Expression of Nucleotide-binding Oligomerization Domain-like Receptor in Oral Lichen Planus

Jin-Kyu Kang

The Graduate School

Yonsei University

Department of Dental Science

Expression of Nucleotide-binding Oligomerization Domain-like Receptor in Oral Lichen Planus

Directed by Professor Jong-Hoon Choi, D.D.S., Ph.D.

A Dissertation Thesis
Submitted to the Department of Dental Science,
the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy of Dental Science

Jin-Kyu Kang

Dec 2013

This certifies that the doctoral dissertation

of Jin Kyu Kang is approved.

Thesis Supervisor : Jong Hoon Choi

H. J. Ahn

Thesis Committee Member : Hyung Joon Ahn

J. S. Kwon

Thesis Committee Member : Jeong Seung Kwon

Thesis Committee Member : Jung Hoon Yoon

Thesis Committee Member : Hyun Dae Lim

The Graduate School

Yonsei University

December 2013

감사의 글

박사학위과정을 마치고 이 논문이 나오기까지 항상 아버지처럼 자상하게 이끌어주신 최종훈 지도교수님께 먼저 깊은 감사를 드립니다. 10년 전 치과대학을 졸업하고 전공을 택하여 처음 구강내과학에 입문했을 때부터 지금까지 학문적인 가르침을 주시고 인생의 멘토가 되어 제가 이 자리에 있도록 해주신 존경하는 은사님이신 김종열 교수님, 신경진 교수님, 김성택 교수님, 안형준 교수님께도 감사의 인사를 드립니다. 또한 마지막까지 논문을 꼼꼼하게 살펴주시고 조언을 아끼지 않았던 권정승 교수님, 임현대 교수님께도 감사드립니다.

이번 논문을 진행하면서 실험을 총괄해 주시고 논문의 집필과정에서도 아낌없는 도움을 주신 원광대학교 대전치과병원 구강병리학교실 윤정훈 교수님과 안미영 교수님, 권성민 박사님, 윤효은 박사님께도 감사드리며, 함께 연구에 동참해 준 구강내과 심영주 교수님과 구강악안면외과 김봉철 교수님, 원광대학교 대전치과병원 구강내과 직원들과 전공의들에도 감사의 말씀을 전합니다.

마지막으로 항상 저를 위해 기도해주시고 물심양면으로 응원해 주시는 가족들에게 진심으로 감사의 마음을 전하며, 사랑하는 아내와 아들 지오와 함께 이 기쁨을 나누고자 합니다.

2013년 12월

저자 씀

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Abstract

Expression of Nucleotide-binding Oligomerization Domain-like Receptor in Oral Lichen Planus

Jin-Kyu Kang, D.D.S., M.S.D.

Department of Oral medicine/Dental Science
The Graduate School, Yonsei University

(Directed by Professor Jong-Hoon Choi, D.D.S., Ph.D.)

Oral lichen planus (OLP) is a common chronic inflammatory disease of the oral mucosa. The etiology is exactly unknown, and there is no specific cure for OLP and the management can be difficult.

The innate immune system is the first line of host defense against pathogens and imbalances in this system contribute to serious infectious disease and to chronic inflammatory and autoimmune disease. Nucleotide-binding oligomerization domain (NOD) is a pivotal sensor protein of the innate immune system, and has been suggested to play a important role in the maintenance of homeostasis and progression of inflammatory oral disease. However, the relationship of NOD and OLP has not been reported yet.

The aim of the present study is to explore the relationship of NOD and OLP. In this study, we examined the expression of NOD1 and NOD2 in OLP patients by RT-PCR and immunohistochemistry.

The result showed that the expression of NOD1 and NOD2 was increased markedly in OLP group compared to control group, especially NOD2. We suggest NOD1 and NOD2 have an important role in the pathogenesis and maintenance of OLP, especially NOD2. We propose that NOD1 and NOD2 could be a new target for diagnosis and treatment of OLP.

Key words : Nucleotide-binding oligomerization domain 1 (NOD1),
Nucleotide-binding oligomerization domain 2 (NOD2),
Innate immune system, Oral lichen planus (OLP)

Expression of Nucleotide-binding Oligomerization Domain-like Receptor in Oral Lichen Planus

Jin-Kyu Kang, D.D.S., M.SD.

Department of Oral Medicine/Dental Science,
The Graduate School, Yonsei University

(Directed by Professor **Jong-Hoon Choi**, D.D.S., Ph.D.)

Ⅰ. Introduction

Oral lichen planus (OLP) is a common chronic inflammatory disease of the oral mucosa (Au et al., 2013). A wide spectrum of mucosal alterations is noted with reticular, atrophic, erosive, papular and occasionally bullous lesions. Sites of involvement most commonly are the buccal mucosa, gingiva, tongue and vermilion portion of the lips, though any intraoral site can be affected (Sciubba, 2011). The lesions may be chronic in nature, and rarely subsided spontaneously (Crincoli et al., 2011). The clinical characteristics is variable; they range from asymptomatic to

debilitating pain provoked by salty, spicy or hot food (Au et al., 2013). OLP predominately affects females, with most patients aged between 30 and 70 years (Jaafari-Ashkavandi et al., 2011). A recent study reported an overall age standardized prevalence of 1.27% (0.96% in men and 1.57% in women) (McCartan & Healy, 2008). The etiology of OLP is exactly unknown to date, but the disease is considered to be an autoimmune disease, which result from apoptosis of basal cells of epithelium induced by auto-cytotoxic $CD8^+$ T lymphocytes (Scully & Carrozzo, 2008). At the present, there is no specific cure for OLP and the management of lesion can be difficult. Most therapeutic modalities, such as systemic and topical corticosteroids, topical retinoids, cyclosporine, tacrolimus and pimecrolimus, aim for symptomatic relief (Au et al., 2013).

