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Oncostatin M Synergistically Induces CXCL10 and ICAM-1 Expression in IL-1β-Stimulated-Human Gingival Fibroblasts

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ABSTRACT

Periodontitis is a chronic bacterial infection of tooth-supporting structures. T-helper type 1 (Th1) cells are related to the exacerbation of periodontal disease. Human gingival fibroblasts (HGFs), the major cell type in periodontal connective tissues, are involved in immunological response in periodontal tissues. However, it is uncertain whether HGFs are related to Th1 response. Chemokine (C-X-C motif) ligand 10 (CXCL10) is a cytokine, that is related to Th1 cells migration. Intercellular adhesion molecule (ICAM)-1 is involved in Th1 cells retention and activation in inflamed tissue. The aim of this study is to examine the effect of oncostatin M (OSM) on CXCL10 and ICAM-1 expression in HGFs. OSM stimulation induced CXCL10 and ICAM-1 expression in HGFs. Moreover, the synergistic effects of CXCL10 release and ICAM-1 expression in HGFs were observed with combined stimulation of interleukin (IL)-1β and OSM. OSM increased type 1 IL-1 receptor (IL-1R1) expression, and IL-1β enhanced OSMRβ expression on HGFs. IL-1β + OSM stimulation enhanced the phosphorylation of inhibitor of nuclear factor κB (IκB)-α, signal transducer and activator of transcription (STAT)3, c-Jun N terminal kinase (JNK), and protein kinase B (Akt) compared to OSM or IL-1β stimulation. CXCL10 production from OSM + IL-1β stimulated HGFs was suppressed by nuclear factor (NF)-κB, STAT3, JNK, and phosphoinositide-3-kinase (PI3K) inhibitors. On the other hand, only NF-κB and STAT3 inhibitors suppressed ICAM-1 expression enhanced by OSM + IL-1β treatment. These effects of OSM and IL-1β may promote Th1 cells infiltration and retention in periodontally diseased tissues and be related to exacerbation of periodontal disease. J. Cell. Biochem. 111: 40–48, 2010.

KEY WORDS: ONCOSTATIN M; CXCL10; ICAM-1; IL-1β; HUMAN GINGIVAL FIBROBLASTS

Periodontitis is a chronic bacterial infection of tooth-supporting structures. It causes destruction of periodontal connective tissues and bone. Oral plaque bacteria including *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Tannerella forsythensis* are recognized as etiologic agents in periodontitis. The disease initiation and progression result from host response to plaque bacteria. Immunohistochemical studies reveal dense inflammatory cells infiltration, including T and B cells and macrophages in periodontitis region [Seymour, 1991; Fujihashi et al., 1993; Page et al., 1997; Hosokawa et al., 2002, 2005; Johnson et al., 2004]. Recently, several studies demonstrated that T-helper type 1 (Th1) cells are involved in bone resorption in oral cavity. Kawai et al. [2000] reported that local *Actinobacillus actinomycetemcomitans* 29-kDa outer membrane protein and lipopolysaccaride (LPS) activation of Th1-type T cells appeared to trigger inflammatory periodontal bone resorption. Stashenko et al. [2007] reported that intrapulpal challenge with viable *Porphyromonas gingivalis* results in massive periapical bone destruction in systemic Th1 response. However, the effects of OSM on Th1 response in periodontal disease is uncertain.

Oncostatin M (OSM) belongs to the interleukin (IL)-6 family of cytokines, which includes IL-6, leukemia-inhibitory factor (LIF), IL-11, cardiotrophin-1 (CT-1), and ciliary neurotrophic factor [Tanaka and Miyajima, 2003]. The IL-6 family of cytokine receptors

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requires dimerization with glycoprotein 130 (gp130), a glycoprotein cell surface receptor, for intracellular signaling. OSM as well as several members of the IL-6 cytokine family are known to activate fibroblasts and to regulate the synthesis of matrix metalloproteinases and their inhibitors in these cells [Korzus et al., 1997; Langdon et al., 1997; Weiss et al., 2005]. In addition, the results of studies in the human, rat, and mouse have supported the notion that OSM is involved uniquely in the regulation of inflammation [Grenier et al., 2001; Hurst et al., 2002; Langdon et al., 2003]. OSM is primarily produced in and released by activated monocytes, T lymphocytes, and neutrophils [Brown et al., 1987; Grove et al., 1991; Sodhi et al., 1997], and it is found in a variety of inflammatory sites. In the human lung during acute lung injury, infiltrating neutrophils secrete OSM. OSM levels also are elevated in the sera of patients with rheumatoid arthritis [Okamoto et al., 1997] as well as in patients with inflamed skin [Tamura et al., 2002] and periodontitis [Lin et al., 2005]. In addition, in vitro studies have demonstrated that OSM not only regulates the remodeling function of fibroblasts but also elicits inflammatory responses in these cells. OSM induces the CC chemokines eotaxin (an eosinophil chemoattractant) and monocyte chemoattractant protein 1 in mouse lung and in synovial fibroblasts, respectively. Moreover, overexpression of OSM in the mouse lung results in increased recruitment of eosinophils. Altogether, these studies suggest that OSM regulates inflammatory function in fibroblasts and that fibroblasts may be implicated in the recruitment of leukocytes upon activation by OSM.

Gingival fibroblasts, the major cell type in periodontal connective tissues, provide tissue framework for tooth anchorage. Until recently, they were presumed to be immunologically inert. Currently, however, researchers recognize their active role in host defense. Upon stimulation with cytokines as well as with bacterial pathogens, human gingival fibroblasts secret various soluble mediators of inflammation such as IL-1β, IL-6, and IL-8 [Takashiba et al., 1992; Sakuta et al., 1998; Daghigh et al., 2002; Wang et al., 2003]; and up-regulate expression of intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1 [Shimabukuro et al., 1996]. These fibroblasts-derived mediators and surface antigens are thought to play an important role in periodontal inflammatory response.

The CXC chemokine CXCL10 was discovered as an IFN-γ-inducible protein of 10 kDa in the monocytic U937 cells [Yen and Chen, 1998] CXCL10 attracts activated Th1 cells through interaction with CXC chemokine receptor 3 (CXCR3) [Yang et al., 1998; Katiyar et al., 1999]. CXCL10 shares this receptor and hence biological activity with two more recently identified CXC chemokines, CXCL9, and CXCL11 [Luster et al., 1985; Rossi and Zlotnik, 2000; Crouvezier et al., 2001]. In vivo, enhanced levels of CXCL10 have been reported in several inflammatory diseases that are predominantly associated with a Th1 phenotype. It is reported that CXCL10 and CXCR3 are detected in inflamed gingival tissues [Farber, 1993; Loetscher et al., 1996]. However, it is unknown whether HGFs are related to CXCL10 production in inflamed gingival tissues.

The aim of this study was to examine the effect of OSM on Th1 type chemokines CXCL10 production and ICAM-1 expression by HGFs. Moreover, we examined the effects of OSM in combination with IL-1β on CXCL10 and ICAM-1 expression by HGFs, because IL-1β is an important inflammatory cytokine in periodontally diseased tissues.

