検　印
省　略

エンドトキシン研究 10
—基礎と臨床の最新知見—

平成 19 年 10 月 31 日　第 1 版第 1 刷発行

編　集　日本エンドトキシン研究会
上西紀夫　小川利久　小玉正智
横地高志　谷　徹

発行者　医学図書出版株式会社
代表者　鈴　木　文　治

発行所　医学図書出版株式会社

東京都文京区本郷 2-28-1 東金ビル
〒 113-0033　電話 (03)3811-8210（代）
FAX (03)3811-8236
振替口座　東京　00130-6-132204

0401
ISBN 978-4-87151-347-0
C 3047　¥4000 E
定価 4,200 円(本体 4,000 円＋税 5%)
(消費税率が変更になった場合，上記定価は税率の差額分変更になります)

Reprint from
Makoto Watanabe, Osamu Okuno (Eds.), K. Sasaki, O. Suzuki, N. Takahashi (Associate Eds
Interface Oral Health Science 2007

Interface Oral Health Science 2007: 225–230

Expression of various Toll-like receptors, NOD1, and NOD2, in human oral epithelial cells, and their function

Yumiko Sugawara[1], Akiko Uehara[2]*, Yukari Fujimoto[3], Koichi Fukase[3], Takashi Sasano[1], and Haruhiko Takada[2]

[1]*Division of Oral Diagnosis, Department of Oral Medicine and Surgery;* [2]*Department of Microbiology and Immunology, Tohoku University Graduate School of Dentistry, Sendai 980-8575;* [3]*Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka 560-0043; Japan*

*kyoro@mail.tains.tohoku.ac.jp

Abstract. Oral epithelium is endowed with innate immune receptors for bacterial components, which play important roles in host defense against bacterial infection. We examined the expression of various Toll-like receptors (TLRs), NOD1 and NOD2 in oral epithelial cells, and the production of β-defensin 2 and peptidoglycan recognition proteins (PGRPs) upon stimulation with their respective chemically synthesized ligands. We found a clear expression of TLR4 as well as TLR2, and a strong expression of NOD1 and NOD2 in normal oral epithelial tissues by immunohistochemical analysis. In the inflamed oral epithelium, cell-surface localizations of TLR2 and TLR4 were more clearly observed than in the healthy tissue. We also showed that oral epithelial cells in culture constitutively expressed TLR3 and TLR7 in addition to TLR2, TLR4, NOD1, and NOD2, and stimulation with synthetic ligands for these receptors (TLR2 agonistic lipopeptide, TLR3 agonistic poly I:C, TLR4 agonistic lipid A, TLR7 agonistic single-stranded RNA, NOD1 agonistic iE-DAP and NOD2 agonistic muramyldipeptide) markedly up-regulated the expression of antibacterial factors, such as β-defensin 2 and PGRPs, but not the proinflammatory cytokines. These findings indicate that these molecules in oral epithelial cells are functional receptors that induce antibacterial responses without excessive inflammatory responses.

Key words. Toll-like receptors, NODs, human oral epithelial cells, β-defensin 2, peptidoglycan recognition proteins

1 Introduction

In the innate immune system, pattern recognition of microorganisms should initiate host defense against invasive pathogens, where pathogen-associated molecular patterns (PAMPs) are recognized by the molecules of hosts. In bacteria,

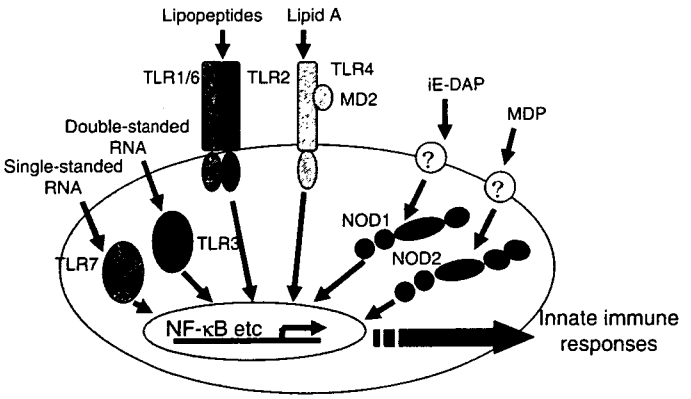

Fig. 1. Recognition of pathogen-associated molecular patterns (PAMPs) by Toll-like receptors (*TLRs*) and NODs molecules

representative PAMPs are distributed mainly on the cell surface such as peptidoglycans (PGNs), lipoproteins and lipopolysaccharides (LPS). Recent studies have demonstrated that, in mammals, these PAMPs are recognized specifically by their respective NODs in addition to Toll-like receptors (TLRs) (Fig. 1) [1, 2]. In this decade, the importance of TLRs in the process of recognition by myeloid cells, such as macrophages or dendritic cells, has been exhaustively studied. Concerning TLR expression on oral epithelial cells, we have reported that primary oral epithelial cells, oral squamous cell carcinoma HSC-2 and HO-1-u-1 cells constitutively expressed TLR2 and TLR4 [3]. In contrast, gingival epithelial cells transfected with HPV-16 constitutively expressed TLR2, but not TLR4 [4]. NOD proteins in human oral epithelium have not been reported so far, except for our report on their function [5]. Here, we will review our findings on the expression of various TLRs and NODs in human oral epithelial cells.