The innate immune system is the first line of host defense against pathogens and imbalances in this system contributes to serious infectious disease and to chronic inflammatory and autoimmune disease (Correa et al., 2012). The innate immune system comprises several classes of pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), the nucleotide-binding oligomerization domain-like receptors (NLRs), the retinoic acid-inducible gene I-like receptors (RLRs) and cytosolic DNA sensors (Thompson et al., 2011). They detect invariant structures of microbes termed pathogen associated molecular patterns (PAMPs), such as peptidoglycan, bacterial lipoprotein, lipopolysaccharide (LPS), flagellin, and viral nucleic acids (Akira et al., 2006).

TLRs are the best characterized PRRs that recognize distinct PAMPs. (Janardhanam et al., 2012). Differential expression of TLRs in epithelial cells has been reported in many oral mucosal diseases including OLP (Srinivasan et al., 2008; Ohno et al., 2011; Janardhanam et al., 2012; Siponen et al., 2012), aphthous ulcers (Gallo et al., 2012), squamous cell carcinoma (Ng et al., 2011) and candidiasis (Netea et al., 2006).

Recently, NLRs have been discovered to play a pivotal roles in the immune response against pathogens. Nucleotide-binding oligomerization domain (NOD) is representative NLRs and it consist of a C-terminal leucine-rich repeat domain that are involved in recognition of conserved microbial patterns or other ligands; an intermediate NOD that mediates self-oligomerization and is essential for NLR activation; and a N-terminal caspase recruitment domain, which is responsible for the interaction with adaptor molecules or downstream effector proteins that mediate the signal transduction functions (Correa et al., 2012; Inohara et al., 2005). The NOD receptor subfamily consists of five members including NOD1, NOD2, NOD3, NOD4 and NOD5, and is involved in recognizing various substructures of pathogens or microbes as ligands (Swain et al., 2013). NOD1 and NOD2 are major immunity proteins, acting primarily as intracellular PRRs involved in the detection of cytoplasmic PAMPs and endogenous products of tissue injury (Philpott & Girardin, 2010). NOD1 senses meso-DAP (meso-diaminopimelic acid)-containing peptidoglycan of Gram-negative bacteria (Chamaillard et al., 2003; Girardin et al., 2003), while NOD2 detects MDP (muramyl dipeptide), the largest peptidoglycan motif common to Gram-negative and Gram-positive bacteria (Girardin et al., 2003; Inohara et al., 2000).

Previous studies reported the expression and function of NODs in oral tissues such as gingival fibroblast (Sugawara et al., 2006; Uehara et al., 2007), dental pulp (Hiaro et al., 2009; Lee et al., 2011), cementoblast (Ahn et al., 2013) and periodontal ligament cells (Tang et al., 2011; Jeon et al., 2012). However, the relationship of NOD and OLP has not yet been reported.

The aim of the present study is to explore the relationship of NOD and OLP. In this study, we examined the expression of NOD1 and NOD2 in OLP patients.

Ⅱ. Materials and methods

1. Study population

The study group consisted of 20 subjects who visited the Department of Oral Medicine and Orofacial Pain, Wonkwang University Daejeon Dental Hospital, Daejeon, Korea. They included 9 male patients and 11 female patients and their mean age was 52.3 years. All the patients were clinically and histologically diagnosed with OLP. A diagnostic criteria was followed by van der Meij and van der Waal (2003). All of them did not have an other immune-related disease. The patients who have previously received steroid therapy or who are currently taking steroid were excluded. The control group consisted of 6 subjects (3 male and 3 female patients, mean age=27.8 years) with no known systemic or oral mucosal disease. The tissue sample was collected from the buccal mucosa of both control and OLP group. This study was approved by the institutional ethical committee of Wonkwang University Daejeon Dental Hospital (IRB No. W-1301/001-001).

2. Reverse transcription-polymerase chain reaction (RT-PCR)

The expression of NOD1 and NOD2 was measured by RT-PCR. Fresh tissues from buccal mucosa were lysed using TRIzol Reagent (Invitrogen, CA, USA). One microgram of total RNA was reverse transcribed into complementary DNA (cDNA), and PCR was performed using the Power cDNA Synthesis kit (Intron Biotechnology, Daejeon, Korea). The primer sequences for each gene, NOD1, NOD2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), are shown in Table 1.

PCR conditions were as follows: the amplifications were performed 40 cycles at 94°C 5 min after an initial denaturation at 94°C 30 s, annealed at 55°C 30 s, extended at 72°C 30 s, and extended at 72°C 10 min in all primers. PCR products were then electrophoresed on a 1.5 % agarose gel and visualized using a gel documentation system (Bio-rad, Hercules, CA, USA). Human cementoblast (HCEM) cells were used as positive control.