MATERIALS AND METHODS

GINGIVAL TISSUE BIOPSIES AND CELL CULTURE

We used HGFs isolated from three clinically healthy gingiva during routine distal wedge surgical procedures. Gingival specimens were cut into small pieces and transferred to culture dishes. The HGFs that grew from the gingivae were primarily cultured on 100 mm^2 uncoated plastic dishes in Dulbecco's modified Eagle medium (DMEM; Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY) and antibiotics (penicillin G; 100 U/ml, streptomycin; 100 mg/ml) at 37°C in humidified air with 5% CO_2. Confluent cells were transferred and cultured for use in the present study. After three to four subcultures by trypsinization, the cultures contained homogeneous, slim and spindle-shaped cells growing in characteristic swirls. The cells were used for experiments after five passages. In selected experiments, HGFs were cultured for 1 h in the presence or in the absence of SB203580 (20 μM: Santa Cruz Biotechnology), PD98059 (20 μM: Santa Cruz Biotechnology), SP600125 (20 μM; Sigma), LY294002 (20 μM; Calbiochem, La Jolla, CA), SC514 (20 μM, Calbiochem), or AG490 (20 μM, Calbiochem) prior to incubation with the various stimuli. Informed consent was obtained from all subjects participating in this study. The study was performed with the approval and compliance of the University of Tokushima Ethical Committee.

CYTOKINE PRODUCTION BY HGFs

HGFs were stimulated with 0.1, 1, 10, or 100 ng/ml OSM (Peprotech, Rocky Hill, NJ) or 10 ng/ml IL-1β (Peprotech) for 24 h. The supernatants from HGFs were collected. To collect cell lysates, HGFs stimulated with OSM for 24 h were washed once with cold PBS, followed by incubation on ice for 30 min with lysis buffer (Cell Signaling Technology, Danvers, MA) supplemented with protease inhibitors (Sigma). After removal of debris by centrifugation, the protein concentrations of the lysates were quantified with the Bradford protein assay using IgG as a standard. The concentrations of CXCL10 in the culture supernatant or cell lysates were measured in triplicate by ELISA (R&D systems, Minneapolis, MN). All assays were performed according to the manufacturer's instructions, and cytokine levels in the supernatants were determined using a standard curve prepared for each assay.

FLOW CYTOMETRIC ANALYSES

Following the required time in culture, the cells were washed twice with ice-cold PBS. HGFs were harvested by incubation with PBS-4 mmol/L EDTA. Most of the cells could be removed by gentle agitation after this treatment. Any cells that failed to detach were removed with gentle scraping. The cells were washed twice with ice-cold PBS and incubated (20 min on ice) in PBS-1%BSA. The cells were incubated with mouse anti-human OSMRβ antibody (Santa Cruz Biotechnology, 5 μg/ml), mouse anti-human ICAM-1 antibody (Sigma, 5 μg/ml), rat anti-human IL-1 Receptor type 1 (IL-1R1) antibody (PBL Biomedical Laboratories, New Brunswick, NJ) or an isotype control antibody on ice for 30 min. After washing three

times with PBS-1% bovine serum albumen (BSA; Sigma), the cells were incubated with the FITC-conjugated rabbit anti-mouse F $(ab')_2$ fragment (DAKO, Kyoto, Japan) or FITC-goat anti-rat IgG(H + L) Conjugate (ZYMED Laboratories, South San Francisco, CA) for 30 min on ice. After washing three times with PBS-1% BSA, the cells were immediately analyzed with flow cytometry (Epics XL-MCL; Coulter, Hialeah, FL).

WESTERN BLOT ANALYSIS

To confirm ICAM-1 production and OSM and/or IL-1β-induced phosphorylation of signal transduction molecules, Western blot analysis was performed. HGFs stimulated with OSM and/or IL-1β were washed once with cold PBS, followed by incubation on ice for 30 min with lysis buffer (Cell Signaling Technology) supplemented with protease inhibitors (Sigma). After removal of debris by centrifugation, the protein concentrations of the lysates were quantified with the Bradford protein assay using IgG as a standard. Protein (20 μg) was loaded onto a 4–20% SDS-PAGE gel, followed by electrotransfer to a PVDF membrane. Expression of ICAM-1 and activations of inhibitor of nuclear factor κB (IκB)-α, signal transducer and activator of transcription (STAT)3, p38 mitogen-activated protein kinase (MAPK), p44/42 MAPK (ERK), c-Jun N