2 Recognition of PAMPs by TLRs and NODs

The innate immune system recognizes microorganisms through a series of pattern recognition receptors that are highly conserved in evolution, specific for common motifs found in microorganisms but not in eukaryotes, and designated as PAMPs [6]. Representative PAMPs are the lipid A moiety of lipopolysaccharides from Gram-negative bacteria, lipopeptides from various bacteria including mycoplasma, and peptidoglycans (PGNs) from either Gram-positive or Gram-negative bacteria. Recent studies have demonstrated that in mammals, these PAMPs are recognized specifically by their respective TLRs (Fig. 1) [1]. More recently, NOD1 and NOD2 were revealed to be an intracellular receptor for a PGN motif containing diaminopimelic acid (DAP) and muramyldipeptide (MDP), respectively (Fig. 1) [2].

3 Oral epithelial cells constitutively expressed functional TLRs and NODs

3.1 Histological analysis showed the expression of TLR2, TLR4, NOD1, and NOD2 in human gingival epithelial tissues

Using immunohistochemistry, we examined whether human oral tissues express TLR2, TLR4, NOD1, and NOD2 molecules. The NOD1 and NOD2 molecules were markedly expressed in the epithelial layer of healthy gingival tissue. Expression of NOD1 and NOD2 was also detected in gingival tissue from adult periodontitis patients, similar to that found in healthy gingival tissue. TLR2 and TLR4 molecules were also detected in healthy and inflamed gingival tissues. It should be noted that cell-surface localizations of TLR2 and TLR4 were more clearly observed in the inflamed gingival tissue than in healthy gingival tissue [7].

3.2 Oral epithelial cells in culture constitutively expressed TLR2, TLR3, TLR4, TLR7, NOD1, and NOD2

We examined whether human epithelial cells in culture expressed TLR2, TLR3, TLR4, TLR7, NOD1, and NOD2 molecules by RT-PCR, flow cytometry, and immunostaining. It was found that human oral HSC-2 and HO-1-u-1 epithelial cells expressed the mRNA of these molecules (Fig. 2a) [7, 8]. Consistent with the results of RT-PCR, tongue, salivary gland epithelial cells, and primary oral epithelial cell lines in addition to these oral epithelial cells constitutively expressed these molecules, as determined by immunostaining assay. In flow cytometry analysis, we could clearly detect cell-surface expression of TL2 and TLR4 and the intracellular expressions of TLR3, TLR7, NOD1, and NOD2 (Fig. 2b) [8].

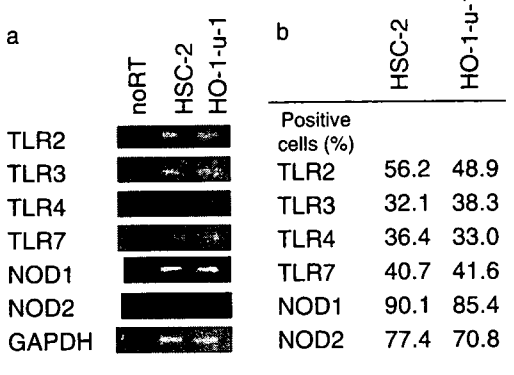

Fig. 2. Expression of TLRs, NOD1 and NOD2 in oral epithelial cells. **a** The mRNA expression of TLRs and NODs were analyzed by PCR. **b** The cells were stained with anti-TLRs and NODs Ab to analyze by flow cytometry [8]

3.3 Oral epithelial cells do not secrete proinflammatory cytokines upon stimulation with PAMPs

Although oral epithelia cells constitutively expressed various TLRs and NODs, these cells did not secrete IL-6, IL-8, and MCP-1 upon stimulation with PAMPs [3, 5, 8].

3.4 Induction of β-defensin 2 triggered by PAMPs in human oral epithelial cells

We examined whether the TLRs and NODs expressed in these oral epithelial cells actually functioned as receptors in terms of β-defensin 2 generation upon stimulation with their respective ligands. It was found that Pam_3CSSNA (TLR2/6 agonist), poly I:C (TLR3 agonist), lipid A (TLR4 agonist), ssPoly U (TLR7 agonist), iE-DAP