Table 1. Primer sequences used in PCR amplification for NODs and GAPDH

Gene		Primer sequence
NOD1	Sense	5′-ACATCCGCAATACTCAGTGTCTG-3′
	Antisense	5′-ACGACTTTGCTCTGAGTGAGCA-3′
NOD2	Sense	5′-GAATGTGCTCTTCACTGCGAGCAA-3′
	Antisense	5′-AGCATGACGTTCTTTGCCAGCA-3′
GAPDH	Sense	5′-CCAAGGTCATCCATGACAACTTTG-3′
	Antisense	5′-GTCATACCAGGAAATGAGCTTGACA-3′

3. Histopathology and immunohistochemistry

The tissue samples from buccal mucosa were fixed in 10% formalin over 24 h. The tissues were then dehydrated in an alcohol-xylene series and embedded in paraffin wax. From each block, sections 2 μm thick were prepared and stained with haematoxylin and eosin for histological examination.

For immunohistochemistry, the sections were incubated in 3% H_2O_2 in methanol for 30 min to remove endogenous peroxidase and blocked with 1% bovine serum

albumin (BSA) in phosphate buffered saline (PBS) for 0.5 h. The sections were then incubated with anti-NOD1 (IMGENEX, CA, USA) and anti-NOD2 antibody (eBioscience, CA, USA) overnight at 4°C. After washed 3 times with PBS, the sections were subjected to avidin-biotin peroxidase complex (ABC) method (Vector, CA, USA) and peroxidase activity was evaluated with 3,3′-diaminobenzidine (Vector, CA, USA). Finally, the sections were counterstained with hematoxylin. Degrees of NOD1 and NOD2 expression were determined by counting the cells positive for NOD1 or NOD2 in control and OLP; sections with more than 50% positive cells were rated as strong (+++), those with 30-50% as moderate (++), those with 10-30% as mild (+) and those with 0-10% as negative (-).

Ⅲ. Results

1. The expression of NOD1 and NOD2 in buccal mucosa

Fresh tissues from buccal mucosa were lysed using TRIzol Reagent. One microgram of total RNA was reverse transcribed into cDNA and PCR was performed.

The gene expression of NOD1 and NOD2 was examined in control and OLP group using RT-PCR. NOD1 and NOD2 gene were almost not expressed in control group. Whereas both of them were markedly expressed in OLP group, in particular, the expression of NOD2 was increased significantly (Figure 1).

2. Histopathologic findings

The specimen was fixed in 10% formalin and routinely processed. Slides were stained with H&E. In OLP group, H&E stained slides showed a parakeratinized epithelium further characterized by basal cell liquefaction, exocytosis of inflammatory cells in the epithelium, and a diffuse infiltration of inflammatory cells, predominantly lymphocytes, in the superficial connective tissue; all together being compatible with OLP (Figure 2).

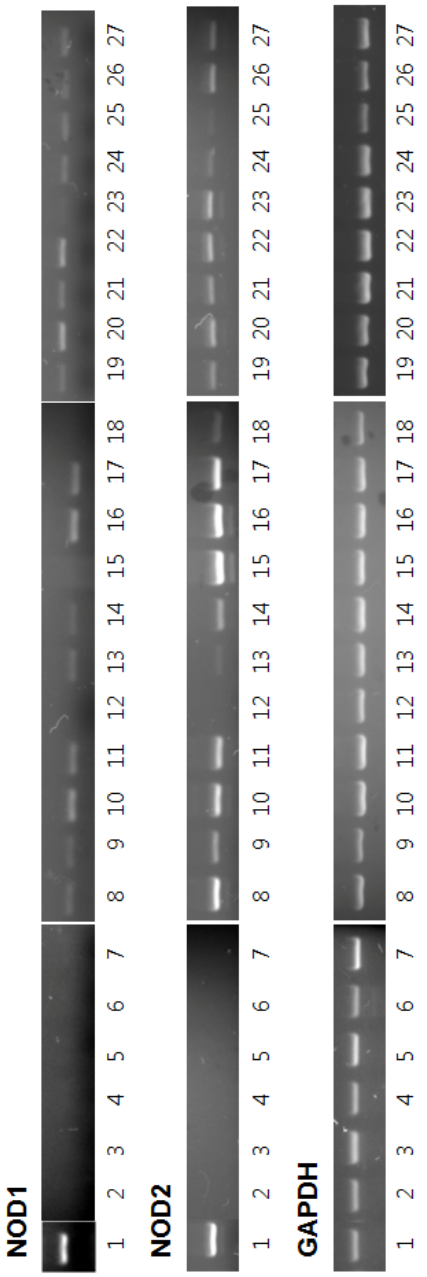

Figure 1. The expression of NOD1 and NOD2 in buccal mucosa. Total RNAs were extracted from individual tissue. cDNA was synthesized by RT-PCR.

