IL-29 enhances CXCL10 production in TNF-α-stimulated human oral epithelial cells

Yoshitaka Hosokawa[a], Ikuko Hosokawa[a], Satoru Shindo[a], Kazumi Ozaki[b], and Takashi Matsuo[a]

[a]Department of Conservative Dentistry, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Tokushima, Japan, and [b]Department of Oral Health Care Promotion, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Tokushima, Japan

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Address correspondence and reprint requests to: Dr. Yoshitaka Hosokawa, Department of Conservative Dentistry, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-cho, Tokushima, Tokushima, Japan, 770-8504

TEL: 81-886-33-7340

FAX: 81-886-33-7340

E-mail: hosokawa@tokushima-u.ac.jp

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Abstract

Interleukin-29 (IL-29) is a cytokine belonging to the type III interferon family. It was recently detected in the gingival crevicular fluid of periodontitis patients. However, the role of IL-29 in the pathogenesis of periodontal disease remains unknown. The aim of this study was to examine the effects of IL-29 on C-X-C motif chemokine ligand 10 (CXCL10) production in human oral epithelial cells. We measured CXCL10 production in TR146 cells, which is a human oral epithelial cell line, using an enzyme-linked immunosorbent assay. We used Western blot analysis to detect IL-29 receptor expression and the phosphorylation levels of signal transduction molecules, including p38 mitogen-activated protein kinases (MAPK), signal transducer and activator of transcription 3 (STAT3), and nuclear factor (NF)-κB p65, in the TR146 cells. The TR146 cells expressed the IL-29 receptor. IL-29 induced CXCL10 production in the TR146 cells. IL-29 significantly enhanced CXCL10 production in tumor necrosis factor (TNF)-α-stimulated TR146 cells. The p38 MAPK, STAT3, and NF-κB pathways were found to be related to the IL-29-induced enhancement of CXCL10 production in TNF-α-stimulated TR146 cells. IL-29 promotes T helper 1 cell accumulation in periodontal lesions by inducing CXCL10 production in oral epithelial cells.

Introduction

Interleukin-29 (IL-29) is a newly discovered cytokine belonging to the type III interferon family [Witte et al., 2010]. Recently, it was reported that IL-29 is involved in the pathogenesis of some diseases including Hashimoto's thyroiditis [Arpaci et al., 2016]. IL-29 is mainly produced by leukocytes, such as dendritic cells [Wolk et al., 2008]. In addition, it was reported that stimulation with IL-4 [Megjugorac et al., 2010] or herpes simplex virus [Melchjorsen et al., 2006] induced IL-29 production in dendritic cells. IL-29 has antiviral and immunomodulatory properties [Li et al., 2009], and IL-29 knockout mice exhibited increased susceptibility to respiratory virus infections, including influenza virus [Qian et al., 2013]. IL-29 was found to decrease the production of T helper 2 (Th2)-type cytokines, including IL-4, IL-5, and IL-13, in some types of cells [Srinivas et al., 2008; Li et al., 2014]. Recently, Shivaprasad and Pradeep reported that patients with chronic/aggressive periodontaitis display higher gingival crevicular fluid IL-29 levels than healthy donors [Shivaprasad et al., 2013]. So, we hypothesized that IL-29 might modulate immune responses in periodontal lesions.

It is known that immune responses in periodontal lesions play an important role in the progression of periodontal disease, and cytokines produced by leukocytes are involved in bone resorption in periodontal lesisons [Souza et al., 2008]. In particular, T-cell phenotypes affect susceptibility to periodontitis, as well as the initiation and severity of the condition [Gaffen et al., 2008]. Chen et al. recently reported that the levels of interferon (IFN)-γ were higher in periodontal lesions than in healthy donors [Chen et al., 2016]. Rajesh et al. also

demonstrated that the expression of T-bet, which is a Th1-cell transcription factor, was elevated in patients with periodontal disease [Rajesh et al., 2015]. These results showed that the number of Th1 cells was increased in periodontal lesions and that Th1 cells are involved in the pathogenesis of periodontitis. So, it is important to examine the mechanism responsible for Th1-cell accumulation in tissues affected by periodontal disease.

