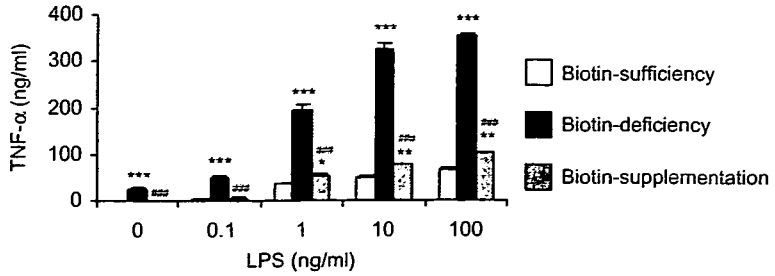

Fig. 2. TNF-α production of biotin-sufficient, -deficient and -supplemented J774.1 cells. Cells (2×10^5 cells/200 μl/well) were stimulated with LPS at 37°C for 24 h. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, compared with biotin-sufficiency. ###, $P < 0.001$, compared with biotin-deficiency

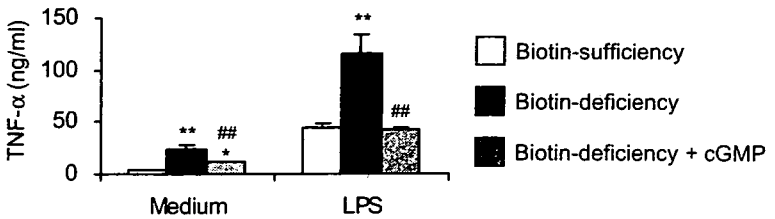

Fig. 3. Effects of cGMP on TNF-α production of biotin-deficient J774.1 cells. Cells (2×10^5cells/200 μl/well) were stimulated with 10 ng/ml of LPS in the presence of 1 mM of 8-Br-cGMP (cGMP) at 37°C for 24 h. * $P < 0.05$, ** $P < 0.01$, compared with biotin-sufficiency. ## $P < 0.01$, compared with biotin deficiency (without cGMP)

To further confirm the effects of biotin deficiency, J774.1 cells were cultured with biotin-deficient medium for 4 weeks and then further incubated with biotin-sufficient medium for 2 weeks (biotin-supplemented cells). The concentrations of TNF-α in the culture supernatants of biotin-supplemented cells with and without LPS stimulation were significantly ($P < 0.01$) reduced to near the levels in the supernatants of biotin-sufficient cells (Fig. 2). These results indicated that biotin-supplementation restored the TNF-α production to the basal level.

3.3 cGMP inhibits TNF-α production of biotin-deficient J774.1 cells

It was reported that cGMP is involved in the biotin-dependent mRNA expressions of holocarboxylase synthetase and biotin-dependent carboxylases [2]. Therefore, we analyzed the effects of cGMP to TNF-α production of biotin-deficient J774.1 cells. TNF-α production of biotin-deficient cells significantly ($P < 0.01$) decreased in the presence of 1 mM of cGMP (Fig. 3). These results indicated that up-regulation of TNF-α production was regulated by cGMP-dependent signaling pathway.

4 Conclusion

It is well known that biotin-deficiency causes cutaneous abnormalities, such as alopecia and scaly erythematous dermatitis [5]. In addition, it was reported that the biotin concentration in serum correlates with inflammatory diseases [5]. Although several studies reported the contribution of abnormalities in lipid metabolism to cutaneous abnormalities [5], the pathological mechanisms of disease conditions caused by biotin-deficiency remain to be clarified. In this study, we clearly demonstrated that biotin-deficiency up-regulated TNF-α production in vivo and in vitro. Moreover, biotin-supplementation inhibits TNF-α production of biotin-deficient cells. Therefore, we considered that the augmentable effect of biotin-deficiency on TNF-α production is reversible.

TNF-α plays important roles in the pathogenesis of atopic dermatitis [9], contact hypersensitivity [10], and pustulosis palmaris et plantaris [11], inflammatory diseases which have been reported to be correlated with biotin. Therefore, it is possible that TNF-α up-regulation caused by biotin-deficiency is involved in the pathological mechanisms of dermatitis and other inflammatory diseases. Our results encourage further investigations on biotin treatment for various inflammatory diseases.