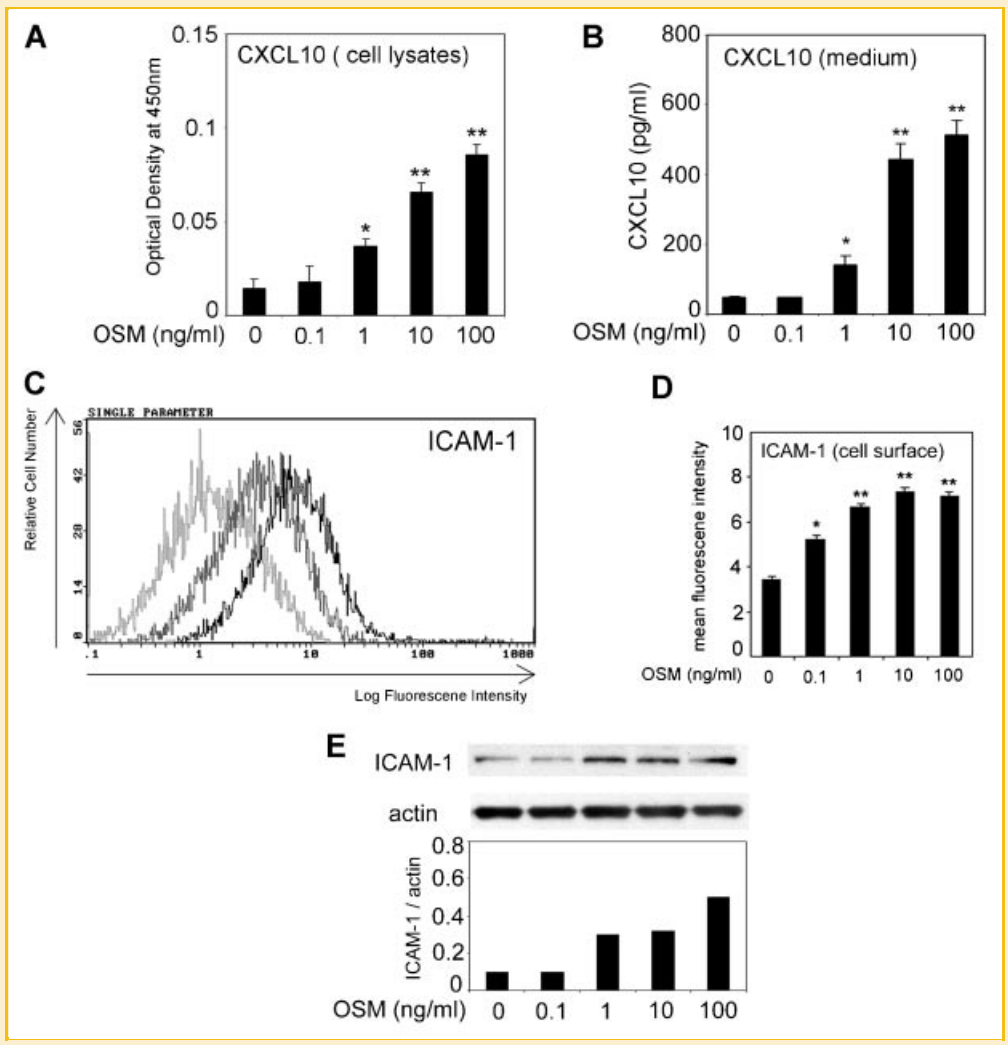

Fig. 1. OSM induced CXCL10 and ICAM-1 expression by HGFs. A: HGFs were treated with OSM (0.1, 1, 10 or 100 ng/ml), and the cell lysates were collected after 24 hours. The expression levels of CXCL10 in the cell lysates were measured using ELISA. Data are representative of three different HGFs samples from three different donors. The results were calculated as the mean and SD of one representative experiment performed in triplicates. Error bars show the SD of the values. B: HGFs were treated with OSM (0.1, 1, 10 or 100 ng/ml), and the supernatants were collected after 24 hours. The expression levels of CXCL10 in the supernatants were measured using ELISA. Data are representative of three different HGFs samples from three different donors. The results were calculated as the mean and SD of one representative experiment performed in triplicates. Error bars show the SD of the values. C: HGFs were treated with OSM (10 ng/ml) and the cells were collected after 24 hours. The expression level of ICAM-1 on the surface of HGFs was measured using flow cytometry. Light gray line represents isotype-control antibody. Dark gray line represents ICAM-1 expression on non-stimulated HGFs. Black line represents ICAM-1 expression on OSM (10 ng/ml)-stimulated HGFs. D: HGFs were treated with OSM (0.1, 1, 10 or 100 ng/ml) and, the cells were collected after 24 hours. The expression levels of ICAM-1 on the surface of HGFs were measured using flow cytometry. The data are representative of three different HGFs samples from three different donors. The results were calculated as mean and SD of one representative experiment performed in triplicate. Error bars show the SD of the values. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from the medium. E: HGFs were treated with OSM (0.1, 1, 10 or 100 ng/ml), and the cell lysates were collected after 24 hours. The cell lysates were subjected to SDS-PAGE followed by western blotting analysis with antibodies against ICAM-1 or actin. Bar graphs of ICAM-1 expression was normalized to actin.

terminal kinase (JNK), and protein kinase B (Akt), and were assessed using anti-human ICAM-1 mouse monoclonal antibody (Biolegend, San Diego, CA), phospho-IκB-α (Ser32/36) mouse monoclonal antibody (Cell Signaling Technology), phospho-STAT3 (Tyr705) mouse monoclonal antibody (Cell Signaling Technology), phospho-STAT3 (Ser727) rabbit monoclonal antibody (Cell Signaling Technology), phospho-p38 MAPK (Thr180/Tyr182) rabbit monoclonal antibody (Cell Signaling Technology), phospho-p44/42 MAPK (Thr202/Tyr204) rabbit monoclonal antibody (Cell Signaling Technology), phospho-SAPK/JNK (Thr183/Tyr185)(81E11) rabbit monoclonal antibody (Cell Signaling Technology), phospho-Akt (Ser473)(193H12) rabbit monoclonal antibody (Cell Signaling Technology), IκB-α mouse monoclonal antibody (Cell Signaling Technology), STAT3 mouse monoclonal antibody (Cell Signaling Technology), p38 MAPK rabbit monoclonal antibody (Cell Signaling Technology), p44/42 MAPK rabbit monoclonal antibody (Cell Signaling Technology), SAPK/JNK rabbit monoclonal antibody (Cell Signaling Technology), or pan-Akt rabbit antibody (Cell Signaling Technology), according to the manufacturer's instructions. Protein bands were visualized by incubation with the HRP-conjugated secondary antibody (Sigma), followed by detection using the ECL system (GE Healthcare, Uppsala, Sweden). The quantitation of the chemiluminescent signal was analyzed using NIH image.

STATISTICAL ANALYSIS

The statistical significance was analyzed using Student's *t* test. *P* values <0.05 were considered significant.

RESULTS

THE EFFECT OF OSM ON CXCL10 AND ICAM-1 PRODUCTION IN HGFs

Since we previously reported cultured HGFs express OSM receptor [Hosokawa et al., 2010], we then investigated whether OSM could stimulate HGFs. We examined CXCL10 and ICAM-1 production in HGFs because CXCL10 and ICAM-1 is related to Th1 cells infiltration and retention in inflamed tissues. Figure 1 shows OSM treatment enhanced CXCL10 production (Fig. 1A) and release (Fig. 1B) from HGFs in a dose dependent manner. Moreover, OSM increased ICAM-1 expression on HGFs (Fig. 1C,D) and ICAM-1 production in HGFs (Fig. 1E) in a dose dependent manner.

OSM SYNERGISTICALLY INDUCED CXCL10 PRODUCTION AND ICAM-1 EXPRESSION BY IL-1β-STIMULATED HGFs

It is known that IL-1β is one of the pathogenic cytokine in periodontally diseased tissues [Graves and Cochran, 2003]. Therefore, we examined the effects of IL-1β in combination with OSM on CXCL10 and ICAM-1 expression on HGFs. OSM synergistically enhanced CXCL10 and ICAM-1 expression induced by IL-1β treatment by HGFs (Fig. 2).

THE EFFECT OF OSM ON IL-1R1 EXPRESSION BY HGFs

We hypothesized OSM might enhance IL-1β receptor expression because OSM enhance Th1 chemokine production and ICAM-1 expression induced by IL-1β treatment. As shown Figure 3, OSM treatment enhanced IL-1R1 expression by HGFs in a concentration-dependent manner.

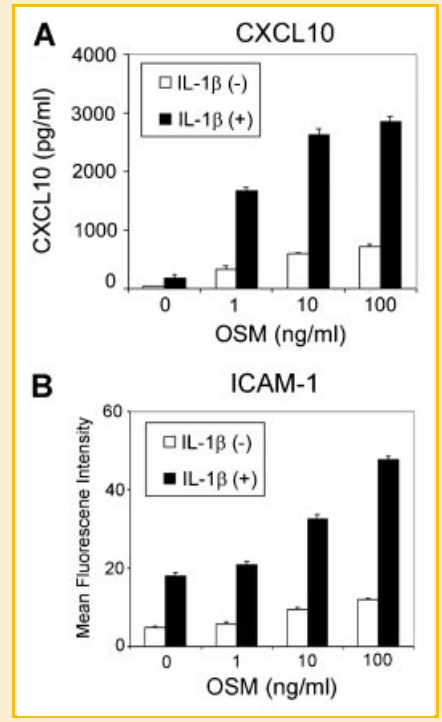

Fig. 2. OSM synergistically enhanced CXCL10 and ICAM-1 expression in IL-1β-stimulated HGFs. A: HGFs were treated with OSM (1, 10, or 100 ng/ml) with or without IL-1β (10 ng/ml), and the supernatants were collected after 24 h. The expression levels of CXCL10 in the supernatants were measured using ELISA. Data are representative of three different HGFs samples from three different donors. The results were calculated as the mean and SD of one representative experiment performed in triplicates. Error bars show the SD of the values. B: HGFs were treated with OSM (1, 10, or 100 ng/ml) with or without IL-1β (10 ng/ml), the cells were collected after 24 h. The expression levels of ICAM-1 on the surface of HGFs were measured using flow cytometry. The data are representative of three different HGFs samples from three different donors. The results were calculated as mean and SD of one representative experiment performed in triplicate. Error bars show the SD of the values.