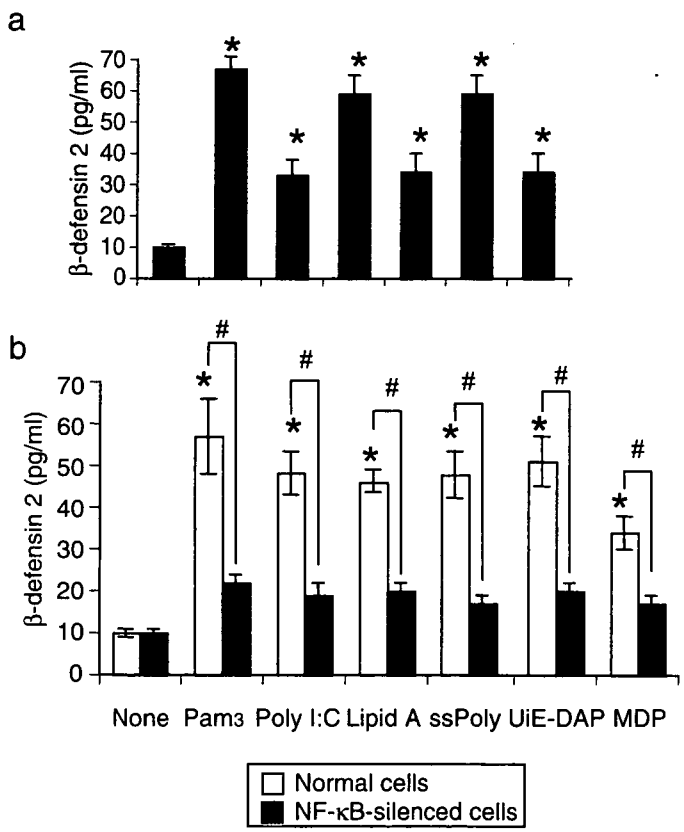

Fig. 3. Induction of β-defensin 2 in oral epithelial cells upon stimulation with synthetic PAMPs via NF-κB. **a** The cells were incubated with PAMPs, and levels of β-defensin 2 were determined by ELISA. **b** HSC-2 cells transfected with siRNA targeting NF-κB were stimulated with PAMPs, and the level of β-defensin 2 in the culture supernatants were determined by ELISA [8]

(NOD1 agonist), and MDP (NOD2 agonist) significantly induced the expression of β-defensin 2 mRNA [8]. In accordance with the results of RT-PCR, β-defensin 2 molecules were significantly up-regulated by stimulation with TLRs and NODs ligands, whereas β-defensin 2 molecules were only slightly expressed on unstimulated cells (Fig. 3a) [7, 8]. These results demonstrated that the TLRs and NODs on oral epithelial cells actively function as pattern recognition receptors and signaling molecules.

3.5 Suppression of β-defensin 2 induction upon stimulation with synthetic PAMPs in oral epithelial cells using siRNA for NF-κB

To elucidate whether NF-κB are responsible for induction of β-defensin 2 with TLR and NOD ligands, we utilized RNA interference assays targeting NF-κB mRNA. Up-regulation of β-defensin 2 induced by synthetic PAMPs was significantly inhibited in NF-κB p65-silenced HSC-2 cells (Fig. 3b) [8]. These results clearly demonstrated that NF-κB are critical molecules for induction of β-defensin 2, triggered by TLR and NOD ligands.

4 Conclusion

Clear expression of TLRs and NODs in oral epithelial cells was found; nevertheless, oral epithelial cells did not secrete proinflammatory cytokines upon stimulation with the PAMPs. Contrary to proinflammatory cytokines, oral epithelial cells secrete β-defensin 2 molecules upon stimulation with respective PAMPs. In addition, up-regulation of β-defensin 2 upon stimulation with PAMPs in oral epithelial cells occurred via NF-κB. These findings indicate that the TLRs and NODs in oral epithelial cells are functional, and epithelial cells might actively participate in bacterial clearance in the mucosa without an accompanying excessive inflammatory response, which might induce tissue destruction.

Acknowledgments. This work was supported in part by a Grant-in-Aid for Science Research from the Japan Society for the Promotion of Science (18390484 to H.T.) (17591959 to Y.S.), from the Ministry of Education, Culture, Sports, Science and Technology, Japan (18689901 to A.U.)

References

1. Akira S, Uematsu S, Takeuchi O (2006) Cell 124:783–801
2. Fritz JH, Ferrero RL, Kahn M, et al (2006) Nat Immunol 7:1250–1257
3. Uehara A, Sugawara S, Tamai R, et al (2001) Med Microbiol Immunol 189:185–192

4. Asai Y, Ohyama Y, Gen K, et al (2001) Infect Immun 69:7387–7395
5. Uehara A, Sugawara Y, Kurata S, et al (2005) Cell Microbiol 7:675–686
6. Janeway Jr CA, Medzhitov R (2002) Annu Rev Immunol 20:197–216
7. Sugawara Y, Uehara A, Fujimoto Y, et al (2006) J Dent Res 85:524–529
8. Uehara A, Fujimoto Y, Fukase K, et al (2007) Mol Immunol 44:3100–3111

Interface Oral Health Science 2007: 237–242

Antibodies against proteinase 3 prime human monocytic cells in culture in a protease-activated receptor 2- and NF-κB-dependent manner for various Toll-like receptor-, NOD1-, and NOD2-mediated activation

Akiko Uehara*, Tadasu Sato, Sou Yokota, Atsushi Iwashiro, and Haruhiko Takada

Department of Microbiology and Immunology, Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan