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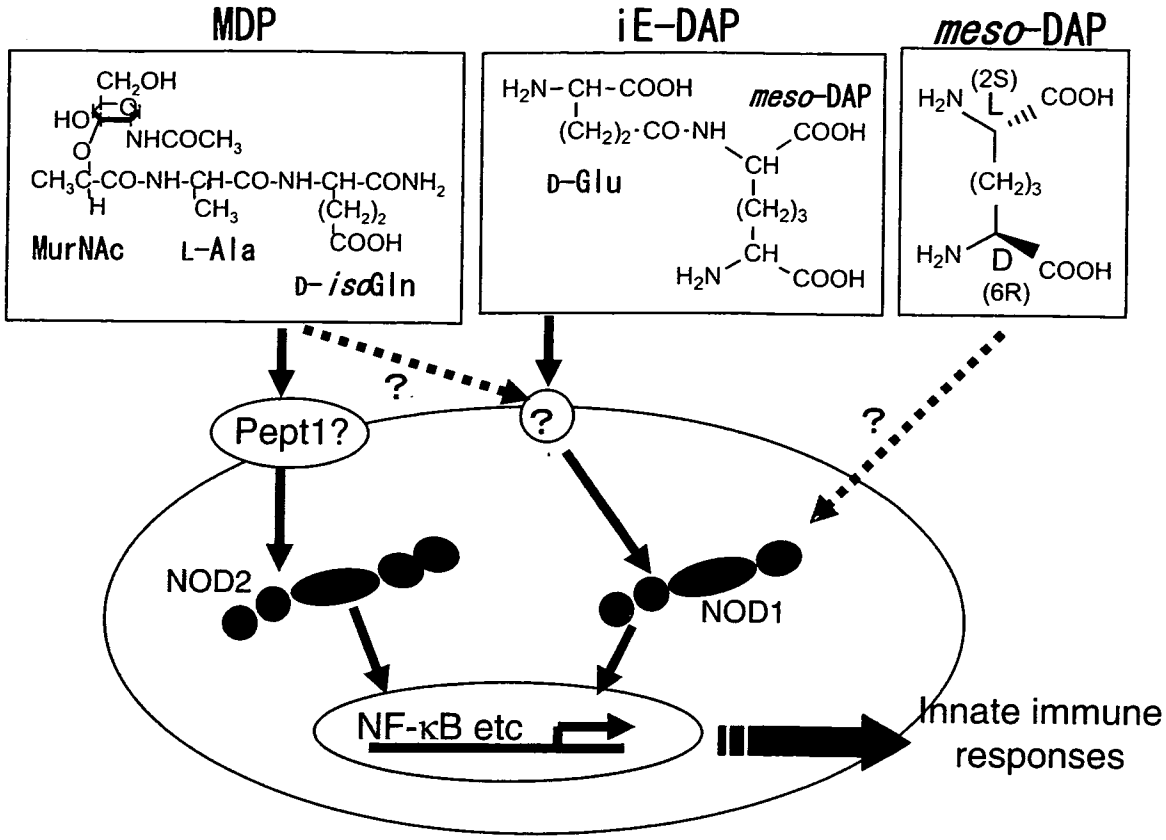

Fig. 1. Which is the minimum structure to activate NOD1? Lipofectamine and cytochalasin D increase the permeability of cells and allow *meso*-diaminopimelic acid (*DAP*) to be internalized into the cytosole. Therefore, even in monocytic cells *meso*-DAP was able to activate NOD1. A plasma membrane transporter PepT1 does not transport NOD1 ligand [6]. The unknown transporter should transport iE-DAP and other NOD1 ligands, but not *meso*-DAP. Therefore, the iE-DAP structure is required to activate NOD1 in most cells such as monocytic cells, whereas epithelial cells might allow *meso*-DAP to permeate the cells. The *meso*-DAP per se is generally capable of activating NOD1 intracellularly

β-defensin 2, and cytokines in specified cases, although the activities of *meso*-DAP were weaker than that of iE-DAP (Fig. 1). Stereoisomers of *meso*-DAP, LL-DAP and LL-DAP were only slightly activated or remained inactive. Synthetic *meso*-lanthionine, which is another PGN component in the specified bacteria such as *Fusobacterium nucleatum*, was also recognized by NOD1. In human monocytic cells, in the presence, but not in the absence, of Lipofectamine or cytochalasin D, *meso*-DAP induced slightly but significantly increased production of cytokines.

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Phagocytic macrophages do not contribute to the induction of serum IL-18 in mice treated with *Propionibacterium acnes* and lipopolysaccharide

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Abstract. Interleukin (IL)-18 is one of the inflammatory cytokine which is expressed not only in activated macrophages but also in non-immune cells, such as keratinocytes and epithelial cells. It is unclear which type of cell is the major source of serum IL-18. We showed that serum levels of IL-18 were increased in mice treated with *Propionibacterium acnes* and lipopolysaccharide (LPS), whereas, administration of clodronate-liposomes (Clo-lip) to induce depletion of macrophages showed no obvious effect on IL-18 levels. IL-18 levels were marginal in the liver, lung and spleen. Treatment with *P. acnes* alone induced IL-18 in each organ, and *P. acnes* and LPS induced increase in IL-18 levels in the liver and spleen, but decrease in the intestines. The administration of Clo-lip in mice showed only a marginal effect on the IL-18 levels in the organs. These results suggest that IL-18 expressed in keratinocytes/epithelial cells contributes to serum IL-18 levels.

Key words. IL-18, keratinocytes, phagocytic macrophages, *Propionibacterium acnes*, lipopolysaccharide

1 Introduction

Interleukin (IL)-18 was known to be originally identified as an interferon-γ (IFN-γ) inducing factor from a murine liver cell cDNA library, generated from mice primed with heat-killed *Propionibacterium acnes* and subsequently challenged with LPS, and to be intracellularly produced as an inactive 24-kDa precursor form (proIL-18) and secreted as an 18-kDa mature form after cleavage by caspase-1, originally designated IL-1β converting enzyme. IL-18 is now recognized as a multifunctional regulator of innate and acquired immune responses through its activation of T helper cell type 1 (Th1) and Th2 responses. A macrophage 'suicide' technique, using liposomes encapsulating dichloromethylene bisphosphonate (clodronate) specifically depletes phagocytic macrophages, but not dendritic cells (DC) or neutrophils, within a day or two of i.v. injection of such liposomes into mice or rats. In addition, Kawase et al. [1] generated Keratin 5/IL-18 transgenic (K5/IL-18 Tg)

mice in which mouse mature IL-18 cDNA was fused with the human K5 promoter. Human K5 promoter is active in murine K5-expressing cells in vivo, and consequently IL-18 is overexpressed in K5-expressing cells in the Tg mice. K5 is mainly expressed in stratified squamous epithelia of skin and mucosa and also expressed in a number of epithelial cells, including thymic reticulum, tracheal and glandular epithelia, whereas K5 is not expressed in other tissues such as liver, muscle, spleen and intestine. This technique and the use of the mice have allowed us to investigate whether the major source of serum IL-18 is activated macrophages or not.