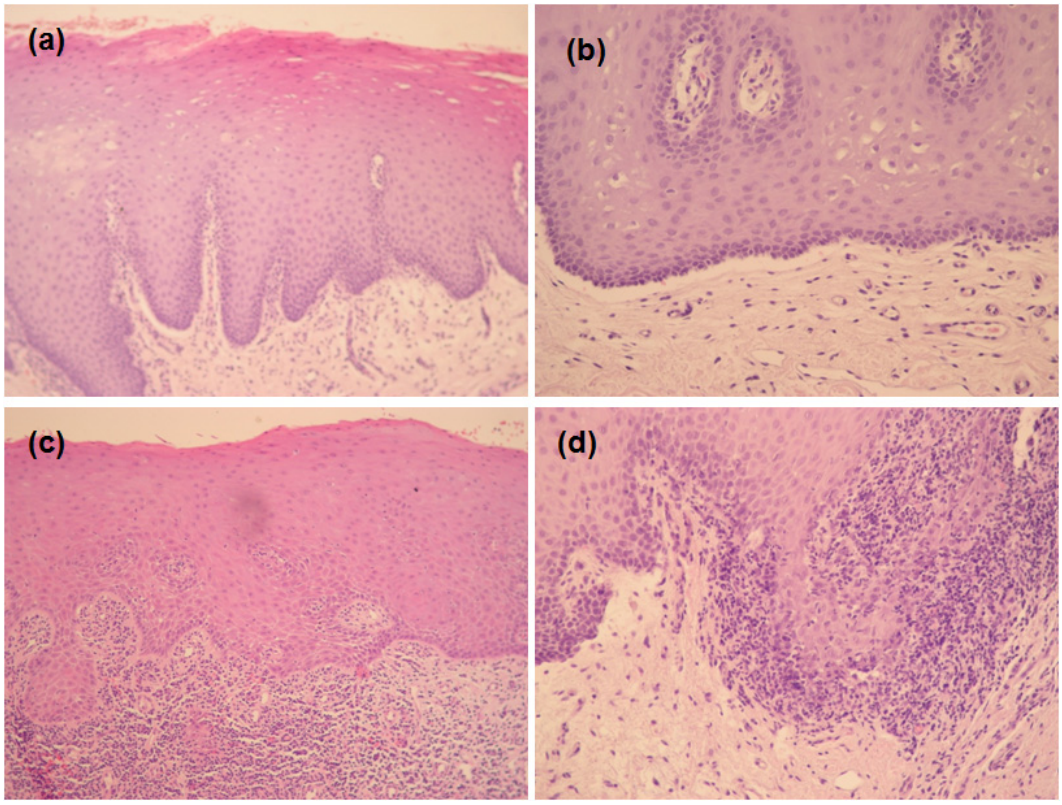

Figure 2. Histopathology of buccal mucosal tissue stained by hematoxylin and eosin (H&E). (a) Control (magnification X100), (b) Control (magnification X200), (c) OLP (magnification X100), (d) OLP (magnification X200). OLP : Oral lichen planus.

3. Immunohistochemical expression of NOD1

To determine the protein expression of NOD1 in buccal mucosa, immunohistochemistry was performed in control and OLP group. NOD1 was broadly expressed in the epithelium of control and OLP group. Further, NOD1 was expressed in destroyed basal cell layer, but not in infrabasal area including inflammatory cells in OLP group (Figure 3). However, the expression of NOD1 was significantly increased in OLP group compared to control group. Also, the number of NOD1-positive basal cells was slightly increased in OLP group compared to control group.

4. Immunohistochemical expression of NOD2

We next examined the expression of NOD2 in buccal mucosa by performing immunohistochemical analysis. NOD2 was not expressed in the control group. However, NOD2 significantly expressed in OLP group (Figure 4). Especially, NOD2 was expressed in infrabasal area including inflammatory cells, as well as in destroyed basal cell layer in OLP group.

These differences in expression pattern of NOD1 and NOD2 are summarized in Table 2.

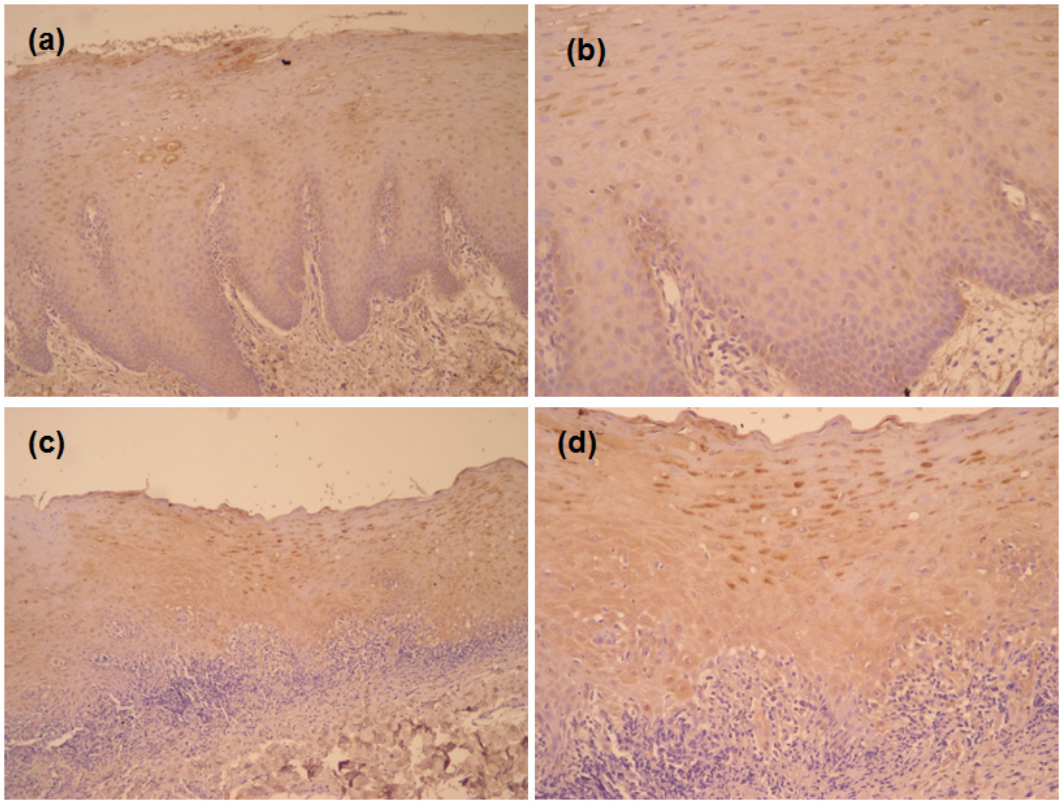

Figure 3. The expression of NOD1 in control and OLP group determined by immunohistochemistry. (a) Control (magnification X100), (b) Control (magnification X200), (c) OLP (magnification X100), (d) OLP (magnification X200).