THE EFFECT OF IL-1β ON OSMRβ EXPRESSION BY HGFs

We also hypothesized IL-1β might enhance OSM receptor expression. Therefore, we examined the effect of IL-1β on OSMRβ expression by HGFs. As shown Figure 4A,B, IL-1β slightly enhanced OSMRβ expression on HGFs.

IL-1β IN COMBINATION WITH OSM SYNERGISTICALLY ENHANCED OSMRβ EXPRESSION

We next examined whether IL-1β in combination with OSM regulate OSMRβ expression, because both cytokines certainly exist in this situation. Figure 4C shows that IL-1β synergistically enhanced OSMRβ expression induced by OSM treatment.

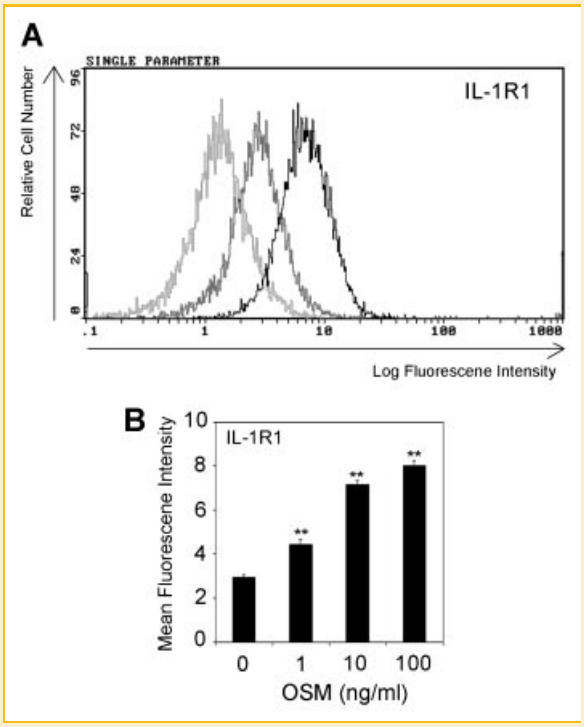

Fig. 3. OSM enhanced IL-1R1 expression on HGFs. A: HGFs were treated with OSM (10 ng/ml) and the cells were collected after 24 h. The expression level of IL-1R1 on the surface of HGFs was measured using flow cytometry. Light gray line represents isotype-control antibody. Dark gray line represents IL-1R1 expression on non-stimulated HGFs. Black line represents IL-1R1 expression on OSM (10 ng/ml)-stimulated HGFs. B: HGFs were treated with OSM (1, 10, or 100 ng/ml) and, the cells were collected after 24 h. The expression levels of IL-1R1 on the surface of HGFs were measured using flow cytometry. The data are representative of three different HGFs samples from three different donors. The results were calculated as mean and SD of one representative experiment performed in triplicate. Error bars show the SD of the values. $^{**}P < 0.01$ significantly different from the medium.

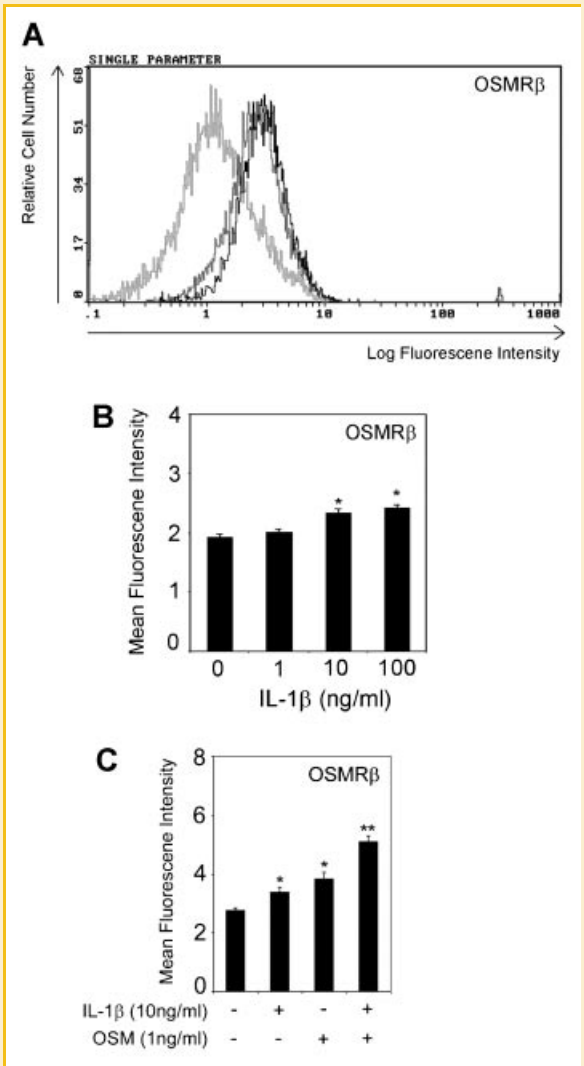

Fig. 4. IL-1β enhanced OSMRβ expression on HGFs. A: HGFs were treated with IL-1β (10 ng/ml) and the cells were collected after 24 h. The expression level of OSMRβ on the surface of HGFs was measured using flow cytometry. Light gray line represents isotype-control antibody. Dark gray line represents OSMRβ expression on non-stimulated HGFs. Black line represents OSMRβ expression on IL-1β (10 ng/ml)-stimulated HGFs. B: HGFs were treated with IL-1β (1, 10, or 100 ng/ml) and, the cells were collected after 24 h. The expression levels of OSMRβ on the surface of HGFs were measured using flow cytometry. The data are representative of three different HGFs samples from three different donors. The results were calculated as mean and SD of one representative experiment performed in triplicate. Error bars show the SD of the values. $^{*}P < 0.05$ significantly different from the medium. C: HGFs were treated with IL-1β (10 ng/ml) with or without OSM (1 ng/ml) and, the cells were collected after 24 h. The expression levels of OSMRβ on the surface of HGFs were measured using flow cytometry. The data are representative of three different HGFs samples from three different donors. The results were calculated as mean and SD of one representative experiment performed in triplicate. Error bars show the SD of the values. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from the medium.

EFFECTS OF OSM AND IL-1β TREATMENT ON NF-κB ACTIVATION IN HGFs

We next examined effects of OSM and IL-1β treatment on NF-κB activation in HGFs because NF-κB pathway is involved in chemokine or adherent molecules expression in various cells. OSM in combination with IL-1β enhanced IκB-α phospholyration and degradation in HGFs compared to OSM or IL-1β stimulation (Fig. 5).

EFFECTS OF OSM AND IL-1β TREATMENT ON STAT3 ACTIVATION IN HGFs

We next examined STAT3 activation in OSM and/or IL-1β stimulated HGFs because OSM is the activator of STAT3 pathway. OSM stimulation induced phosphorylation of both tyrosine and serine STAT3 phosphorylation in HGFs. On the other hand, IL-1β stimulation did not modulate STAT3 activation. However, IL-1β treatment enhanced serine STAT3 phosphorylation in OSM-treated HGFs (Fig. 6).