2 Materials and Methods

Wild type (female C57BL/6) mice and K5/IL-18 Tg mice were used. The mice were administered with heat-killed *P. acnes* (1 mg dry weight/mouse) i.p. injection, and LPS was injected i.v. 7 days later. Levels of IL-18 in sera and internal organs were collected 2 h later and analyzed using enzyme-linked immunosorbent assay (ELISA) kit and Western Blotting assay.

3 Results and discussion

Serum levels of mature IL-18 with 18 kDa were markedly increased in mice treated with *P. acnes* and LPS, whereas administration of Clo-lip showed no obvious effect on serum IL-18 levels. IL-18 levels were marginal in the liver, lung and spleen, and more pronounced in the intestines, especially in the duodenum. Treatment with *P. acnes* alone induced IL-18 more than two fold in each organ, and *P. acnes* and LPS induced a marked increase in IL-18 levels in the liver and spleen, but decreased in the intestines. Furthermore, serum liver enzyme levels and liver injury induced by *P. acnes* and LPS were moderately reduced by Clo-lip. In untreated K5/IL-18 Tg mice, serum IL-18 levels were already extremely high. Treatment of K5/IL-18 Tg mice with *P. acnes* and LPS induced further increase in serum IL-18 levels comparable to those in WT mice. These results suggest that phagocytic macrophages do not actively contribute to the induction of serum IL-18 and liver injury in mice treated with *P. acnes* and LPS, and the major source of serum IL-18 is non-immune cells, such as keratinocytes and epithelial cells, in mice treated with *P. acnes* and LPS. Part of details was shown in our report [2].

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Epigenetic regulation of susceptibility to anti-cancer drugs in HSC-3 cells

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Abstract. In this study, we investigated the effects of DNA methyltransferases inhibitor zebularine (ZEB) and histone deacetylases inhibitor suberoylanilide hydroxamic acid (SAHA) on the apoptosis induced by cisplatin (CDDP) or 5-fluorouracil (5-FU) in human oral squamous cell carcinoma (HSC)-3 cells. HSC-3 cells were incubated with CDDP (5 μg/ml) or 5-FU (250 μg/ml) with or without ZEB (120 μM) and/or SAHA (1.5 μM). CDDP or 5-FU alone induced apoptosis in about 30% of cells. The combination of CDDP/SAHA or CDDP/ZEB led to a significant increase in apoptotic cells up to 80% after 48 h incubation, and the triple combination of CDDP/SAHA/ZEB showed a synergetic effect on apoptosis induction. Although the combination of 5-FU/SAHA showed a moderate increase in apoptosis after 72 h, the combination of 5-FU/ZEB inhibited apoptosis rather than that of 5-FU alone. These results indicate that epigenetic active agents (ZEB and SAHA) could sensitize HSC-3 cells to apoptosis induced by these anti-cancer drugs, which may be an important characteristic of solid cancer treatment.

Key words. epigenetics, apoptosis, zebularine, SAHA, oral squamous cell carcinoma

1 Introduction

Epigenetic alterations, including the histone acetylation and DNA methylation, play an important role in the regulation of gene expression associated with cell cycles and apoptosis that may affect the chemosensitivity of cancers. Inhibitors of DNA methyltransferases and histone deacetylases can reactivate epigenetically silenced genes for tumor suppression and thereby decreasing tumor cell growth in vitro and in vivo [1, 2]. However, the precise mechanism of their inhibitors is little known in terms of drug susceptibility and apoptosis induction of oral cancers. Furthermore, chemotherapeutic potential of cisplatin (CDDP) and 5-fluorouracil (5-FU) that are widely used for chemotherapy of oral cancers is low, and remains unsatisfactory. In this study, we investigated the effects of zebularine (ZEB) and suberoylanilide hydroxamic acid (SAHA) on the enhancement of susceptibility to anti-cancer agents in oral squamous cell carcinoma cells.