OLP : Oral lichen planus.

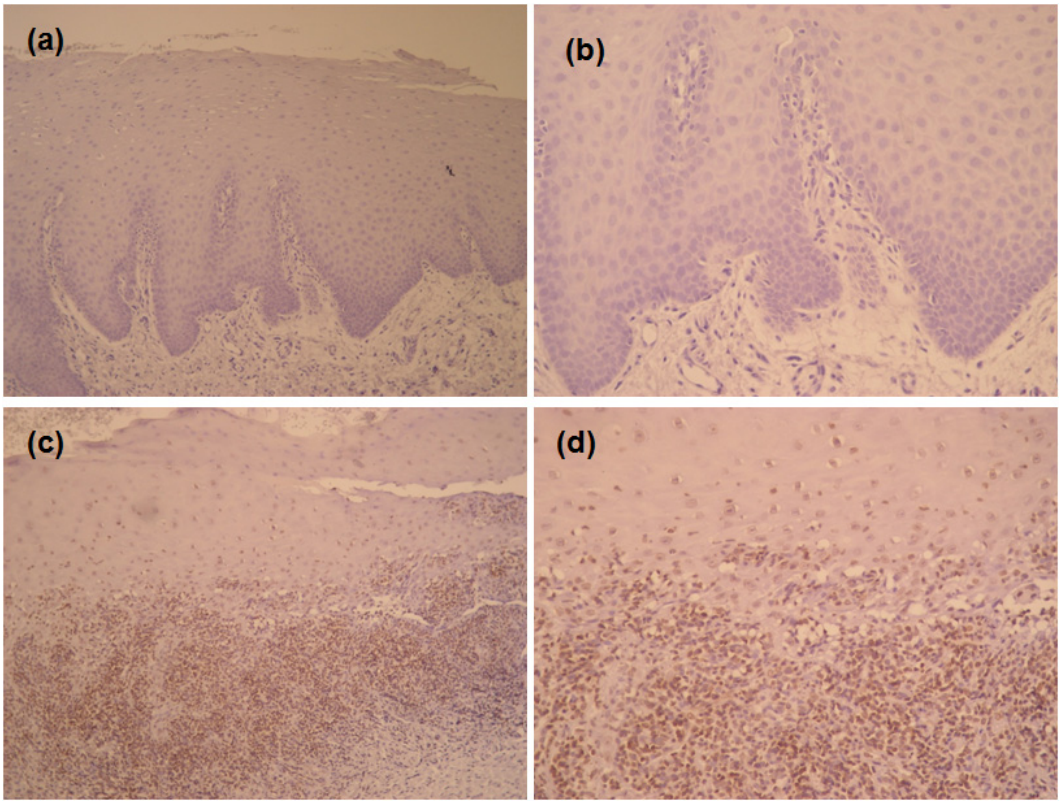

Figure 4. The expression of NOD2 in control and OLP group determined by immunohistochemistry. (a) Control (magnification X100), (b) Control (magnification X200), (c) OLP (magnification X100), (d) OLP (magnification X200).

OLP : Oral lichen planus.

Table 2. The expression pattern of NOD1 and NOD2 in control and OLP group

	Control	OLP*
NOD1	+	++
NOD2	-	+++

*OLP : Oral lichen planus

-; negative, +; mild expression, ++; moderate expression, +++; strong expression.

Ⅳ. Discussion

The family of NLRs was first identified in vertebrates and comprises a group of pivotal sensor protein of the innate immune system for microbial cell wall components or danger signals (Lange et al., 2011). The function of NLRs has been described in a variety of maladies, including chronic inflammation, autoimmunity and cancer predisposition (Antosz and Osiak, 2013).

The function of NOD1 and NOD2 in the pathogenesis of several diseases has been reported in previous studies. The disruption of NOD1 and NOD2 may contribute to the pathogenesis of inflammatory bowel disease (Boughan et al., 2006; Zilbauer et al., 2007). It was reported that NOD1 was related to several types of malignancy (Kutikhin, 2011) and allergic disease, such as asthma and dermatitis atopia (Hysi et al., 2005; Eder et al., 2006). Also, NOD2 polymorphism have been associated with multiple inflammatory disorders, including Crohn's disease, Blau syndrome, early-onset sarcoidosis and atopic disease (Correa et al., 2012).

Recently, several studies have been investigated the expression and function of NOD in oral epithelium. Almost of them revealed the marked expression of NOD, and the authors suggested that NOD have an important role in the maintenance of homeostasis and progression of inflammatory oral diseases, such as pulpitis and periodontitis, via innate immune system (Sugawara et al., 2006; Lee et al., 2011; Tang et al., 2011; Jeon et al., 2012).