CXC chemokine ligand (CXCL) 10 is a chemokine that plays a crucial role in Th1-cell accumulation in periodontal lesions [Taubman et al., 2001]. We previously reported that periodontal resident cells produce CXCL10. Tumor necrosis factor (TNF)-α, IFN-γ, oncostatin M, and IL-1β can induce CXCL10 production in human gingival fibroblasts and human periodontal ligament cells [Hosokawa et al., 2009; Hosokawa et al., 2010; Hosokawa et al., 2015]. However, it is unclear if IL-29 has the ability to induce CXCL10 production in periodontal tissue.

The aim of this study was to investigate the effects of IL-29 on CXCL10 production in oral epithelial cells. Moreover, we examined which intracellular signal transduction pathways are involved in CXCL10 production in IL-29-stimulated human oral epithelial cells.

Materials and Methods

Cell culture

The human oral epithelial cell line TR146, which was isolated from a human buccal carcinoma, was kindly provided by Dr Mark Herzberg (University of Minnesota, MN, USA). It is reported that TR146 cells show some morphological similarities to the human buccal

epithelial cells [Jacobsen et al., 1999] TR146 cells were used as human oral epithelial cell line in some previous papers [Hiroshima et al., 2016; Kohli et al., 2014; Sorenson et al., 2012]. TR146 cells were grown in Ham's F12 medium (Nacalai 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 μg/ml; Gibco) at 37°C in humidified air with 5% CO_2. When the cells reached subconfluence, they were harvested and subcultured.

CXCL10 production in TR146 cells

TR146 cells were stimulated with recombinant human IL-29 (1, 10, or 100 ng/ml; Peprotech, Rocky Hill, NJ, USA) with or without TNF-α (10 ng/ml: Peprotech) for 24 hours. The supernatants of the TR146 cells were collected, and their CXCL10 concentrations were measured in triplicate using enzyme-linked immunosorbent assays (ELISA). Duoset ELISA kits (R&D Systems, Minneapolis, MN, USA) were used to obtain these measurements. All assays were performed according to the manufacturer's instructions, and the CXCL10 level was determined using the standard curve prepared for each assay. In selected experiments, the TR146 cells were cultured for 1 hour in the presence or absence of SB203580 (10 μM; Santa Cruz Biotechnology, Santa Cruz, CA, USA), PD98059 (10 μM; Santa Cruz Biotechnology), SP600125 (10 μM; Santa Cruz Biotechnology), fludarabine (50 μM; Cayman Chemical, Ann Arbor, MI, USA), WP1066 (5 μM; Santa Cruz Biotechnology), 10-DEBC hydrochloride (5 μM; Santa Cruz Biotechnology), or SC514 (10 μM; Santa Cruz Biotechnology), prior to the

incubation of the cells with IL-29.

Western blot analysis

Western blot analysis was performed to detect IL-29 receptor expression and the IL-29 with or without TNF-α-induced phosphorylation of signal transduction molecules. TR146 cells were stimulated with IL-29 (100 ng/ml) or TNF-α (10 ng/ml) for 15, 30, or 60 minutes; 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 removing any debris via centrifugation, the protein concentrations of the lysates were quantified with the Bradford protein assay using IgG as a standard. Twenty-μg protein samples were loaded onto 4-20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, before being electrotransferred to polyvinylidene difluoride membranes. The membranes were then blocked with 1% non-fat dried milk for 1 hour and reacted with the anti-human IL-10 receptor β mouse monoclonal antibody (R&D Systems, catalog number MAB874, 1 μg/ml), anti-human IL-28 receptor α mouse monoclonal antibody (BioLegend, San Diego, CA, catalog number 337802, 1 μg/ml), anti-phospho-p38 MAPK rabbit monoclonal antibody (Cell Signaling Technology, catalog number 8632, 1000 times dilution), anti-phospho-STAT3 rabbit monoclonal antibody (Cell Signaling Technology, catalog number 9145, 2000 times dilution), anti-phospho-NF-κB p65 rabbit monoclonal antibody (Cell Signaling Technology, catalog number 3033, 1000 times dilution), anti-p38 MAPK

rabbit monoclonal antibody (Cell Signaling Technology, catalog number 8690, 1000 times dilution), anti-STAT3 mouse monoclonal antibody (Cell Signaling Technology, catalog number 9139, 2000 times dilution), anti-NF-κB p65 rabbit monoclonal antibody (Cell Signaling Technology, catalog number 8242, 1000 times dilution), or anti-GAPDH rabbit monoclonal antibody (Cell Signaling Technology, catalog number 5174, 6000 times dilution) overnight. The membranes were incubated with the horseradish peroxidase-conjugated secondary antibody (Sigma) for 1 hour at room temperature, and then detection was performed using the ECL prime Western blotting detection system (GE Healthcare, Uppsala, Sweden). The quantitation of the chemiluminescent signals was carried out using ImageJ (version 1.44).