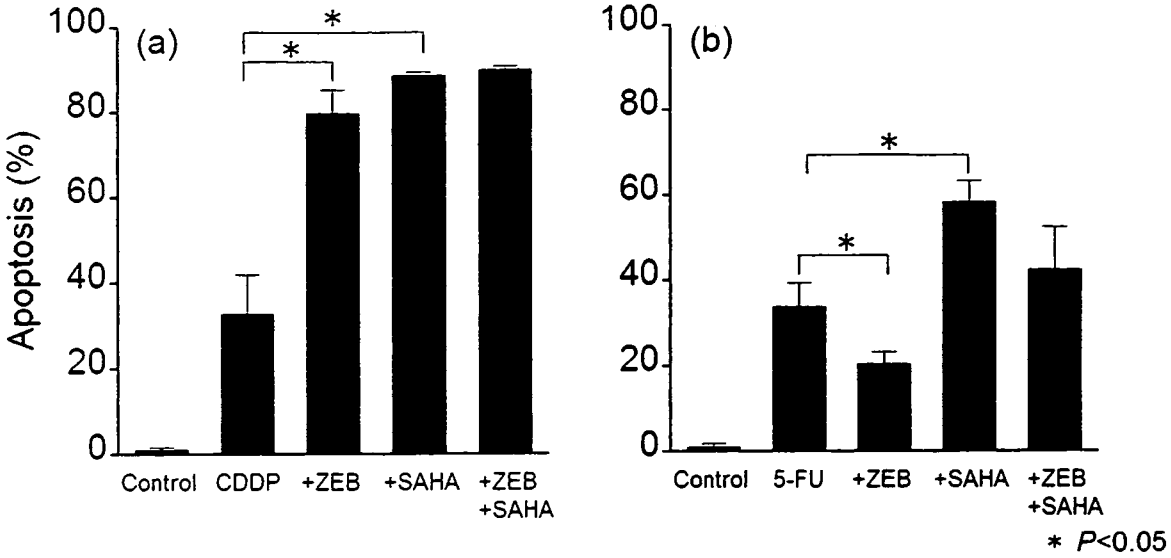

Fig. 1. Apoptosis induced by the combination of anti-cancer drugs with suberoylanilide hydroxamic acid (*SAHA*) or zebularine (*ZEB*). Cells (2×10^4 cells/well) were seeded in a 12-well flat bottomed culture plate and incubated in RPMI-1640 medium with 10% *FBS* (fetal bovine serum) for 24 h. Cells were treated with ZEB (120 μM) for 48 h, followed by treatment with cisplatin (CDDP, 5 μg/ml) for 48 h or 5-fluorouracil (*5-FU*, 250 μg/ml) for 72 h, or with SAHA (1.5 μM) concomitantly with CDDP or 5-FU. Apoptotic cells induced by CDDP (**a**) or 5-FU (**b**) in combination with SAHA or ZEB were determined by a *TUNEL* (terminal deoxynucleotidyl transferase mediated dUTP Mick End Labeling) assay. *HSC*, human oral squamous cell carcinoma

2 Results and conclusions

The combination of CDDP/SAHA or CDDP/ZEB led to a significant increase in apoptotic cells up to 80% after 48 h incubation (Fig. 1a). The combination of 5-FU/SAHA showed a moderate increase in apoptosis, whereas the combination of 5-FU/ZEB showed a decrease in apoptosis compared with the treatment of 5-FU alone after 72 h (Fig. 1b). Although the triple combination of CDDP/SAHA/ZEB showed synergetic effect on apoptosis induction, the combination of 5-FU/SAHA/ZEB decreased apoptosis rather than that of 5-FU/SAHA. These results indicate that SAHA could sensitize human oral squamous cell carcinoma (HSC)-3 cells to apoptosis induced by both anti-cancer drugs (CDDP, 5-FU). The action of ZEB in combination with 5-FU may be complex and associated with the drug metabolism cascade, which is distinct from combination with CDDP.

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Histamine amplifies proinflammatory signaling cascade in human gingival fibroblasts

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Abstract. As histamine is an important mediator in immune responses, histamine, inflammatory cytokines, and bacterial components released in inflamed periodontal tissues may be synergistically involved in inflammatory processes. The present study showed that human gingival fibroblasts (HGF) express histamine receptors (Rs) H1R and H2R, and responded to histamine to produce interleukin (IL)-8. The stimulation of HGF with tumor necrosis factor-α, IL-1α, and lipopolysaccharide markedly induced IL-8 production, and the IL-8 production was synergistically augmented in the presence of or pretreatment with histamine. The histamine response and the synergistic effect were reproduced by an H1R agonist. Selective inhibitors of mitogen-activated protein kinases (MAPKs), nuclear factor (NF)-κB, and phospholipase C (PLC) significantly inhibited the synergistic effect. These results indicate that HGF are capable of secreting IL-8 in response to histamine through H1R, and that histamine synergistically augments the inflammatory stimuli by the amplification of the MAPK and NF-κB pathway through H1R-linked PLC.

Key words. histamine, fibroblasts, inflammation, MAPK, NF-κB

1 Introduction

Periodontitis is caused by gram-negative periodontopathic bacteria, and inflamed gingival epithelial cells, as well as infiltrated lymphocytes, appear to express several inflammatory cytokines, interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α. It is reported that histamine is more released in the inflamed gingiva of periodontitis patients, but it is unclear whether periodontal tissues express histamine receptors and are able to respond to histamine. Therefore, we used TNF-α, IL-1α, and lipopolysaccharide (LPS) as major inflammatory stimuli and measured the secretion of IL-8, one of the major chemokines, from human gingival fibroblasts (HGF) in response to the inflammatory stimuli with or without histamine. We also used specific signaling inhibitors to elucidate the signaling pathway.

2 Materials and methods

After incubation for 1 day, confluent HGF were washed with medium three times, and test stimulants were added for the time indicated. For the inhibition experiments, HGF were preincubated with inhibitors for 30 min–1 h at 37°C and were then stimulated with test stimulants at 37°C. After the incubation, the levels of IL-8 in the supernatants were determined with OptEIA human IL-8 enzyme-linked immunosorbent assay kit (BD Biosciences, San Jose, CA, USA).

3 Results and discussion

The present study showed that HGF express H1R and H2R and responded to histamine to produce IL-8. The IL-8 levels induced by inflammatory stimuli, TNF-α, IL-1, and LPS, with histamine were comparable with those induced by a tenfold concentration of the inflammatory stimuli alone. The result indicates that histamine augments the sensitivity tenfold to the inflammatory stimuli at the site of inflammation. H1R are linked Gαq/11 protein, and Gαq/11 stimulate NF-κB and mitogen-activated protein kinases (MAPKs), and signaling through TNFR, IL-1R, and the LPS receptor also activates both cascades. This study showed that histamine activates MAPK and NF-κB signaling cascades via H1R using specific inhibitors and histamine receptor agonists. The synergistic IL-8 production was also significantly suppressed by the inhibition of NF-κB and MAPKs. These observations indicate that the amplification of MAPKs and NF-κB are equally involved in the synergism in HGF. The principal mechanism of H1R activation is through Gαq/11, resulting in the activation of phospholipase C (PLC). The inhibition of PLC suppressed the production of IL-8 induced by histamine and TNF-α to the levels of TNF-α alone. The results indicate that histamine activates PLC through H1R, and consequently amplifies the MAPK and NF-κB pathway induced by the inflammatory stimuli. In conclusion, the present study showed that HGF secrete IL-8 in response to histamine through H1R, and that histamine synergistically augments IL-8 secretion induced by TNF-α, IL-1α, and LPS by the amplification of the MAPK and NF-κB pathway through H1R-linked PLC. It was suggested that the histamine participated in the amplification of the inflammatory reaction in periodontitis. Therefore, control of histamine receptors at inflammatory sites might be beneficial in the regulation of periodontitis. Details were shown in our report [1].