In the present study, we investigated the expression of NOD1 and NOD2 in OLP. Firstly, we examined the mRNA levels of NOD1 and NOD2 by RT-PCR. The result showed no mRNA expression of NOD1 and NOD2 in control group and marked expression of NOD1 and NOD2 in OLP group, especially NOD2 expression was significantly higher. This is the first report on the expression of NOD1 and NOD2 in buccal mucosa.

Previous studies showed that the expression levels of NOD2 varies between cell types. The mRNA and protein of NOD2 was markedly expressed in hepatocytes, oral epithelial cells and renal tubular epithelial cells (Sugawara et al., 2006; Scott et al., 2010; Shigeoka et al., 2010), but was not expressed or was weakly expressed in intestinal epithelial cells (Begue et al., 2006). We suspected that there were the differences in NOD2 expression among oral tissues including gingiva, dental pulp, periodontal ligament, cementum and buccal mucosa, etc.

Next, we performed immunohistochemistry to elucidate the expression levels and the location of NODs. In immunohistochemistry, NOD1 showed the weak expression in the epithelium of buccal mucosa in control group and increased expression in OLP group. It was observed that NOD1 was expressed in the destroyed basal cell layer of OLP tissue, but not in infrabasal area including inflammatory cells. This result means NOD1 expression may be partially related to the pathogenesis of OLP. On the other hand, NOD2 showed almost no expression in control group and more significantly increased expression in OLP group. It should be noted that NOD2 was expressed in infrabasal area including inflammatory cells, as well as in destroyed basal cell layer. These results suggest that NOD2 is thought to be strongly associated with OLP.

Studies on the expression of NOD in OLP has so far not been reported, however, several studies have been reported on the relationship of TLRs, well known characterized PRR, and OLP (Srinivasan et al., 2008; Ohno et al., 2011; Janardhanam et al., 2012; Siponen et al., 2012). Although the results are variable, almost of them showed marked expression of TLR family in OLP.

Some studies reported the expression of TLRs and NODs in oral tissues. Sugawara et al. (2006) reported that TLR2, TLR4, NOD1 and NOD2 were expressed simultaneously in healthy gingival tissue and Tang et al. (2011) reported the same results in periodontal ligament fibroblast. Furthermore, several studies

suggested that TLRs and NODs were functionally activated altogether and can induce inflammatory process in oral tissues (Uehara et al., 2005; Uehara & Takada, 2007; Uehara & Takada, 2008; Tang et al., 2011; Jeon et al., 2012). Although the association of TLR and OLP was not included in this study, as considering the results of existing studies comprehensively, the elevated expression of NOD was suggested to be related with TLR, and NOD was shown to play an important role in the pathogenesis of OLP with TLR.

NOD triggers innate immune responses by inducing signalling pathways, such as nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPK), stress kinases, inflammatory caspases and autophagy. This results in the activation of inflammatory cytokines and/or chemokines (Correa et al., 2012).

Santoro et al. (2003) reported the increased expression of NF-κB in OLP compared with cutaneous lichen planus (CLP). Clinically, OLP is more chronic and recalcitrant than CLP and may persist for very long periods. The authors suggested that the chronic course of OLP may be suggested result from the activation of the inflammatory mediator NF-κB. NF-κB is known to be the most important signal in inflammatory process induced by TLRs and NODs. TLRs were already found to contribute to the pathogenesis of OLP in the previous studies (Srinivasan et al., 2008; Ohno et al., 2011; Siponen et al., 2012). Although in this study, we did not evaluate the level of NF-κB, similarly with previous studies performed in TLRs, the overexpression of NOD2 may be associated with NF-κB elevation in OLP.

In conclusion, we clearly demonstrated that NOD1 and NOD2 were expressed in the buccal mucosal tissue of OLP patients and NOD2 expression was more significant compared with NOD1. In addition, NOD1 was expressed in epithelium and basal cell layer, whereas, NOD2 was expressed in basal cell layer and infrabasal area including inflammatory cells. We suggest that NOD1 and NOD2

have an important role in the pathogenesis and maintenance of OLP, especially NOD2.

We propose that NOD1 and NOD2 could be a new target for diagnosis and treatment of OLP. Further studies should investigate the function of NOD in OLP and treatment modalities regulating NODs for clinical benefit.

References

Ahn MY, Yoon HE, Park JH, Lee J, Min SK, Ahn SG, Yoon JH. Characterization of NODs and TLRs in innate immune response of human cementoblast cells. Oral Dis 19: 374-380, 2013.

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 124: 783-801, 2006.

Antosz H, Osiak M. NOD1 and NOD2 receptors: integral members of the innate and adaptive immunity system. Acta Biochim Pol 60: 351-360, 2013.

Au J, Patel D, Campbell JH. Oral lichen planus. Oral Maxillofac Surg Clin North Am 25: 93-100, 2013.

Begue B, Dumant C, Bambou JC, Beaulieu JF, Chamaillard M, Hugot JP, Goulet O, Schmitz J, Philpott DJ, Cerf-Bensussan N, Ruemmele FM. Microbial induction of CARD15 expression in intestinal epithelial cells via toll-like receptor 5 triggers an antibacterial response loop. J Cell Physiol 209: 241-252, 2006.