Statistical analysis

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

Results

IL-29 receptor expression in TR146 cells

It is reported that both IL-10 receptor β and IL-28 receptor α are required to recognize IL-29 [Sheppard et al., 2003]. Therefore, we examined the expression of IL-10 receptor β, and IL-28 receptor α in TR146 cells. Fig. 1 shows that both IL-10 receptor β and IL-28 receptor α are expressed in TR146 cells.

The effect of IL-29 on CXCL10 production in TR146 cells

Th1 cells are involved in the pathogenesis of periodontal disease [Taubman et al., 2001]. So, it is important to examine CXCL10 production because CXCL10 is a key chemokine for Th1-cell accumulation in inflammatory lesions. We examined whether IL-29 was able to induce CXCL10 production in TR146 cells. Fig. 2 shows that IL-29 enhanced CXCL10 production in TR146 cells in a concentration-dependent manner.

The effect of IL-29 on CXCL10 production in TNF-α-stimulated TR146 cells

It is known that TNF-α is able to induce CXCL10 production in epithelial cells [Wu et al., 2015]. Therefore, we hypothesized that IL-29 might modulate the CXCL10 production induced by TNF-α in TR146 cells. Fig. 3 shows that TNF-α (10 ng/ml) induced CXCL10 production (245.12±6.33 pg/ml) and IL-29 (100 ng/ml) enhanced CXCL10 production in TNF-α-stimulated TR146 cells (1633.9±255.65 pg/ml, p=0.0101).

Effects of signal transduction inhibitors on CXCL10 production in IL-29-stimulated TR146 cells

Next, we examined which signal transduction pathways are involved in CXCL10 production in IL-29-treated TR146 cells using chemical inhibitors. We focused on the MAPK, STAT, Akt, and NF-κB pathways because it is known that CXCL10 production is modulated by these pathways [Sauty et al., 1999]. IL-29 (100 ng/ml) could induce CXCL10 production (926.95 ±37.78 pg/ml). SB203580 (a p38 MAPK inhibitor), WP1066 (a STAT3 inhibitor), and SC514 (an NF-κB inhibitor) suppressed CXCL10 production (560.79 ±24.59 pg/ml p=0.0019, 562.63 ±31.87 pg/ml p=0.00026, 400.13 ±81.08 pg/ml p=0.0056, respectively).

On the other hand, PD98059 (an extracellular signal-regulated kinase [ERK] inhibitor), SP600125 (a c-Jun N-terminal kinase [JNK] inhibitor), and 10-DEBC hydrochloride (an Akt inhibitor) increased CXCL10 production (1453.34 ±22.95 pg/ml p=0.00035, 1899.61 ± 88.01 pg/ml p=0.0017, 1455.69 ±156.23 pg/ml p=0.015, respectively). Fludarabine (STAT1 inhibitor) did not modulate CXCL10 production (918.67 ±21.81 pg/ml p=0.075). These findings indicate that the p38 MAPK, STAT3, and NF-κB pathways have positive effects on CXCL10 production, and the ERK, JNK, and Akt pathways negatively modulate CXCL10 production in IL-29-stimulated TR146 cells.

The effects of IL-29 on p38 MAPK, STAT3, and NF-κB p65 phosphorylation in TNF-α-stimulated TR146 cells

Fig. 4 shows that the p38 MAPK, STAT3, and NF-κB pathways have positive effects on CXCL10 production in TR146 cells. Therefore, we examined the levels of p38 MAPK, STAT3, and NF-κB phosphorylation in TNF-α/IL-29-stimulated TR146 cells and compared them with those seen in IL-29-stimulated TR146 cells to elucidate which signal pathways are involved in the enhancement of CXCL10 production. Fig. 5 clearly shows that the TNF-α/IL-29-stimulated TR146 cells exhibited greater p38 MAPK, STAT3, and NF-κB phosphorylation levels than the IL-29-stimulated TR146 cells.