EFFECTS OF OSM AND IL-1β TREATMENT ON MAPKs AND Akt ACTIVATION IN HGFs

We next examined effects of OSM and IL-1β treatment on MAPKs and Akt activation in HGFs. OSM in combination with IL-1β did not

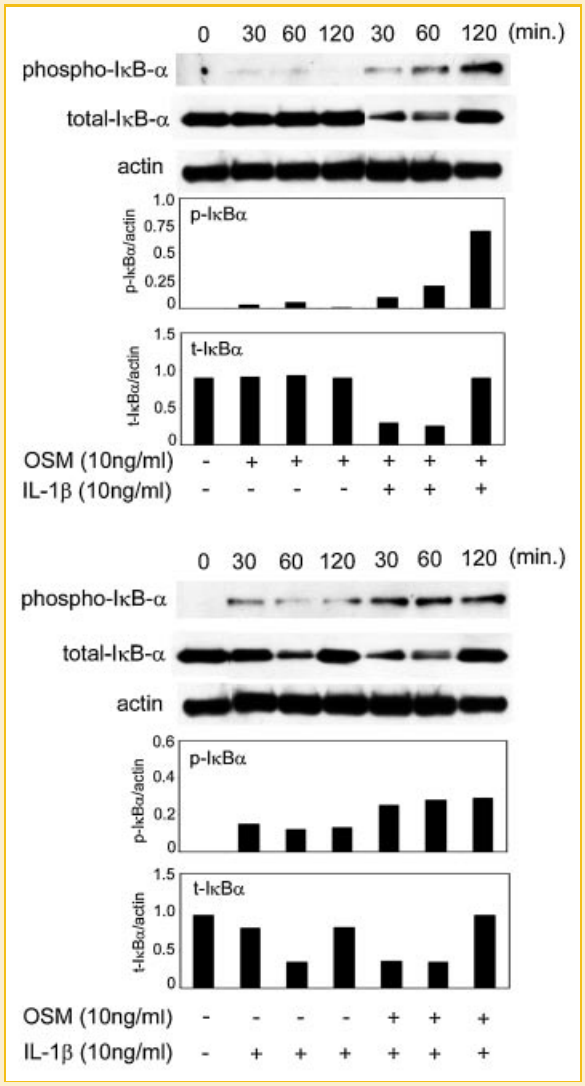

Fig. 5. Effects of OSM and IL-1β on phosphorylation and degradation of IκB-α in HGFs. The cultured cells were stimulated with 10 ng/ml OSM and/or 10 ng/ml IL-1β for 15, 30, or 60 min. The cell extracts were subjected to SDS-PAGE followed by Western blotting analysis with antibodies against phospho-specific IκB-α, IκB-α, or actin. Bar graphs of phospho-IκB-α or total-IκB-α expressions were normalized to actin.

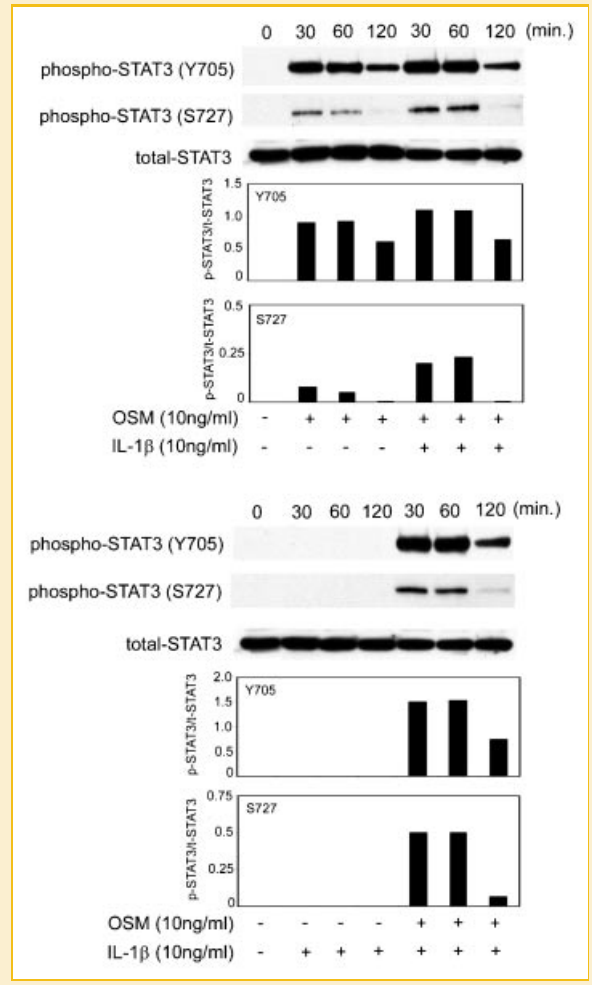

Fig. 6. Effects of OSM and IL-1β on phosphorylation of STAT3 in HGFs. The cultured cells were stimulated with 10 ng/ml OSM and/or 10 ng/ml IL-1β for 15, 30, or 60 min. The cell extracts were subjected to SDS-PAGE followed by Western blotting analysis with antibodies against tyrosine phospho-specific STAT3, serine phospho-specific STAT3, or STAT3. Bar graphs of phospho-STAT3 expressions were normalized to total-STAT3.

modulate p38 MAPK and ERK activation in OSM or IL-1β-treated HGFs. On the other hand, OSM and IL-1β stimulation enhanced JNK and Akt phosphorylation in HGFs compared to HGFs treated with OSM or IL-1β stimulation (Fig. 7).

EFFECTS OF SIGNAL INHIBITORS ON CXCL10 OR ICAM-1 EXPRESSION IN OSM AND IL-1β-STIMULATED HGFs

Finally, we examined which signal pathway is involved in CXCL10 or ICAM-1 expression in OSM and IL-1β stimulated HGFs. CXCL10 production from OSM and IL-1β treated HGFs were inhibited by SB203580 (a p38 MAPK inhibitor), SP600125 (a JNK inhibitor), LY294002 (a PI3K inhibitor), SC514 (a NF-κB inhibitor), and AG490 (a STAT3 inhibitor). PD98059 (a MEK inhibitor) did not modulate CXCL10 production (Fig. 8). On the other hand, ICAM-1 expression on OSM and IL-1β-treated HGFs were suppressed by only NF-κB and STAT3 inhibitors.