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An antibacterial protein CAP18/LL-37 enhanced production of hepatocyte growth factor in human gingival fibroblast cultures

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Abstract. Human cationic antibacterial protein CAP18/LL-37 exhibits bactericidal and various immunobiological activities. The constitutive expression of CAP18/LL-37 in oral epithelial cells was demonstrated, and that CAP18/LL-37 activated human gingival fibroblasts to enhance production of hepatocyte growth factor, which has been shown to exert multiple biological activities. These findings might be related to restoration and regeneration of periodontal tissues.

Key words. CAP18/LL-37, hepatocyte growth factor, oral epithelial cells, gingival fibroblasts, innate immunity

1 Background

Human cationic antibacterial protein CAP18/LL-37 belongs to the cathelicidin family, which plays an important role in the innate host defense system; the cathelicidin possesses potent sterilizing activities against Gram-negative and Gram-positive bacteria. CAP18/LL-37 is produced by hematopoietic cells and epithelial cells, and is found in a number of tissues and body fluids such as saliva, plasma, and airway surface liquid [1]. Fibroblasts are capable of producing hepatocyte growth factor (HGF), and we have found that human gingival fibroblasts produce HGF upon stimulation with cytokines such as interleukin (IL)-1α [2]. HGF has been shown to exert multiple biological activities as a mitogen, a motogen and a morphogen for various cells [3].

2 CAP18/LL-37 enhanced production of HGF in human gingival fibroblasts

We demonstrated the constitutive expression of CAP18/LL-37 in oral epithelial cells. Therefore, we examined whether CAP18/LL-37 regulated HGF production in human gingival fibroblasts. We used IL-1α as a positive control according to our

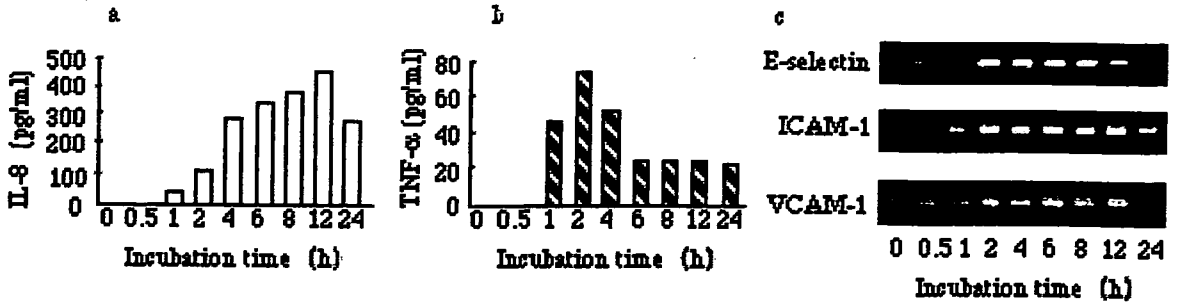

Fig. 1. *Abiotrophia defectiva*-induced cytokine production and leukocyte adhesion molecules expression of HUVEC. HUVEC (1×10^5 cells) were stimulated with *A. defectiva* ATCC 49176 (10^7 CFU) at 37°C. After incubation, IL-8 (**a**) and TNF-α (**b**) in the supernatants were analyzed by ELISA. The expression of the leukocyte adhesion molecules (*E-selectin*, *ICAM-1* and *VCAM-1*) specific mRNA in *A. defectiva*-stimulated HUVEC were detected by RT-PCR (**c**).

To elucidate further the pathogenic ability of *A. defectiva* in infective endocarditis, *A. defectiva*-induced proinflammatory cytokine productions and leukocyte adhesion molecule expressions were examined. The results indicate that *A. defectiva* induce HUVEC to produce IL-8 and TNF-α (Fig. 1a, b), and subsequently to express E-selectin, ICAM-1 and VCAM-1 (Fig. 1c). Furthermore, the stimulation with *A. defectiva* induced IκB degradation in HUVEC. Thus, NF-κB activation could be involved in *A. defectiva*-induced activation of HUVEC.

The present findings indicate that *A. defectiva* possesses a relatively higher adhesive ability to endothelial cells as well as ECM than other oral streptococci, and could induce endothelial cells to produce IL-8 and TNF-α, and subsequently to express E-selectin, ICAM-1 and VCAM-1. Thus, *A. defectiva* entering into blood streams could adhere to endothelial cells and induce proinflammatory responses through cytokine productions and leukocyte adhesion molecule expressions, which may account for the potential pathogenic traits of the organisms in infective endocarditis.