*kyoro@mail.tains.tohoku.ac.jp

Abstract. Anti-neutrophil cytoplasmic antibodies (ANCA) against proteinase 3 (PR3) have been detected under a wide range of inflammatory conditions, and the interaction of anti-PR3 antibodies (Abs) with leukocytes provoked cell activation. Flow cytometric analysis revealed an increase of CD14, various Toll-like receptors (TLRs), NOD1, and NOD2 expressions during the anti-PR3 priming in human monocytic THP-1 cells. Anti-RP3 Abs resulted in a markedly enhanced interleukin (IL)-8, tumor necrosis factor-α, and monocyte chemoattractant protein-1 liberation on chemically synthesized TLR2-agonistic lipopeptide (FSL-1), TLR3-agonistic Poly I:C, TLR4-agonistic lipid A, TLR7/8-agonistic ssPoly U, TLR9-agonistic bacterial CpG DNA, NOD1-agonistic FK156, and NOD2-agonistic muramyldipeptide in human monocytic THP-1 cells and human peripheral blood mononuclear cells. RNA interference assays revealed that anti-PR3 Abs primed THP-1 cells in a PR3- and protease-activated receptor-2-dependent manner. Furthermore, anti-PR3 Ab-mediated priming was significantly abolished by inhibition of phospholipase C and nuclear factor-κB. These results suggest that anti-PR3 Abs prime human monocytic cells to produce cytokines on stimulation with various microbial components by the up-regulation of the TLR and NOD signaling pathway, and that these mechanisms may actively participate in the inflammatory process in ANCA-related autoimmune diseases.

Key words. proteinase 3, protease-activated receptor-2, Toll-like receptors, NODs, human monocytic cells

1 Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) were first identified in patients with necrotizing glomerulonephritis [1]. ANCA are autoantibodies directed against the enzymes located in the primary granules of neutrophils and lysosomes of

monocyte. An established association does exist between the occurrence of ANCA, especially those targeting proteinase 3 (PR3), and the development of active Wegener's granulomatosis [2]. ANCA have since been detected under a wide range of inflammatory, infectious, and neoplasmtic conditions [3]. In isolated monocytes, anti-PR3 antibodies (Abs) stimulate the release of proinflammatory cytokines [4, 5], and Nowack et al. [6] reported that the expression of CD14 and CD18 was up-regulated on monocytes by ANCA in vitro, as well as by monoclonal Abs against PR3.

Recently, we revealed that proinflammatory cytokines induced the production of PR3 in membrane-bound and secretory forms in human oral epithelial cells, and the addition of anti-PR3 Abs to cytokine-primed oral epithelial cells induced the aggregation of PR3 followed by the activation of protease-activated receptor (PAR)-2, which resulted in remarkable secretion of interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 [7].

In this article, we review the priming effects on human monocytic cells by anti-PR3 Abs through PR3 and PAR-2 for Toll-like receptors (TLRs)-, NOD1-, and NOD2, as well as CD-14-dependent activation that we have obtained to date.

2 Recognition of pathogen-associated molecular patterns by TLRs and NODs

The innate immune system recognizes microorganisms through a series of pattern recognition receptors (PRRs) that are highly conserved in evolution, specific for common motifs found in microorganisms but not in eukaryotes, and designated as pathogen-associated molecular patterns (PAMPs) [8]. Representative PAMPs are the lipid A moiety of lipopolysaccharides from Gram-negative bacteria, lipopeptides from various bacteria, including mycoplasma and peptidoglycans (PGNs) from either Gram-positive or Gram-negative bacteria. Recent studies have demonstrated that in mammals, these PAMPs are recognized specifically by their respective TLRs [9]. More recently, NOD1 and NOD2 were revealed to be an intracellular receptor for a PGN motif containing diaminopimelic acid and muramyldipeptide, respectively [10].

3 Activation of human cells through the PAR family

Protease-activated receptor family members are G protein-coupled receptors characterized by a proteolytic cleavage of the N terminus that exposes tethered ligands and autoactivates the receptor function [11]. There are four members of this family. Because PARs are expressed in a wide variety of cell types, they are believed to play important roles in several pathophysiological processes, including growth,

development, inflammation, tissue repair, and pain [11]. As oral epithelial cells and gingival fibroblasts also constitutively express PARs, these cells are activated to produce inflammatory cytokines through PARs [12, 13]. In addition, we revealed that the addition of anti-PR3 Abs to cytokine-primed oral epithelial cells induced the aggregation of PR3 followed by the activation of PAR-2, which resulted in remarkable secretion of IL-8 [7].

4 What are ANCA?

Anti-neutrophil cytoplasmic antibodies were first identified in patients with necrotizing glomerulonephritis [1]. It is known that there are two types of ANCA (cANCA and pANCA), and cANCA are autoantibodies against PR3. ANCA have since been detected under a wide range of inflammatory, infectious, and neoplasmtic conditions [3]. Novo et al. [14, 15] described a high rate of the occurrence of ANCA in serum of patients with periodontal disease.