DISCUSSION

In this study, we demonstrated that OSM can induce CXCL10 from HGFs. It has been reported that CXCL10 is related to the migration of Th1 cells, because Th1 cells preferentially express CXCR3 [Annunziato et al., 1998]. Moreover, Kawai et al. [2000] reported that Th1 cells are involved in bone resorption in periodontally diseased tissues. Therefore, OSM might be related to periodontal tissue destruction to induce CXCL10 production by HGFs. Moreover, we revealed OSM could enhance ICAM-1 expression on HGFs. Fearon et al. [2006] demonstrated that OSM induced ICAM-1 expression on endothelial cells. Their reports agree with our results. It has been reported that ICAM-1 antibody altered the

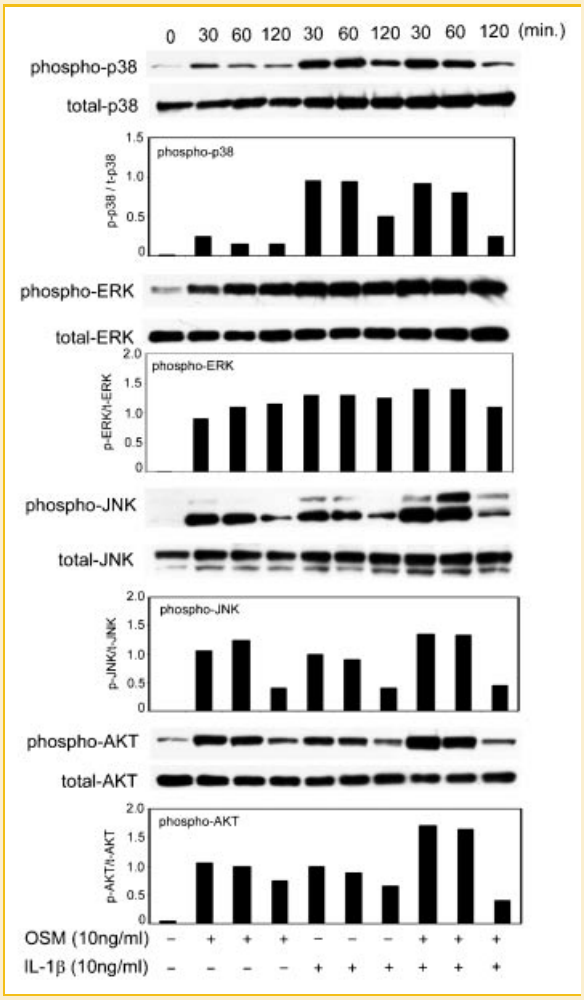

Fig. 7. Effects of OSM and IL-1β on p38 MAPK, ERK, JNK, and Akt phosphorylation in HGFs. The cultured cells were stimulated with 10 ng/ml OSM and/or 10 ng/ml IL-1β for 15, 30, or 60 min. The cell extracts were subjected to SDS-PAGE followed by Western blotting analysis with antibodies against phospho-specific p38 MAPK, p38 MAPK, phospho-specific ERK, ERK, phospho-specific JNK, JNK, phospho-specific Akt, and Akt. Bar graphs of phospho-p38 MAPK, phosphor-ERK, phosphor-JNK, or phosphor-Akt expressions were normalized to total-p38 MAPK, total-ERK, total-JNK, or total-Akt, respectively.

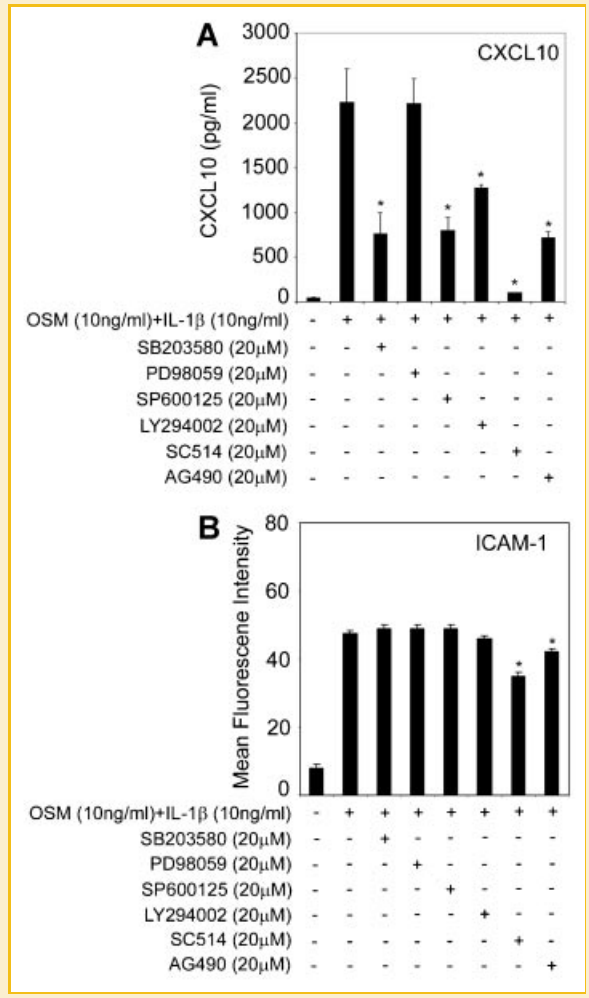

Fig. 8. Effects of signal transduction inhibitors on the OSM and IL-1β-stimulated CXCL10 and ICAM-1 expression by HGFs. A: Cells were pre-incubated with SB203580 (20 μM), PD98059 (20 μM), SP600125 (20 μM), LY294002 (20 μM), SC514 (20 μM), or AG490 (20 μM) for 1 h and then incubated with OSM (10 ng/ml) and IL-1β (10 ng/ml). After 24 h incubation, the supernatants were collected, and CXCL10 expression was measured by ELISA. Data are representative of HGFs from three different donors. The results were calculated as the mean and SD of one representative experiment performed in triplicate. The error bars show the SD of the values. $^{*}P < 0.05$ significantly different from the OSM and IL-1 β-stimulated HGFs without inhibitors. B: Cells were pre-incubated with SB203580 (20 μM), PD98059 (20 μM), SP600125 (20 μM), LY294002 (20 μM), SC514 (20 μM), or AG490 (20 μM) for 1 h and then incubated with OSM (10 ng/ml) and IL-1β (10 ng/ml). After 24 h incubation, cells were collected after 24 h. The expression levels of ICAM-1 on the surface of HGFs were measured using flow cytometry. The data are representative of three different HGFs samples from three different donors. The results were calculated as mean and SD of one representative experiment performed in triplicate. Error bars show the SD of the values. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from the OSM and IL-1β-stimulated HGFs.

recruitment of activated Th1-like cells into the synovium of arthritis [Schulze-Koops et al., 1995]. Therefore, ICAM-1 induced by OSM might be related to Th1 cells infiltration in periodontally diseased tissues.

In this report, we demonstrated that OSM synergistically enhanced IL-1β-induced CXCL10 production and ICAM-1 expression by HGFs. Some researchers reported that the synergistic effects between OSM and IL-1β [Repovic et al., 2003; Faffe et al., 2005]. Faffe et al. [2005] reported that OSM enhanced VEGF production by IL-1β-stimulated human airway smooth muscle cells. Repovic et al. [2003] demonstrated that OSM striking synergy prostagrandin E2 production by astrocytes in combination with IL-1β. Our reports agree with theirs. Moreover, Faffe et al. [2005] also reported that OSM enhanced IL-1R1 expression by human airway smooth muscle cells. They explained the phenomenon might be related to synergistic effects between OSM and IL-1β. We also demonstrated that OSM could up-regulate IL-1R1 expression by HGFs. This result is similar with Faffe's report. Moreover, we showed IL-1β enhanced OSMRβ expression by HGFs. To our knowledge, this is

the first report that the effects of IL-1β on OSMRβ expression. This fact might be related to the synergy between OSM and IL-1β by HGFs.

In this study, we show OSM and IL-1β enhanced phosphorylation and degradation of IκB-α in HGFs compared to single stimulation of OSM or IL-1β. Kasza et al. [2002] previously reported that OSM and IL-1β strongly activated NF-κB in human astrocytes though the level of NF-κB activation was same as the single stimulation of IL-1β. Therefore, the synergistic effects of OSM and IL-1β on NF-κB activation might be seen in limited types of cells.