Boughan PK, Argent RH, Body-Malapel M, Park JH, Ewings KE, Bowie AG, Ong SJ, Cook SJ, Sorensen OE, Manzo BA, Inohara N, Klein NJ, Nuñez G, Atherton JC, Bajaj-Elliott M. Nucleotide-binding oligomerization domain-1 and epidermal growth factor receptor: critical regulators of beta-defensins during Helicobacter pylori infection. J Biol Chem 281: 11637-11648, 2006.

Chamaillard M, Hashimoto M, Horie Y, Masumoto J, Qiu S, Saab L, Ogura Y, Kawasaki A, Fukase K, Kusumoto S, Valvano MA, Foster SJ, Mak TW, Nuñez G, Inohara N. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol 4: 702-707, 2003.

Correa RG, Milutinovic S, Reed JC. Roles of NOD1 (NLRC1) and NOD2 (NLRC2) in innate immunity and inflammatory diseases. Biosci Rep 32: 597-608, 2012.

Crincoli V, Di Bisceglie MB, Scivetti M, Lucchese A, Tecco S, Festa F. Oral lichen planus: update on etiopathogenesis, diagnosis and treatment. Immunopharmacol Immunotoxicol 33: 11-20, 2011.

Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrländer C, Nowak D, Holst O, Martinez FD; ALEX-Team. Association between exposure to farming, allergies and genetic variation in CARD4/NOD1. Allergy 61: 1117-1124, 2006.

Gallo C, Barros F, Sugaya N, Nunes F, Borra R. Differential expression of toll-like receptor mRNAs in recurrent aphthous ulceration. J Oral Pathol Med 41: 80-85, 2012.

Girardin SE, Boneca IG, Carneiro LA, Antignac A, Jéhanno M, Viala J, Tedin K, Taha MK, Labigne A, Zähringer U, Coyle AJ, DiStefano PS, Bertin J, Sansonetti PJ, Philpott DJ. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science 300: 1584-1587, 2003.

Hirao K, Yumoto H, Takahashi K, Mukai K, Nakanishi T, Matsuo T. Roles of TLR2, TLR4, NOD2, and NOD1 in pulp fibroblasts. J Dent Res 88: 762-767, 2009.

Hysi P, Kabesch M, Moffatt MF, Schedel M, Carr D, Zhang Y, Boardman B, von Mutius E, Weiland SK, Leupold W, Fritzsch C, Klopp N, Musk AW, James A, Nunez G, Inohara N, Cookson WO. NOD1 variation, immunoglobulin E and asthma. Hum Mol Genet 14: 935-941, 2005.

Inohara N, Koseki T, Lin J, del Peso L, Lucas PC, Chen FF, Ogura Y, Núñez G. An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. J Biol Chem 275: 27823-27831, 2000.

Inohara N, Chamaillard M, McDonald C, Nuñez G. NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. Annu Rev Biochem 74: 355-383, 2005.

Jaafari-Ashkavandi Z, Mardani M, Pardis S, Amanpour S. Oral mucocutaneous diseases: clinicopathologic analysis and malignant transformation. J Craniofac Surg 22: 949-951, 2011.

Janardhanam SB, Prakasam S, Swaminathan VT, Kodumudi KN, Zunt SL, Srinivasan M. Differential expression of TLR-2 and TLR-4 in the epithelial cells in oral lichen planus. Arch Oral Biol 57: 495-502, 2012.

Jeon DI, Park SR, Ahn MY, Ahn SG, Park JH, Yoon JH. NOD1 and NOD2 stimulation triggers innate immune responses of human periodontal ligament cells. Int J Mol Med 29: 699-703, 2012.

Kutikhin AG. Role of NOD1/CARD4 and NOD2/CARD15 gene polymorphisms in cancer etiology. Hum Immunol 72: 955-968, 2011.

Lange C, Hemmrich G, Klostermeier UC, López-Quintero JA, Miller DJ, Rahn T, Weiss Y, Bosch TC, Rosenstiel P. Defining the origins of the NOD-like receptor system at the base of animal evolution. Mol Biol Evol 28: 1687-1702, 2011.

Lee YY, Chan CH, Hung SL. Chen YC, Lee YH, Yang SF. Up-regulation of nucleotide-binding oligomerization domain 1 in inflamed human dental pulp. J Endod 37: 1370-1375, 2011.

McCartan BE, Healy CM. The reported prevalence of oral lichen planus: a review and critique. J Oral Pathol Med 37: 447-453, 2008.

Netea MG, Gow NA, Munro CA, Bates S, Collins C, Ferwerda G, Hobson RP, Bertram G, Hughes HB, Jansen T, Jacobs L, Buurman ET, Gijzen K, Williams DL, Torensma R, McKinnon A, MacCallum DM, Odds FC, Van der Meer JW, Brown AJ, Kullberg BJ. Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. J Clin Invest 116: 1642-1650, 2006.

Ng LK, Rich AM, Hussaini HM, Thomson WM, Fisher AL, Horne LS, Seymour GJ. Toll-like receptor 2 is present in the microenvironment of oral squamous cell carcinoma. Br J Cancer 104: 460-463, 2011.

Ohno S, Tateishi Y, Tatemoto Y, Morishita K, Sasabe E, Yamamoto T. Enhanced expression of Toll-like receptor 2 in lesional tissues and peripheral blood monocytes of patients with oral lichen planus. J Dermatol 38: 335-344, 2011.