5 Anti-PR3 Abs amplified innate immune responses in human monocytic cells via PAR-2

5.1 Treatment with anti-PR3 Abs up-regulated the expression of CD14, TLRs, NOD1, and NOD2 in human monocytic cells

THP-1 cells constitutively expressed various TLRs and NODs, and the incubation of the cells with anti-PR3 Abs resulted in the up-regulated expression of TLR2 and TLR4, as well as CD14, on the cell surface (Fig. 1). Furthermore, intracellular levels of TLR3, 7, 8, 9, NOD1, and NOD2 also clearly increased (Fig. 1). In addition, THP-1 cells constitutively expressed PAR-1, -2, -3, and PR3 on their surface, and the addition of anti-PR3 Abs also up-regulated the expression of PAR-2 and PR3 (Fig. 1).

5.2 Anti-PR3 Abs enhanced TLR and NOD ligand-induced secretion of proinflammatory cytokines in monocytic cells

We examined whether the up-regulated expression of TLRs and NODs evoked by anti-PR3 Abs induced amplified responses to respective ligands. Stimulation with TLR2-agoinstic FSL-1, TLR3-agonistic poly I:C, and TLR9-agonistic CpG DNA significantly induced the production of MCP-1 and IL-8, and anti-PR3 Abs also

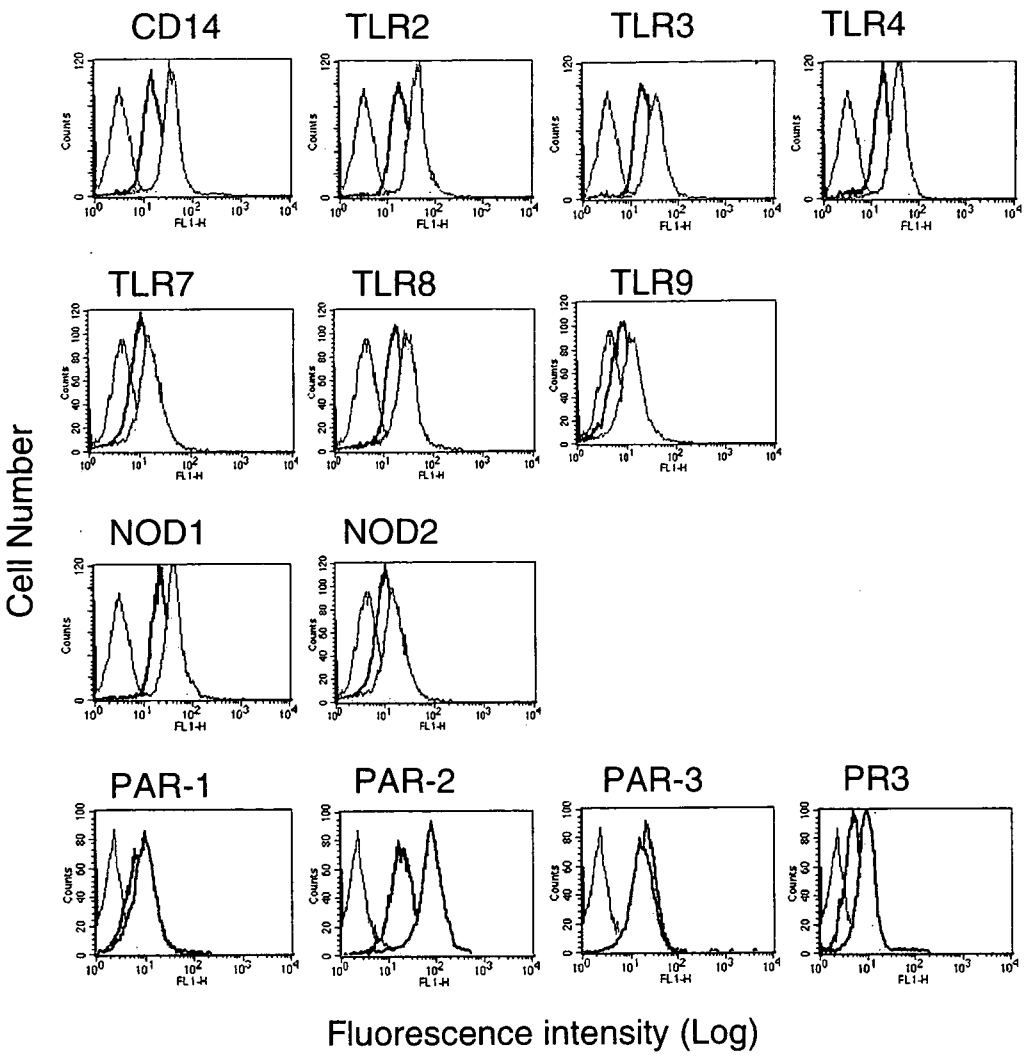

Fig. 1. Up-regulation of the expression of CD14, Toll-like receptors (*TLRs*), NODs, protease-activated receptor (*PAR*)-2, and proteinase 3 (*PR3*) in monocytic cells in response to anti-PR3 antibodies (Abs). THP-1 cells were stimulated with anti-PR3 Abs (*filled graphs*) or isotype-matched control IgG (*bold line*). After 6 h of incubation, the expression of CD14, TLRs, NODs, PARs, and PR3 were assessed by flow cytometry. The *thin lined curve* is the staining with a control Ab [16]