Discussion

In this study, we showed that IL-29 induced CXCL10 production in human oral epithelial cells. This is the first study to demonstrate that IL-29 has the ability to induce Th1-type

chemokine production in periodontal resident cells. Witte et al. recently reported that IL-29 induced CXCL9, CXCL10, and CXCL11 production in human keratinocytes [Witte et al., 2016]. We examined CXCL9 and CXCL11 production in TR146 cells using ELISA. However, we could not detect either of these molecules. They also stated that IL-29 induced the production of much smaller amounts of CXCL9 in human keratinocytes. Therefore, it is possible that IL-29 induces the production of small amounts of CXCL9 and CXCL11 in TR146 cells. However, these chemokines might have almost no effect on Th1-cell accumulation in periodontal lesions. Pekarek et al. detected IL-29 increased mRNA levels of CXCL9, CXCL10, and CXCL11 in peripheral blood mononuclear cells [Pekarek et al., 2007], and leukocytes are known to be are present in periodontal lesions. So, IL-29 might induce CXCL10 production in epithelial cells and leukocytes and increase the number of Th1 cells in periodontal lesions. However, the amounts of CXCL9, CXCL10, and CXCL11 in IL-29-induced human leukocytes are unknown, and it is also uncertain which types of leukocytes contribute to CXCL9, CXCL10, and CXCL11 production. We should examine the effects of IL-29 on leukocytes to address these questions.

We clearly showed that IL-29 (100 ng/ml) synergistically increased CXCL10 production in TNF-α (10 ng/ml)-stimulated TR146 cells. This is the first study to find that IL-29 enhances TNF-α-induced inflammatory mediator expression. On the other hand, other studies have demonstrated that IL-29 is able to modulate the cytokine expression induced by other molecules. For example, Xu et al. reported that IL-29 increased IL-6 and IL-8 production in

peptidoglycan, polycytidylic acid, or lipopolysaccharide-stimulated human synovial fibroblasts [Xu et al., 2013]. They also reported that IL-29 (100 ng/ml) enhanced IL-6, IL-8, and TNF-α (10 ng/ml) production in lipopolysaccharide-stimulated RAW264.7 cells [Xu et al., 2015]. These reports and our results indicate that IL-29 might exacerbate inflammatory reactions in cooperation with other inflammatory mediators, such as proinflammatory cytokines or toll-like receptor ligands.

We found that IL-29 activated various signal transductions pathways, including the MAPK, STAT1, Akt, and NF-κB pathways. Some previous studies have examined the signal pathways that are activated by IL-29 stimulation. Xu et al. reported that IL-29 induced p38 MAPK, ERK, JNK, STAT1, and NF-κB p65 phosphorylation in human synovial fibroblasts although they also showed that IL-29 did not induce STAT3 or Akt phosphorylation [Xu et al., 2016]. Guenterberg et al. demonstrated that IL-29 enhanced STAT1 phosphorylation in a human melanoma cell line although it did not enhance the basal phosphorylation of STAT3 [Guenterberg et al., 2010]. Brands et al. reported that IL-29 induced p38 MAPK, ERK, JNK, and Akt phosphorylation in human intestinal epithelial cells [Brand et al., 2005]. Judging from previous reports and ours, we consider that IL-29 induces p38 MAPK, ERK, JNK, STAT1, and NF-κB p65 phosphorylation although STAT3 phosphorylation is not modulated by IL-29 stimulation. We suggest that IL-29 might activate the Akt pathway in a cell type-dependent manner.

In this study, we demonstrated that the p38 MAPK, STAT3, and NF-κB pathways positively

regulate CXCL10 production in IL-29-stimulated TR146 cells. We previously showed that a p38 MAPK inhibitor and a STAT3 inhibitor significantly decreased CXCL10 production in oncostatin M-treated human gingival fibroblasts [Hosokawa et al., 2010]. We recently reported that the p38 MAPK and NF-κB pathways are involved in CXCL10 production in IL-1β-stimulated human periodontal ligament cells [Hosokawa et al., 2016]. Taken together, our previous studies and this study demonstrate that the p38 MAPK, STAT3, and NF-κB pathways are essential for CXCL10 production. A JNK inhibitor enhanced CXCL10 production in this study, whereas a JNK inhibitor decreased CXCL10 production in IL-1β-stimulated human periodontal ligament cells in our previous studies. We consider that the role of JNK in CXCL10 production is dependent on the kind of cell or irritant involved. Moreover, we showed that an ERK inhibitor increased CXCL10 production in TR146 cells. Zaheer et al. reported that PD98059 enhanced human rhinovirus-induced CXCL10 production in human airway epithelial cells [Zaheer et al., 2009]. Based on the results of our previous and present studies, we suggest that the ERK pathway negatively regulates CXCL10 production.