Santoro A, Majorana A, Bardellini E, Festa S, Sapelli P, Facchetti F. NF-kappaB expression in oral and cutaneous lichen planus. J Pathol 201: 466-472, 2003.

Sciubba JJ. Autoimmune oral mucosal diseases: clinical, etiologic, diagnostic, and treatment considerations. Dent Clin North Am 55: 89-103, 2011.

Scott MJ, Chen C, Sun Q, Billiar TR. Hepatocytes express functional NOD1 and NOD2 receptors: a role for NOD1 in hepatocyte CC and CXC chemokine production. J Hepatol 53: 693-701, 2010.

Scully C, Carrozzo M. Oral mucosal disease: Lichen planus. Br J Oral Maxillofac Surg 46: 15-21, 2008.

Shigeoka AA, Kambo A, Mathison JC, King AJ, Hall WF, da Silva Correia J, Ulevitch RJ, McKay DB. Nod1 and nod2 are expressed in human and murine renal tubular epithelial cells and participate in renal ischemia reperfusion injury. J Immunol 184: 2297-2304, 2010.

Siponen M, Kauppila JH, Soini Y, Salo T. TLR4 and TLR9 are induced in oral lichen planus. J Oral Pathol Med 41: 741-747, 2012.

Srinivasan M, Kodumudi KN, Zunt SL. Soluble CD14 and toll-like receptor-2 are potential salivary biomarkers for oral lichen planus and burning mouth syndrome. Clin Immunol 126: 31-37, 2008.

Sugawara Y, Uehara A, Fujimoto Y, Kusumoto S, Fukase K, Shibata K, Sugawara S, Sasano T, Takada H. Toll-like receptors, NOD1, and NOD2 in oral epithelial cells. J Dent Res 85: 524-529, 2006.

Swain B, Basu M, Samanta M. NOD1 and NOD2 receptors in mrigal (Cirrhinus mrigala): inductive expression and downstream signalling in ligand stimulation and bacterial infections. J Biosci 38: 533-548, 2013.

Tang L, Zhou XD, Wang Q, Zhang L, Wang Y, Li XY, Huang DM. Expression of TRAF6 and pro-inflammatory cytokines through activation of TLR2, TLR4, NOD1, and NOD2 in human periodontal ligament fibroblasts. Arch Oral Biol 56: 1064-1072, 2011.

Thompson MR, Kaminski JJ, Kurt-Jones EA, Fitzgerald KA. Pattern recognition receptors and the innate immune response to viral infection. Viruses 3: 920-940, 2011.

Uehara A, Sugawara Y, Kurata S, Fujimoto Y, Fukase K, Kusumoto S, Satta Y, Sasano T, Sugawara S, Takada H. Chemically synthesized pathogen-associated molecular patterns increase the expression of peptidoglycan recognition proteins via toll-like receptors, NOD1 and NOD2 in human oral epithelial cells. Cell Microbiol 7: 675-686, 2005.

Uehara A, Takada H. Functional TLRs and NODs in human gingival fibroblasts. J Dent Res 86: 249-254, 2007.

Uehara A, Takada H. Synergism between TLRs and NOD1/2 in oral epithelial cells. J Dent Res 87: 682-686, 2008.

van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med 32: 507-512, 2003.

Zilbauer M, Dorrell N, Elmi A, Lindley KJ, Schüller S, Jones HE, Klein NJ, Núnez G, Wren BW, Bajaj-Elliott M. A major role for intestinal epithelial nucleotide oligomerization domain 1 (NOD1) in eliciting host bactericidal immune responses to Campylobacter jejuni. Cell Microbiol 9: 2404-2416, 2007.

Abstract (In Korean)

구강편평태선에서 NOD 유사수용체의 발현

<지도 최 종 훈 교수>

연세대학교 대학원 치의학과

강 진 규

구강편평태선은 구강점막에 흔히 발생하는 만성염증성 질환으로 현재까지 그 원인이 명확히 밝혀지지 않았으며, 완벽한 치료법이 없고, 대증요법에 한정되어 있어 잘 치료되지 않는다.

선천면역반응은 인체가 병원균에 대항하는 1차 방어기전으로, 이러한 선천면역반응의 이상은 심각한 감염성 질환이나 만성염증성 질환, 자가면역 질환을 유발할 수 있다. NOD 유사수용체는 선천면역반응에 관여하는 단백질로, 구강내에서 항상성 유지와 염증성 질환의 발병에 중요한 역할을 하는 것으로 알려져 있지만, NOD와 구강편평태선의 연관성에 대하여는 아직 보고된 바가 없다.

본 연구의 목적은 NOD와 구강편평태선의 연관성을 알아보는 것이며, RT-PCR과 면역조직화학염색을 통하여, 구강편평태선 환자에서 NOD1과 NOD2의 발현을 관찰하였다.

구강편평태선 환자에서 대조군에 비해 NOD1과 NOD2의 발현이 현저하게 증가되었으며, 특히 NOD2가 더욱 높게 발현되었다. NOD1과 NOD2는 구강편평태선의 발병과 유지에 중요한 역할을 하는 것으로 보이고, 특히 NOD2가 더욱 연관되어 있을 것으로 생각되며, NOD1과 NOD2는 구강편평태선의 진단과 치료에 새로운 목표가 되리라 사료된다.

핵심되는 말 : NOD 유사수용체 1, NOD 유사수용체 2, 선천면역반응, 구강편평태선