weakly induced the production of MCP-1 and IL-8. When THP-1 cells were pre-incubated with anti-PR3 Abs for 6 h, and subsequently challenged with the various TLR, and NOD ligand-induced production of IL-8 and MCP-1 was observed in THP-1 cells [16]. Next, we examined whether similar priming effects of anti-PR3 Abs were observed in human peripheral blood mononuclear cells (PBMCs). Consistent with the results for THP-1 cells, anti-PR3 Abs promoted the TLR and NOD ligand-induced secretion of IL-8, MCP-1, and tumor necrosis factor-α in PBMCs [16].

5.3 Priming effect of anti-PR3 Abs occurred in a PAR-2-, PR3-, and NF-κB-dependent manner

To examine the signaling molecules of the priming effects of anti-PR3 Abs on CD14, TLR, and NOD expression in THP-1 cells, we used siRNA to diminish the expression of PAR-2, PR3, and nuclear factor (NF)-κB. In transfected cells, the anti-PR3 Ab-mediated priming effect was significantly reduced by PAR-2-, PR3-, and NF-κB-specific siRNA [16]. These results indicated that the secretion of cytokines induced by anti-PR3 Abs occurred through NF-κB, which are located downstream of the PAR-2 and PR3 signaling pathway.

6 Conclusion

Anti-PR3 Abs (ANCA), being weak direct activators of monocytes and neutrophils to release cytokines per se, exert a major priming effect on these leukocytes, enhancing their responsiveness to secondary stimulation with bacterial PAMPs. Up-regulation of various PRRs, including TLRs and NODs, acting as respective PAMPs, was characterized as one mechanism underlying the anti-PR3-elicited priming response. Such cooperation between anti-PR3 Abs and bacterial PAMPs may well trigger exacerbations of disease activity during infections and contribute to the persistence of inflammatory lesions, which might be a novel model for the pathogenesis of autoimmune diseases (Fig. 2).

Acknowledgments. This study was supported in part by Grants-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (18689901), and by the Exploratory Research Program for Young Scientists from the President of Tohoku University.

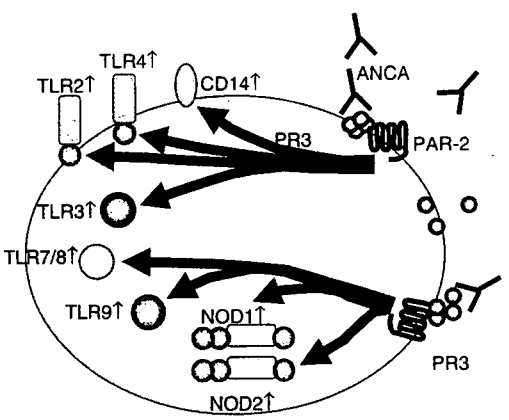

Fig. 2. Anti-PR3 Abs prime human monocytic cells to produce cytokines on stimulation with various bacterial components by up-regulating the TLR and NOD expression. These mechanisms may actively participate in the inflammatory process

References

1. Davies DJ, Moran JE, Niall JF, et al (1982) Br Med J 285:606
2. van der Woude FJ, Rasmussen N, Lobatto S, et al (1985) Lancet 1:425–429
3. Ardiles LG, Valderrama G, Moya P, et al (1997) Clin Nephrol 47:1–5
4. Casselman BL, Kilgore KS, Miller BF, et al (1995) J Lab Clin Med 126:495–502
5. Ralston DR, Marsh CB, Lowe MP, et al (1997) J Clin Invest 100:1416–1424
6. Nowack R, Schwalbe K, Flores-Suárez L-F, et al (2000) J Am Soc Nephrol 11:1639–1646
7. Uehara A, Sugawara Y, Sasano T, et al (2004) J Immunol 173:4179–4189
8. Janeway CA Jr, Medzhitov R (2002) Annu Rev Immunol 20:197–216
9. Akira S, Uematsu S, Takeuchi O (2006) Cell 124:783–801
10. Fritz JH, Ferrero RL, Kahn M, et al (2006) Nat Immunol 7:1250–1257
11. Coughlin SR (2000) Nature 407:258–264
12. Uehara A, Sugawara S, Muramoto K, et al (2002) J Immunol 169:4594–4603
13. Uehara A, Muramoto K, Takada H, et al (2003) J Immunol 170:5690–5696
14. Novo E, Viera N (1996) J Periodont Res 31:365–368
15. Novo E, Gregor EG-M, Nava S, et al (1997) P R Health Sci J 16:369–373
16. Uehara A, Iwashiro A, Sato T, et al (2007) Mol Immunol 44:3552–3562

Reprint from

Makoto Watanabe, Osamu Okuno (Eds.), K. Sasaki, O. Suzuki, N. Takahashi (Associate Eds.)