As shown previously, OSM can induce the activation of STAT3 in some types of cells [Boulton et al., 1995; Matthews et al., 2005]. In this report, we revealed OSM activated STAT3 in HGFs, though single stimulation of IL-1β could not activate STAT3. Moreover, we revealed STAT3 inhibitor reduced CXCL10 production and ICAM-1 expression by IL-1β + OSM-stimulated HGFs, though did not modulate CXCL10 production and ICAM-1 expression by IL-1β-stimulated HGFs. Wung et al. [2005] reported that the inhibition of STAT3 phosphorylation reduced ICAM-1 expression by IL-6-stimulated endothelial cells. Our results agree with theirs. There is no report on the role of STAT3 on CXCL10 production. However, McLoughlin et al. [2005] reported IL-6 selectively governs T cell infiltration by regulating CXCL10 secretion and CXCR3 expression on the $CD3^+$ infiltrate through analysis of mononuclear cell infiltration in WT and IL-6-deficient mice during peritoneal inflammation, and they explained STAT3 might be related to this fact because T cell migration was related to STAT3 activity, because monoallelic deletion of *Stat3* in $gp130^{Y757F/Y757F}$ mice ($gp130^{Y757F/Y757F}$:$Stat3^{+/-}$) corrected the exaggerated responses observed in $gp130^{Y757F/Y757F}$ mice that is presenting hyperactivation of STAT3. Our in vitro results might testify their in vivo results.

In this report, we show that OSM + IL-1β stimulation enhanced JNK and Akt phosphorylation in HGFs compared with the OSM or IL-1β stimulation. It has been reported OSM or IL-1β stimulation can induce the phosphorylation of JNK [O'Neill and Greene, 1998; Chattopadhyay et al., 2007]. However, no one examined effects of OSM + IL-1β stimulation on the activation of JNK. Therefore, this is the first report that OSM + IL-1β stimulation strongly enhanced phosphorylation of JNK. It has been reported that Akt is involved in collagenase expression in OSM + IL-1β-stimulated cells [Litherland et al., 2008]. However, they did not examine the phosphorylation of Akt. We showed that Akt is related to CXCL10 production in OSM + IL-1β-stimulated HGFs in this report.

In summary, the current study demonstrates that OSM causes CXCL10 release and ICAM-1 expression by cultured HGFs. OSM also synergizes with IL-1β to increase CXCL10 and ICAM-1, at least in part, as a result of OSM-induced increases of IL-1R1 on HGFs. We speculate that IL-1β-induced increases in OSMRβ expression may also contribute. Moreover, our data suggest that these effects of OSM are related to the activation of NF-κB, STAT3, JNK, and Akt. The observation that OSM has the capacity to drive and amplify production of Th1 chemokines and ICAM-1 by HGFs, related to Th1 cells migration into periodontally diseased tissues, indicates that OSM could contribute to the destruction of periodontal tissues in periodontal disease, and OSM might be a target for periodontal disease treatment.

REFERENCES

Annunziato F, Galli G, Cosmi L, Romagnani P, Manetti R, Maggi E, Romagnani S. 1998. Molecules associated with human Th1 or Th2 cells. Eur Cytokine Netw 9:12–16.

Boulton TG, Zhong Z, Wen Z, Darnell JE, Jr., Stahl N, Yancopoulos GD. 1995. STAT3 activation by cytokines utilizing gp130 and related transducers involves a secondary modification requiring an H7-sensitive kinase. Proc Natl Acad Sci USA 92:6915–6919.

Brown TJ, Lioubin MN, Marquardt H. 1987. Purification and characterization of cytostatic lymphokines produced by activated human T lymphocytes. Synergistic antiproliferative activity of transforming growth factor beta 1, interferon-gamma, and oncostatin M for human melanoma cells. J Immunol 139:2977–2983.

Chattopadhyay S, Tracy E, Liang P, Robledo O, Rose-John S, Baumann H. 2007. Interleukin-31 and oncostatin-M mediate distinct signaling reactions and response patterns in lung epithelial cells. J Biol Chem 282:3014–3026.

Crouvezier S, Powell B, Keir D, Yaqoob P. 2001. The effects of phenolic components of tea on the production of pro- and anti-inflammatory cytokines by human leukocytes in vitro. Cytokine 13:280–286.

Daghigh F, Borghaei RC, Thornton RD, Bee JH. 2002. Human gingival fibroblasts produce nitric oxide in response to proinflammatory cytokines. J Periodontol 73:392–400.

Faffe DS, Flynt L, Mellema M, Whitehead TR, Bourgeois K, Panettieri RA, Jr., Silverman ES, Shore SA. 2005. Oncostatin M causes VEGF release from human airway smooth muscle: Synergy with IL-1beta. Am J Physiol Lung Cell Mol Physiol 288:L1040–L1048.

Farber JM. 1993. HuMig: A new human member of the chemokine family of cytokines. Biochem Biophys Res Commun 192:223–230.

Fearon U, Mullan R, Markham T, Connolly M, Sullivan S, Poole AR, FitzGerald O, Bresnihan B, Veale DJ. 2006. Oncostatin M induces angiogenesis and cartilage degradation in rheumatoid arthritis synovial tissue and human cartilage cocultures. Arthritis Rheum 54:3152–3162.

Fujihashi K, Kono Y, Beagley KW, Yamamoto M, McGhee JR, Mestecky J, Kiyono H. 1993. Cytokines and periodontal disease: Immunopathological role of interleukins for B cell responses in chronic inflamed gingival tissues. J Periodontol 64:400–406.

Graves DT, Cochran D. 2003. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol 74:391–401.

Grenier A, Combaux D, Chastre J, Gougerot-Pocidalo MA, Gibert C, Dehoux M, Chollet-Martin S. 2001. Oncostatin M production by blood and alveolar neutrophils during acute lung injury. Lab Invest 81:133–141.

Grove RI, Mazzucco C, Allegretto N, Kiener PA, Spitalny G, Radka SF, Shoyab M, Antonaccio M, Warr GA. 1991. Macrophage-derived factors increase low density lipoprotein uptake and receptor number in cultured human liver cells. J Lipid Res 32:1889–1897.

Hosokawa Y, Nakanishi T, Yamaguchi D, Takahashi K, Yumoto H, Ozaki K, Matsuo T. 2002. Macrophage inflammatory protein 3alpha-CC chemokine receptor 6 interactions play an important role in CD4+ T-cell accumulation in periodontal diseased tissue. Clin Exp Immunol 128:548–554.

Hosokawa Y, Nakanishi T, Yamaguchi D, Nakae H, Matsuo T. 2005. Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, in periodontal diseased tissue. Clin Exp Immunol 139:506–512.

Hosokawa Y, Hosokawa I, Ozaki K, Nakanishi T, Nakae H, Matsuo T. 2010. Catechins inhibit CXCL10 production from oncostatin-M-stimulated human gingival fibroblasts. (in press).

Hurst SM, McLoughlin RM, Monslow J, Owens S, Morgan L, Fuller GM, Topley N, Jones SA. 2002. Secretion of oncostatin M by infiltrating neutrophils: Regulation of IL-6 and chemokine expression in human mesothelial cells. J Immunol 169:5244–5251.