In conclusion, IL-29 enhanced CXCL10 production in human oral epithelial cells via the p38 MAPK, STAT3, and NF-κB pathways, which might control Th1-cell accumulation in periodontal lesions and be involved in pathological processes in periodontal disease.

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Conflicts of interest

The authors confirm that they have no conflicts of interest.

Figure legends

Fig. 1. IL-10 receptor β and IL-28 receptor α expression in TR146 cells

The extracts of non-stimulated TR146 cells were subjected to SDS-PAGE. Western blot analysis with antibodies against IL-10 receptor β, IL-28 receptor α, and GAPDH was performed.

Fig. 2. The effect of IL-29 on CXCL10 production in TR146 cells

TR146 cells were incubated with human recombinant IL-29 (1, 10, or 100 ng/ml) for 24 hours, and then the supernatant was collected. The concentration of CXCL10 in each supernatant was measured using ELISA. The results are 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 cells

Fig. 3. Effect of IL-29 on CXCL10 production in TNF-α-stimulated TR146 cells

TR146 cells were stimulated with TNF-α (10 ng/ml) with or without IL-29 (1, 10, or 100 ng/ml). Their supernatants were collected after 24 hours. The concentrations of CXCL10 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 TNF-α-stimulated TR146 cells that were not treated with

IL-29

Fig. 4. Effects of signal transduction inhibitors on CXCL10 production in IL-29-stimulated TR146 cells

TR146 cells were incubated with SB203580 (10 μM), PD98059 (10 μM), SP600125 (10 μM), fludarabine (50 μM), WP1066 (5 μM), 10-DEBC hydrochloride (5 μM), or SC514 (10 μM) for 1 hour and then stimulated with human recombinant IL-29 (100 ng/ml). Their supernatants were collected after 24 hours. The concentrations of CXCL10 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-29-stimulated TR146 cells that were not pretreated with signal transduction inhibitors

Fig. 5. p38 MAPK, STAT3, and NF-κB p65 phosphorylation in TR146 cells treated with IL-29 with or without TNF-α

The cultured cells were treated with IL-29 (100 ng/ml) alone or IL-29 (100 ng/ml) and TNF-α (10 ng/ml) for 15, 30, or 60 min. The cells were lysed in lysis buffer containing protease inhibitors, and the phosphorylation of p38 MAPK, STAT3, and NF-κB p65 was assessed using Western blot analysis. Representative Western blot data that indicates the phospho-p38 MAPK, total p38 MAPK, phospho-STAT3, total STAT3, phospho-NF-κB p65, total NF-κB p65, and GAPDH levels detected in the TR146 cells during three independent experiments are shown.

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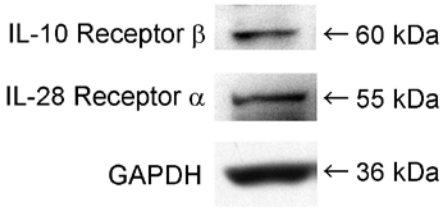

Fig. 1

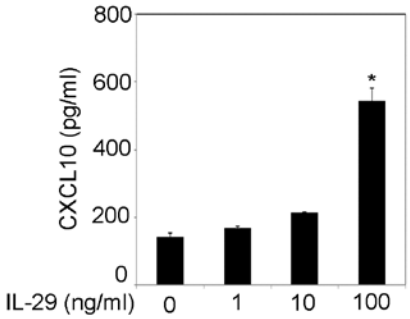

Fig. 2

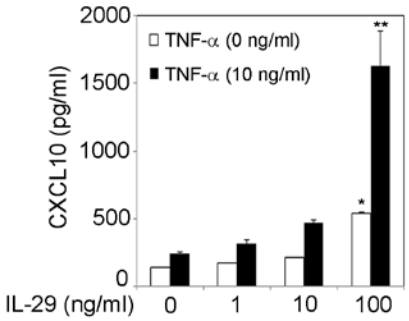
2000
1500
1000
500
0
CXCL10 (pg/ml)
TNF-α (0 ng/ml)
TNF-α (10 ng/ml)
*
**
IL-29 (ng/ml)
0
1
10
100