Interface Oral Health Science 2007

Interface Oral Health Science 2007: 275–276

Meso-diaminopimelic acid and *meso*-lanthionine, amino acids peculiar to bacterial cell-wall peptidoglycans, activate human epithelial cells in culture via NOD1

A. Uehara[1], Y. Fujimoto[2], A. Kawasaki[2], K. Fukase[2], and H. Takada[1]*
[1]*Department of Microbiology and Immunology, Tohoku University Graduate School of Dentistry, Sendai 980–8575;* [2]*Department of Chemistry, Graduate School of Science, Osaka University, Osaka 560–0043; Japan*
*dent-ht@mail.tains.tohoku.ac.jp

Abstract. NOD1 recognizes the diaminopimelic acid (DAP)-containing peptide moiety of bacterial peptidoglycans (PGNs) intracellularly, and a minimum NOD1 ligand has been reported to be γ-D-glutamyl-*meso*-DAP (iE-DAP). In this study, we demonstrated that chemically synthesized *meso*-DAP and *meso*-lanthionine by themselves activated various human epithelial cells through NOD1 to generate anti-bacterial factors and cytokines in specified cases. In human monocytic cells, in the presence of Lipofectamine or cytochalasin D, *meso*-DAP induced production of cytokines. Our findings suggest that *meso*-DAP is a sufficient structure to activate NOD1 when incorporated intracellularly.

Key words. NOD1, peptidoglycans, *meso*-diaminopimelic acid, *meso*-lanthionine, epithelial cells

Peptidoglycans (PGNs) are ubiquitous constituents of the cell walls of Gram-positive and Gram-negative bacteria. The PGNs exhibit various immunobioactivities, most of which have been reproduced by a chemically synthesized muramyldipeptide (MDP; MurNAc-L-Ala-D-isoGln). Another type of PGN fragment, *meso*-DAP-containing peptide moiety also exerted similar bioactivities to MDP, and iE-DAP was reported to be the minimum structure [1]. In 2003, iE-DAP and MDP were demonstrated to be recognized by their respective intracellular proteins carrying a nucleotide-binding oligomerization domain (NOD), NOD1 and NOD2, respectively [2, 3]. It must be noted that the DAP alone was reported to be completely inactive using a commercial (Sigma) DAP, a mixture of *meso*-DAP, LL-DAP and DD-DAP [2], or *meso*-DAP purified from commercial DAP [4].

In the course of a study examining the innate immune system in oral epithelium, we unexpectedly found that a commercial DAP specimen (Sigma) activated human oral epithelial cells. Therefore, we have carried out exhaustive studies using three chemically synthesized stereoisomers of DAP; *meso*-DAP, LL-DAP and DD-DAP, in human epithelial and monocytic cells in culture [5]. We found that the *meso*-DAP itself activated human oral, pharyngeal, esophageal, colonic and cervical epithelial cells through NOD1 to generate anti-bacterial factors, PGN recognition proteins and