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IL-18 expressed in salivary gland cells induces IL-6 and IL-8 in the cells in synergy with IL-17

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Abstract. Interleukin (IL)-18, an immunoregulatory and proinflammatory cytokine, has been shown to play an important pathogenic role in inflammatory and autoimmune disorders. In the present study, immunohistochemical examination showed that the expression of IL-18 was detected in acinar and ductal epithelial cells in the salivary glands of patients with Sjögren's syndrome (SS) but not in those of healthy subjects. Human salivary gland human parotid gland cell lines (HSY) cells constitutively expressed mRNA of IL-18 and caspase-1. Receptors for IL-18 and IL-17 were expressed on the cell surface. IL-18 induced the secretion of IL-6 and IL-8 in the presence of low amount of IL-17, a T cell-derived proinflammatory cytokine. These results suggest that IL-18 expressed in salivary gland cells is associated with pathogenesis of SS in microenvironment of salivary glands in synergy with IL-17.

Key words. IL-18, IL-17, salivary gland, Sjögren's syndrome

1 Introduction

SS is a chronic autoimmune disease of the exocrine glands with infiltration of lymphocytes. It is reported that IL-18 actively contributes to the modulation of immune regulation in the exocrine glands. Recently, over expression of IL-18 in salivary glands with keratin 5 promoter in mice results in SS-like massive cell infiltration into salivary glands, and atrophy of glandular cells was observed. Furthermore, the expression of IL-17 mRNA in the salivary glands was prominent by DNA microarray analysis in the mice. Therefore, it is supposed that the infiltrating $CD4^+$ T cells into the salivary glands of SS patients secrete IL-17, and that there are some relations between IL-17 and IL-18 in pathogenesis of SS. In the present study, we examined the expression of IL-18 in salivary glands and IL-18-induced production of proinflammatory mediators, IL-6 and IL-8, in synergy with IL-17 using human salivary gland cells in culture.

2 Materials and methods

Labial salivary gland and parotid saliva were extirpated and gathered, respectively, from patients with SS and healthy volunteers as controls. All subjects were informed and consented with this study. The Ethical Review Board of Tohoku University Graduate School of Dentistry approved the experimental procedures. Expression of IL-18 was analyzed by immunohistochemistry assay. Human salivary gland cell lines (HSY) was used to analyses the expression of IL-18 by RT-PCR and Western blotting, secretion of IL-18 and other cytokine by ELISA, and manifestation of IL-18 receptor (IL-18R) and IL-17 receptor (IL-17R) by Flow cytometry.

3 Results and discussion

The expression of IL-18 was detected in acinar and ductal epithelial cells in the salivary glands of SS patients, and slightly detected in some ducts of the salivary glands in normal. No IL-18 expression was detected in infiltrating mononuclear cells in the salivary glands of SS patients, indicating that activated macrophages or antigen-presenting dendritic cells were not infiltrated in the field. These results indicated that expression of IL-18 in the salivary gland epithelial cells in acinar is correlated with SS.

HSY cells constitutively expressed IL-18 and caspase-1 mRNA. Western blotting showed that the cells constitutively expressed a precursor form of IL-18 but not an 18-kDa active form in the cells. Incubation of the proIL-18 containing cell lysate of HSY cells with caspase-1 converted to an 18-kDa mature form, indicating that the IL-18 expressed in salivary gland cell was properly processed in the presence of caspase-1. Elevation of intracellular Ca^{2+} by A23817 significantly induced IL-18 secretion in a dose-dependent manner in HSY cells.

Flow cytometric analyses showed that HSY cells expressed IL-18R and IL-17R on the cell surface. The results indicate that IL-18 secreted by salivary gland cells is able to bind own cells and to activate themselves in an autocrine manner, and that IL-18 and IL-17 may synergistically activate salivary gland cells. Stimulation of HSY cells with IL-18 alone did not induce secretion of IL-6 and IL-8. However, in the presence of IL-17 at 10 mg/ml or 1 ng/ml, IL-18 induced the secretion of IL-6 or IL-8 in a dose-dependent manner. These results indicate that IL-18 synergy with IL-17 is involved in pathology of salivary gland disorder.

4 Conclusion

It is suggested by the present study that IL-18 could play an important role in occurrence, formation, and promoting inflammation in salivary gland, cooperating with IL-17 by inducing proinflammatory cytokines and chemokines.

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Infiltration of immune cells in salivary gland by IL-18 overexpression in mice

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Abstract. Sjögren's syndrome (SS) is a chronic autoimmune disease of the exocrine glands with infiltration of lymphocytes and destruction of glandular cells. We previously reported that interleukin (IL)-18, an inflammatory cytokine that is involved in autoimmune diseases, is produced in salivary gland (SG) of SS patients. In this study, histological changes and lymphocytes subpopulations in SG of keratin 5 (K5)/IL-18 transgenic (Tg) mice overexpressing mature IL-18 using human K5 promoter were examined. Histological analysis revealed severe infiltration of lymphocytes and atrophy of glandular duct cells in SG. Flow cytometric analyses showed that T, B, NK cells and Macrophages are infiltrated in SG of the mice. These results indicated that overexpression of IL-18 with K5 promoter induced SS-like feature.

Key words. IL-18, Sjögren's syndrome, immune cells

1 Background

Sjögren's syndrome (SS) is a chronic autoimmune disease of the exocrine glands. Extensive lymphocytic infiltration predominantly with $CD4^+$ T cells is detected in salivary and lachrymal glands from SS patients. A predominant T helper cell type 1 (Th1) pattern of cytokines was expressed in minor salivary glands from patients with primary SS. Dryness of the mouth and eyes results from destruction of the salivary and lachrymal glands. Interleukin (IL)-18 is a multifunctional regulator of innate and acquired immune response. IL-18 is identified not only in immune cells such as activated macrophages and dendritic cells, but also in non-immune cells such as keratinocytes and epithelial cells of various organs. IL-18 is expressed in duct cells and mononuclear cells infiltrated in periductal area of SS salivary glands. Serum IL-18 levels are significantly higher in SS patients than in healthy subjects, indicating that IL-18 is critically involved in SS pathogenesis. In this study, we examined the alteration of SG in K5/IL-18 transgenic (Tg) mice.