Johnson RB, Wood N, Serio FG. 2004. Interleukin-11 and IL-17 and the pathogenesis of periodontal disease. J Periodontol 75:37–43.

Kasza A, Kiss DL, Gopalan S, Xu W, Rydel RE, Koj A, Kordula T. 2002. Mechanism of plasminogen activator inhibitor-1 regulation by oncostatin M and interleukin-1 in human astrocytes. J Neurochem 83:696–703.

Katiyar SK, Matsui MS, Elmets CA, Mukhtar H. 1999. Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. Photochem Photobiol 69:148–153.

Kawai T, Eisen-Lev R, Seki M, Eastcott JW, Wilson ME, Taubman MA. 2000. Requirement of B7 costimulation for Th1-mediated inflammatory bone resorption in experimental periodontal disease. J Immunol 164:2102–2109.

Korzus E, Nagase H, Rydell R, Travis J. 1997. The mitogen-activated protein kinase and JAK-STAT signaling pathways are required for an oncostatin M-responsive element-mediated activation of matrix metalloproteinase 1 gene expression. J Biol Chem 272:1188–1196.

Langdon C, Leith J, Smith F, Richards CD. 1997. Oncostatin M stimulates monocyte chemoattractant protein-1- and interleukin-1-induced matrix metalloproteinase-1 production by human synovial fibroblasts in vitro. Arthritis Rheum 40:2139–2146.

Langdon C, Kerr C, Tong L, Richards CD. 2003. Oncostatin M regulates eotaxin expression in fibroblasts and eosinophilic inflammation in C57BL/6 mice. J Immunol 170:548–555.

Lin SJ, Chen YL, Kuo MY, Li CL, Lu HK. 2005. Measurement of gp130 cytokines oncostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. Cytokine 30:160–167.

Litherland GJ, Dixon C, Lakey RL, Robson T, Jones D, Young DA, Cawston TE, Rowan AD. 2008. Synergistic collagenase expression and cartilage collagenolysis are phosphatidylinositol 3-kinase/Akt signaling-dependent. J Biol Chem 283:14221–14229.

Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, Baggiolini M, Moser B. 1996. Chemokine receptor specific for IP10 and mig: Structure, function, and expression in activated T-lymphocytes. J Exp Med 184:963–969.

Luster AD, Unkeless JC, Ravetch JV. 1985. Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. Nature 315:672–676.

Matthews VB, Knight B, Tirnitz-Parker JE, Boon J, Olynyk JK, Yeoh GC. 2005. Oncostatin M induces an acute phase response but does not modulate the growth or maturation-status of liver progenitor (oval) cells in culture. Exp Cell Res 306:252–263.

McLoughlin RM, Jenkins BJ, Grail D, Williams AS, Fielding CA, Parker CR, Ernst M, Topley N, Jones SA. 2005. IL-6 trans-signaling via STAT3 directs T cell infiltration in acute inflammation. Proc Natl Acad Sci USA 102:9589–9594.

Okamoto H, Yamamura M, Morita Y, Harada S, Makino H, Ota Z. 1997. The synovial expression and serum levels of interleukin-6 interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. Arthritis Rheum 40:1096–1105.

O'Neill LA, Greene C. 1998. Signal transduction pathways activated by the IL-1 receptor family: Ancient signaling machinery in mammals, insects, and plants. J Leukoc Biol 63:650–657.

Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. 1997. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. Periodontology 2000 14:216–248.

Repovic P, Mi K, Benveniste EN. 2003. Oncostatin M enhances the expression of prostaglandin E2 and cyclooxygenase-2 in astrocytes: Synergy with interleukin-1beta, tumor necrosis factor-alpha, and bacterial lipopolysaccharide. Glia 42:433–446.

Rossi D, Zlotnik A. 2000. The biology of chemokines and their receptors. Annu Rev Immunol 18:217–242.

Sakuta T, Tokuda M, Tamura M, Jimi E, Ikebe T, Koba T, Nagaoka S, Takada H. 1998. Dual regulatory effects of interferon-alpha, -beta, and -gamma on interleukin-8 gene expression by human gingival fibroblasts in culture upon stimulation with lipopolysaccharide from Prevotella intermedia, interleukin-1alpha, or tumor necrosis factor-alpha. J Dent Res 77:1597–1605.

Schulze-Koops H, Lipsky PE, Kavanaugh AF, Davis LS. 1995. Elevated Th1- or Th0-like cytokine mRNA in peripheral circulation of patients with rheumatoid arthritis. Modulation by treatment with anti-ICAM-1 correlates with clinical benefit. J Immunol 155:5029–5037.

Seymour GJ. 1991. Importance of the host response in the periodontium. J Clin Periodontol 18:421–426.

Shimabukuro Y, Murakami S, Okada H. 1996. Antigen-presenting-cell function of interferon gamma-treated human gingival fibroblasts. J Periodontal Res 31:217–228.

Sodhi A, Shishodia S, Shrivastava A. 1997. Cisplatin-stimulated murine bone marrow-derived macrophages secrete oncostatin M. Immunol Cell Biol 75:492–496.

Stashenko P, Goncalves RB, Lipkin B, Ficarelli A, Sasaki H, Campos-Neto A. 2007. Th1 immune response promotes severe bone resorption caused by Porphyromonas gingivalis. Am J Pathol 170:203–213.

Takashiba S, Takigawa M, Takahashi K, Myokai F, Nishimura F, Chihara T, Kurihara H, Nomura Y, Murayama Y. 1992. Interleukin-8 is a major neutrophil chemotactic factor derived from cultured human gingival fibroblasts stimulated with interleukin-1 beta or tumor necrosis factor alpha. Infect Immun 60:5253–5258.

Tamura S, Morikawa Y, Tanaka M, Miyajima A, Senba E. 2002. Developmental expression pattern of oncostatin M receptor beta in mice. Mech Dev 115:127–131.

Tanaka M, Miyajima A. 2003. Oncostatin M, a multifunctional cytokine. Rev Physiol Biochem Pharmacol 149:39–52.

Wang PL, Ohura K, Fujii T, Oido-Mori M, Kowashi Y, Kikuchi M, Suetsugu Y, Tanaka J. 2003. DNA microarray analysis of human gingival fibroblasts from healthy and inflammatory gingival tissues. Biochem Biophys Res Commun 305:970–973.

Weiss TW, Kvakan H, Kaun C, Zorn G, Speidl WS, Pfaffenberger S, Maurer G, Huber K, Wojta J. 2005. The gp130 ligand oncostatin M regulates tissue inhibitor of metalloproteinases-1 through ERK1/2 and p38 in human adult cardiac myocytes and in human adult cardiac fibroblasts: A possible role for the gp130/gp130 ligand system in the modulation of extracellular matrix degradation in the human heart. J Mol Cell Cardiol 39:545–551.

Wung BS, Hsu MC, Wu CC, Hsieh CW. 2005. Resveratrol suppresses IL-6-induced ICAM-1 gene expression in endothelial cells: Effects on the inhibition of STAT3 phosphorylation. Life Sci 78:389–397.

Yang F, de Villiers WJ, McClain CJ, Varilek GW. 1998. Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. J Nutr 128:2334–2340.

Yen GC, Chen HY. 1998. Scavenging effect of various tea extracts on superoxide derived from the metabolism of mutagens. Biosci Biotechnol Biochem 62:1768–1770.