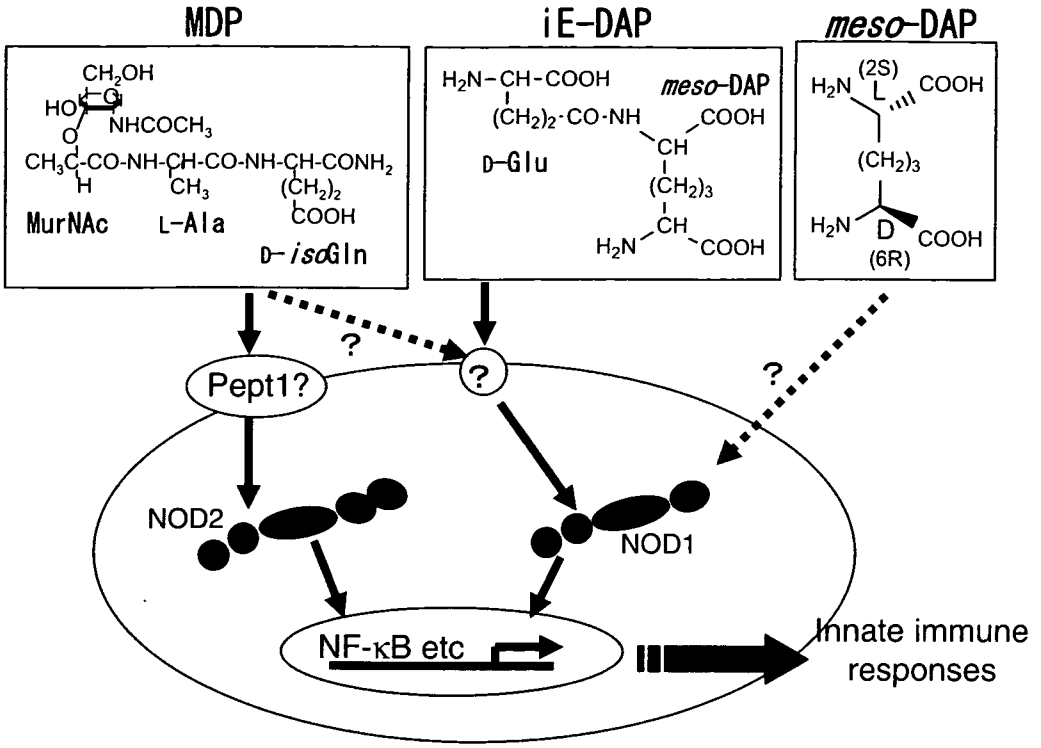

Fig. 1. Which is the minimum structure to activate NOD1? Lipofectamine and cytochalasin D increase the permeability of cells and allow *meso*-diaminopimelic acid (*DAP*) to be internalized into the cytosole. Therefore, even in monocytic cells *meso*-DAP was able to activate NOD1. A plasma membrane transporter 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.

References

1. Kitaura Y, Nakaguchi O, Takeno H, et al (1982) J Med Chem 25:335–337
2. Chamaillard M, Hashimoto M, Horie Y, et al (2003) Nat Immunol 4:702–707
3. Girardin SE, Boneca IG, Carneiro LA, et al (2003) Science 300:1584–1587
4. Girardin SE, Travassos LH, Herve M, et al (2003) J Biol Chem 278:41702–41708
5. Uehara A, Fujimoto Y, Kawasaki A, et al (2006) J Immunol 177:1796–1804
6. Ismair MG, Vavricka SR, Kullak-Ublick GA, et al (2006) Can J Physiol Pharmacol 84:1313–1319

Reprint from

Makoto Watanabe, Osamu Okuno (Eds.), K. Sasaki, O. Suzuki, N. Takahashi (Associate Eds.)

Interface Oral Health Science 2007

Interface Oral Health Science 2007: 283–284

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

Hitomi Maeda[1,2,3]*, Akiko Uehara[1], Takashi Saito[3], Hideaki Mayanagi[2], Isao Nagaoka[4], and Haruhiko Takada[1]

[1]*Department of Microbiology and Immunology; and* [2]*Division of Pediatric Dentistry, Graduate School of Dentistry;* [3]*Dentistry for Disabled, Dental Hospital, Tohoku University, Sendai 980–8575;* [4]*Department of Biochemistry, Juntendo University School of Medicine, Tokyo 113–8421; Japan*

*mail-to-ekht@mail.tains.tohoku.ac.jp

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

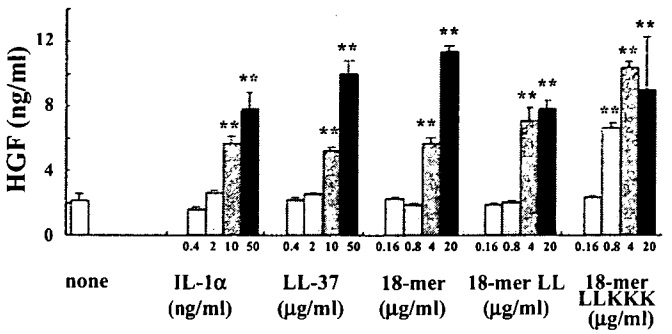

Fig. 1. Enhanced hepatocyte growth factor (*HGF*) production in human gingival fibroblasts by *LL-37* and its *18-mer* derivatives. * $P < 0.05$, ** $P < 0.01$

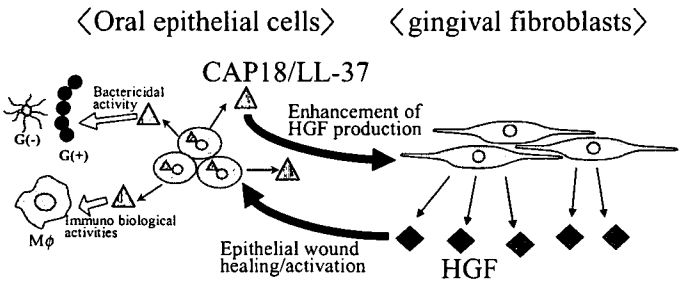

Fig. 2. Possible interaction between *oral epithelial cells* and *gingival fibroblasts* through *CAP18/LL-37* and *HGF*

previous report [2]. We found that human gingival fibroblasts exhibited enhanced HGF production upon stimulation with CAP18/LL-37 in a dose-dependent manner (Fig. 1). Furthermore, we revealed that 18-mer derivatives of CAP18/LL-37, especially 18-mer LLKKK exhibited stronger HGF production than CAP18/LL-37.

3 Conclusion

Human oral epithelial cells produced CAP18/LL-37, and CAP18/LL-37 enhanced HGF production in human gingival fibroblasts. HGF might be involved in restoration and regeneration of periodontal tissues (Fig. 2).

References

1. Bowdish DME, Davidson DJD, Hancodk REW (2006) Curr Top Microbiol Immunol 306:27–66
2. Tamura M, Arakaki N, Tsubouchi H, et al (1993) J Biol Chem 268:8140–8145
3. Ohnishi T, Daikuhara Y (2003) Arch Oral Biol 48:797–804