2 Materials and methods

Labial salivary gland and parotid saliva were extirpated and gathered, respectively, from patients with SS and healthy volunteers as controls. All subjects were informed and consented with this study. The Ethical Review Board of Tohoku University Graduate School of Dentistry approved the experimental procedures. Expression of IL-18 was analyzed by immunohistochemistry assay. Human salivary gland cell lines (HSY) was used to analyses the expression of IL-18 by RT-PCR and Western blotting, secretion of IL-18 and other cytokine by ELISA, and manifestation of IL-18 receptor (IL-18R) and IL-17 receptor (IL-17R) by Flow cytometry.

3 Results and discussion

The expression of IL-18 was detected in acinar and ductal epithelial cells in the salivary glands of SS patients, and slightly detected in some ducts of the salivary glands in normal. No IL-18 expression was detected in infiltrating mononuclear cells in the salivary glands of SS patients, indicating that activated macrophages or antigen-presenting dendritic cells were not infiltrated in the field. These results indicated that expression of IL-18 in the salivary gland epithelial cells in acinar is correlated with SS.

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2 Materials and methods

Mice: female K5/IL-18 Tg mice (C57BL/6 background, 2–12 month old), n = 13.

Histological analysis: formalin-fixed samples were embedded in paraffin and stained with hematoxylin and eosin.

Flow cytometry: cells were purified from SG, submandibular lymph nodes (SML), thymus and spleen. Cells were stained with antibodies for various immune cell markers, and flow cytometric analyses were performed using FACSCalibur cytometer (BD Biosciences, San Jose, CA, USA).

3 Results and discussion

Hypertrophy of SG, SML and thymus was observed in 6–8-month-old K5/IL-18 Tg mice with female predominance. Histological analysis revealed severe infiltration of lymphocytes and atrophy of glandular duct cells in SG of K5/IL-18 Tg mice but not in wild type mice. Flow cytometric analyses with 12-month-old female K5/Il-18 Tg mice showed that the percentages of $CD3^+$ T cells were 15.0% ($CD3^+CD4^+$, 5.0%; $CD3^+CD8^+$, 5.5%) and 22.2% ($CD3^+CD4^+$, 8.4%; $CD3^+CD8^+$, 12.7%) in SG and SML, respectively. On the other hand, the percentages of $CD3^+$ T cells were similar between thymus and spleen, 38.9% ($CD3^+CD4^+$, 21.7%; $CD3^+CD8^+$, 15.2%) and 29.3% ($CD3^+CD4^+$, 17.3%; $CD3^+CD8^+$, 10.0%), respectively. No $CD4^+CD8^+$ double positive T cells were detected in thymus. The percentages of $CD19^+$ B cells were higher in SG (67.6%) and SML (56.3%) than in thymus (41.3%) and spleen (46.0%). The percentages of $NK1.1^+$ cells were higher in SG (6.0%) than in other tissues (SML, 1.5%; thymus, 1.9%; spleen, 1.5%). The percentages of $F4/80^+$ cells were higher in SG (14.3%) and spleen (5.7%) than in SML (0.2%) and thymus (1.0%).

4 Conclusion

These results suggest that overexpression of IL-18 in SG showed SS-like feature with infiltrations of immune cells and alterations of glandular cells in SG in vivo. Therefore, the mice could be beneficially used for further analysis of precise mechanisms of SS pathogenesis.

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Gelatinase activity in human saliva and its fluctuation in the oral cavity

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Abstract. This study aimed to determine the activities of gelatinase in whole saliva before and after a meal. Paraffin-stimulated whole saliva was collected from seven healthy volunteers (male, age: 27.3 ± 1.3) at 30 min before a meal and 30 min after a meal. Gelatinase activity and collagenase activity were measured by the Gelatinase assay kit and the Collagenase assay kit, respectively. Furthermore, the saliva samples were incubated at 37°C for 2 h and the activities were measured again. All the saliva samples before a meal had gelatinase (1.07 ± 0.28 U/ml) and collagenase (0.11 ± 0.14 U/ml) activities. The gelatinase activity decreased to 10 ± 13% after a meal ($P < 0.05$). At 30 min after a meal, the activity increased again and reached 56 ± 46% of the activity before a meal. The present study confirmed that whole saliva contains gelatinase and collagenase activities. The gelatinase activity in the saliva samples, especially samples obtained after a meal, increased during a 2-h incubation. It was suggested that whole saliva has an activating system for gelatinase, which may activate a latent type of gelatinase in saliva.

Key words. gelatinase activity, whole saliva

Introduction

Human proteases contribute to the growth and the turnover of human tissues by degrading extracellular matrices, while the proteases are also involved in inflammatory diseases and tumorous diseases. Recent studies suggest that the proteases in the oral cavity are related to the etiology of dentin caries [1] and periodontitis [2]. However, their activity and fluctuation in the oral cavity have not been investigated well.

Therefore, this study aimed to investigate (1) the activities of gelatinase and collagenase in whole saliva, (2) the fluctuation of the activity of gelatinase in whole saliva before and after a meal, and (3) the activation of gelatinase activity in whole saliva.

Measurement of gelatinase and collagenase activities

After informed consent was obtained, whole saliva was collected from seven healthy volunteers (male, age: 27.3 ± 1.3) by chewing a sheet of paraffin film for 1 min at 30 min before a meal and 30 min after a meal. Furthermore, the saliva samples were incubated at 37°C for 2 h and the activities were measured again.

