCL9, CXCL10, and CXCL11 in the supernatants were measured using ELISA. The results are shown as the mean and SD of a representative experiment performed in triplicate. The error bars indicate the SD. \* = P<0.05, significantly different from the IL-27 and TNF- $\alpha$ -stimulated TR146 that were not pretreated with signal transduction inhibitors.

IL-8 and CCL20 production. Meanwhile, IL-27 increased CXCL9, CXCL10, and CXCL11 in TNF- $\alpha$ -stimulated TR146. Therefore, IL-27 could be involved in Th1 cells accumulation. The role of Th1 cells in periodontal lesion is controversial. In some studies, IFN- $\gamma$ , which is a Th1 cytokine, is related to bone destruction in response to *Pgingivalis* [31]. On the other hand, Takayanagi et al. reported that IFN- $\gamma$  could inhibit osteoclastogenesis [32]. So, the role of Th1 cells in periodontal lesion is uncertain. However, IL-27 might be related with Th1 cells accumulation in periodontal lesion at least. Further study about the role of Th1 cells in periodontal tissues should be necessary.

We revealed IL-27 could inhibit IL-8 production in TNF- $\alpha$ -stimulated oral epithelial cells by STAT1 activation. Other researchers examined the effect of IL-27 on IL-8 production. Su et al. reported that IL-27 priming enhanced IL-8 production from LPS-stimulated lung fibroblasts and bronchial epithelial cells [33]. On the other hand, Kachroo et al. showed IL-

27 could inhibit IL-8 production in human non-small cell lung carcinoma cells [34]. They also showed that STAT1 inhibition increased IL-8 expression. The result of this article is similar with ours. So, the influence of IL-27 on IL-8 production depends on the kind of cells or stimulation, and STAT1 pathway might be related with IL-8 production.

We appeared IL-27 could decrease CCL20 production in TNF- $\alpha$ -stimulated oral epithelial cells by STAT1 activation. It is known that IL-27 inhibits Th17 response. Therefore, the inhibitory effect of IL-27 on CCL20 is reasonable. We found one report about the effect of IL-27 on CCL20 production. Shibata et al. reported that IL-27 could inhibit CCL20 production TNF- $\alpha$ -treated human keratinocytes [35]. Their report totally agrees with our results. However, they did not examine the signal transduction pathway. There are no reports that examined the role of STAT1 on CCL20 production. However, it has been reported that IL-27 could inhibit IL-17 expression in Th17 cells through a STAT1-mediated mechanism [36]. So, STAT1 activation induced by IL-27 might be involved in suppression of Th17 response including IL-17 or CCL20 expression.

We also found that IL-27 could increase CXCR3 ligands production in TNF- $\alpha$ -stimulated TR146. It is certain that IL-27 is involved in Th1 response, and there are several reports about the effect of IL-27 on CXCR3 ligands production. Qiu et al. reported that IL-27 could enhance CXCL10 production in TNF- $\alpha$ -treated human coronary artery endothelial cells [37]. Dong et al. also reported that IL-27 increased CXCL10 expression in TNF- $\alpha$ -stimulated human lung fibroblasts [38]. Judging from our report and previous reports, we think both IL-27 and TNF- $\alpha$  promote accumulation of Th1 cells in periodontal lesion by inducing CXCR3 ligands production to epithelial cells, endothelial cells, and fibroblasts. We revealed that STAT1 and STAT3 pathways are related with the enhancement of CXCR3 ligands expression in IL-27 and TNF- $\alpha$ -treated TR146. It is known that STAT1 inhibition suppressed CXCL10 and CXCL11 production in IL-27-stimulated human dermal lymphatic endothelial cells [39]. However, there are no reports about the role of STAT3 pathway on CXCR3 ligands production in IL-27-stimulated cells though it is known that IL-27 could activate STAT3 pathway [21]. We previously reported that a STAT3 inhibitor could decrease CXCL10 production in oncostatin M-treated human gingival cells [40]. Xu also reported that CXCL10 production by IL-6-stimulated human macrophage is dependent on STAT3 phosphorylation [41]. So, we think STAT3 activation is positively involved in CXCR3 ligands production in human cells.

In conclusion, IL-27 could enhance Th1 chemokine production in human oral epithelial cell line. On the other hand, IL-27 decreased IL-8 and CCL20, which could induce accumulation of neutrophils and Th17 cells. STAT1 and STAT3 pathways are related with this phenomenon. Therefore, IL-27 might change the population of leukocytes in periodontal lesion, and be related with the pathogenesis of periodontal disease.

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## Disclosure Statement

The authors confirm that they have no conflicts of interest.

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