Fig. 3

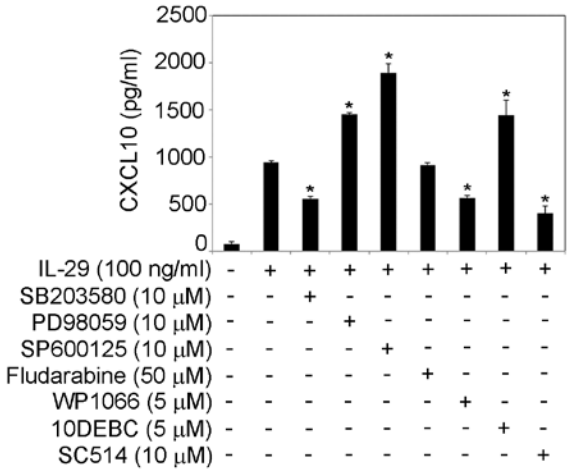
2500
2000
1500
1000
500
0
CXCL10 (pg/ml)
IL-29 (100 ng/ml) - + + + + + + + +
SB203580 (10 μM) - - + - - - - - -
PD98059 (10 μM) - - - + - - - - -
SP600125 (10 μM) - - - - + - - - -
Fludarabine (50 μM) - - - - - + - - -
WP1066 (5 μM) - - - - - - + - -
10DEBC (5 μM) - - - - - - - + -
SC514 (10 μM) - - - - - - - - +

Fig. 4

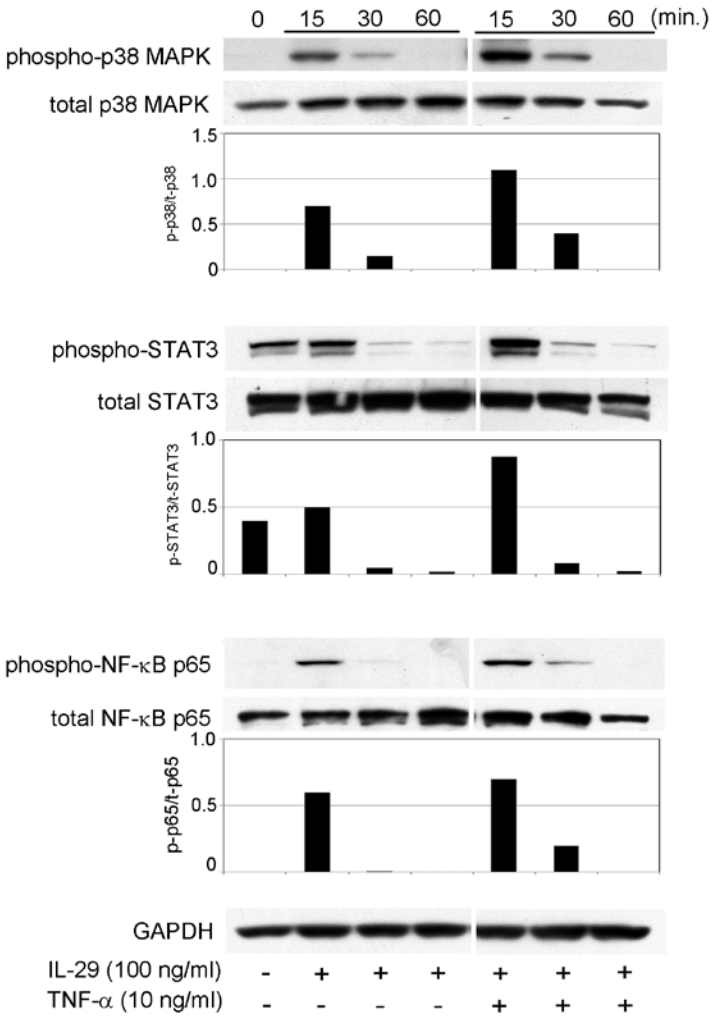
0 15 30 60 15 30 60 (min.)
phospho-p38 MAPK
total p38 MAPK
p-p38/t-p38
1.5
1.0
0.5
0
phospho-STAT3
total STAT3
p-STAT3/t-STAT3
1.0
0.5
0
phospho-NF-κB p65
total NF-κB p65
p-p65/t-p65
1.0
0.5
0
GAPDH
IL-29 (100 ng/ml) - + + + + + +
TNF-α (10 ng/ml) - - - - + + +

Fig. 5