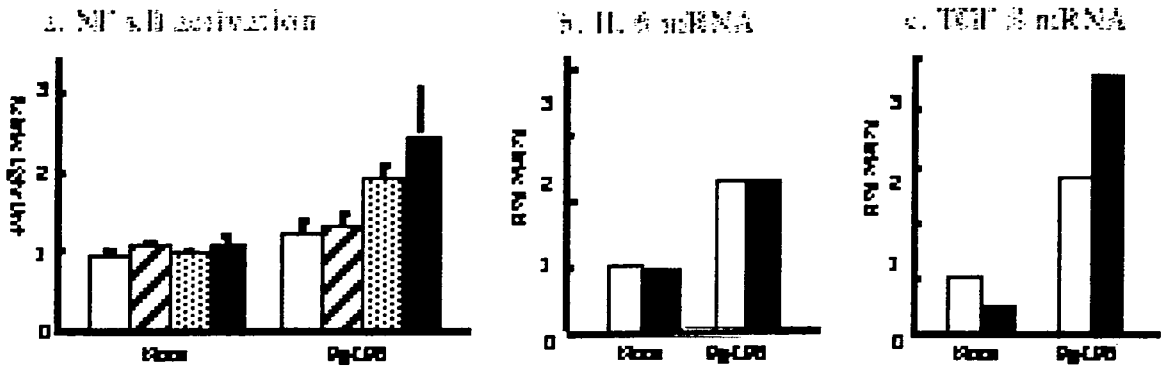

Fig. 1. *Porphyromonas gingivalis* lipopolysaccharide (*Pg-LPS*)-induced nuclear factor (*NF*)-κB activation and accumulation of IL-6 and TGF-β mRNA in CD14- (*closed bar*), TLR2- (*hatched bar*), and TLR4-transfected (*dotted bar*), and control (*open bar*) CH12.LX. NF-κB activation was detected by the luciferase activities in CH12.LX transfected with the CD14 expression plasmid and/or the TLR expression plasmid, together with pRL-TK and pGL3-mELAM-1 luciferase reporter plasmids for 48 h, either untreated or stimulated with Pg-LPS (1 μg/ml) for 6 h before harvest. Relative quantifications of IL-6 and TGF-β mRNA in CH12.LX were detected by real-time PCR

After stimulation with Pg-LPS, CD14- and TLR4-transfected, but not TLR2-transfected, CH12.LX showed higher induction of NF-κB activation, suggesting that Pg-LPS could induce B cell activation in a CD14-dependent pathway through TLR4 and NF-κB activation (Fig. 1a). Pg-LPS also induced cell proliferation of control (a parent vector-transfected) CH12.LX, accompanied by up-regulations of TGF-β and IL-6 mRNA, and the moderate induction of luciferase activity. However, the overexpression of membrane CD14 resulted in enhancement of the up-regulations of TGF–β mRNA (Fig. 1c), but not IL-6 mRNA (Fig. 1b) nor proliferative responses (data not shown) to Pg-LPS. Furthermore, the treatment of B cells with an MAP kinase inhibitor, herbimycin A, abrogated the Pg-LPS-induced proliferation as well as the tyrosine phosphorylation.

Thus, the present findings suggest that Pg-LPS could induce B cell activation in both CD14-dependent and independent pathways. In the CD14-dependent pathway, TGF-β production could be induced upon stimulation with Pg-LPS through NF-κB activation. The CD14-independent pathway mediated by tyrosine phosphorylation could also exist in the Pg-LPS-induced activation of B cells, leading to cell proliferation and IL-6 production.

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Priming effects of microbial or inflammatory agents in metal allergies

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Abstract. Although T-cells are thought to play central roles in Ni-allergy, this idea is based largely on in vitro studies, because in vivo studies have been limited by the paucity of adequate murine models of Ni-allergy (and indeed it has been said that it is difficult to induce Ni-allergy in mice). However, we recently found that a lipopolysaccharide (LPS) of *Escherichia coli* acted as a potent adjuvant, sensitizing mice to Ni. LPS also sensitized mice to other metals (Cr, Co, Pd, and Ag). Here, we report that in addition to LPS, a variety of microbial or inflammatory substances sensitize mice to Ni. Our findings suggest that a microbial or an inflammatory milieu is an important factor leading to metal allergies.

Key words. metal allergy, nickel allergy, adjuvant, inflammation, infection

1 Introduction

Metals are thought to cause various types of allergic reactions (including dermatitis, lichen planus, palmoplantar pustulosis, asthma, and rhinitis), and even to cause carcinomas. Ni, a constituent of many alloys, is the most frequent contact allergen. Unlike classical haptens, metal ions form geometrically highly defined, but reversible, coordination complexes with partner molecules. Thus, the host may recognize metal ions in complicated ways. The knowledge that the partner molecules are intact self-proteins led us to speculate that metal allergies might actually be forms of autoimmune disease. Although T-cells are thought to play central roles in Ni-allergy, this idea is largely based on in vitro studies, because the in vivo studies have been limited by the paucity of adequate murine models of Ni-allergy (and indeed it has been said that it is difficult to induce Ni-allergy in mice). However, we recently found that an *Escherichia coli* lipopolysaccharide (LPS) [a ligand of Toll-like receptor 4 (TLR4)] acted as a potent adjuvant, sensitizing mice to Ni, and that the Ni(+LPS)-allergy was fully induced even in nude mice lacking T-cells. LPS also sensitized mice to other metals (Cr, Co, Pd, and Ag). Here, the effects of a number of inflammatory agents derived from various sources, including microbial and chemical substances, were examined.