Up-regulation of Nucleotide-binding Oligomerization Domain 1 in Inflamed Human Dental Pulp

Ya-Yun Lee, DDS, PhD, Chi-Hang Chan, DDS,† Shan-Ling Hung, PhD,*‡ Yi-Chen Chen, DDS,‡ Yuan-Ho Lee, PhD,§ and Shue-Fen Yang, DDS, MS†‡*

Abstract

Introduction: The innate immune response is activated by recognition of microbial components through specific pattern recognition receptors including nucleotide-binding oligomerization domain (NOD)-like receptors. However, the regulation of NOD-1 in inflamed human dental pulp remains poorly understood. This study aimed to evaluate the expression of NOD-1 in healthy and inflamed human dental pulps. In addition, the secretion of chemokines induced by NOD-1 and the related signaling pathways were studied. **Methods:** Samples of human dental pulp tissues were obtained from freshly extracted wisdom teeth. The protein localization of NOD-1 in the pulp tissues was detected by immunohistochemistry. In addition, human dental pulp fibroblasts were stimulated with NOD-1 agonist γ-D-glutamyl-meso-diaminopimelic acid. Production of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) was determined by an enzyme-linked immunosorbent assay. The involvement of mitogen-activated protein kinase (MAPK) signaling pathways was examined by Western blot analysis, and the association of MAPK signaling with chemokine production was determined. **Results:** The results demonstrated the expression of NOD-1 in normal dental pulp, and up-regulated NOD-1 expression was observed in inflamed dental pulp. On stimulation with NOD-1 agonist, production of IL-8 and MCP-1 was induced in a dose-dependent manner. Moreover, phosphorylation of p38 MAPK and Jun N-terminal kinase (JNK) was enhanced by stimulation of NOD-1. With the treatment of p38 MAPK and JNK inhibitors, the NOD-1–induced IL-8 production was suppressed. **Conclusions:** In response to microbial invasion, the expression of NOD-1 can be regulated in a ligand-inducible manner. NOD-1 might participate in pulp inflammation through chemokine production via MAPK signaling pathways. *(J Endod 2011;37:1370–1375)*

Key Words

Caries, dental pulp, interleukin-8, monocyte chemoattractant protein-1, nucleotide-binding oligomerization domain 1

The oral cavity is an environment constantly challenged with microorganisms. More than 1200 bacteria species have been identified in the oral cavity (1). In addition to the protection provided by surrounding hard tissues, the host immunity plays an important role to prevent the invasion of microbial pathogens in the dental pulp (2). Innate immunity stands as the first line of host defense, which recognizes pathogen-derived molecular signatures via pattern recognition receptors (PRRs). PRRs have been demonstrated to respond to a wide range of exogenous structures such as peptidoglycans, lipopeptides, and lipopolysaccharides (LPS) from bacteria. Toll-like receptor family (TLR) is the best-studied family of pattern recognition molecules. It has been shown that TLRs are present in the dental pulp, and elevated levels of TLR-2 and TLR-4 expression have been demonstrated after bacterial infection (3).

Recently, another set of cytosolic PRRs, named nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, has been detected (4). NLRs share common characteristics with TLRs in that ligand binding is mediated through a leucine-rich repeat domain. In humans, 22 members in different structures are now being characterized in the NLR family (5). Among them, NOD-1 and NOD-2 mainly detect peptidoglycan on the cell walls of bacteria. Specifically, NOD-1 detects diaminopimelic acid–type peptidoglycan, which is mostly found in gram-negative bacteria; NOD-2 detects muramyl dipeptide, which is a common motif found in both gram-positive and gram-negative bacteria (4). It has been shown that NOD-1 and NOD-2 participate in the innate immune response through the activation of nuclear factor kappa B signaling pathway (6). In addition, NLRs are associated with many chronic inflammatory disorders such as asthma (7), Crohn's disease (6), and atopic dermatitis (8). These findings indicate that in conjunction with complex regulation of environmental and genetic factors, these receptors might play important roles in regulating immune responses.

Pulpitis is a disease primarily caused by mixed infection of gram-positive and gram-negative bacteria (9), and it is a dynamic process because both bacterial virulence and host immunity are the determining factors of the outcome (10). The chemokines, including interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1), direct the migration of polymorphonuclear leukocytes, monocytes, and macrophages to the site of infection (11). IL-8 and MCP-1 production can be stimulated by endodontic pathogens (12). Elevated levels of IL-8 have been reported in the pulp tissues and gingival crevicular fluid from patients with pulpitis (13, 14). Moreover, neurogenic inflammation induces the production of IL-8 and MCP-1 in human dental pulp cells (15).

NOD1/2 signaling has been reported to induce IL-8 expression in various cell types, including oral epithelial cells and monocytic cells (16, 17). NOD-2 has been detected in normal dental pulp (18). However, little is known about the expression and the regulation of NOD-1 in healthy and inflamed dental pulps. The purpose of this study was to evaluate the expression of NOD-1 in healthy and inflamed dental pulps. In

From the *Institute of Oral Biology and †School of Dentistry, National Yang-Ming University, Taipei; ‡Department of Stomatology, Division of Endodontics, Taipei Veterans General Hospital, Taipei; and §Department of Economics, Fo-Guang University, Yilan, Taiwan.

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Address requests for reprints to Dr Shue-Fen Yang, Department of Stomatology, Taipei Veterans General Hospital, No. 201, Sec. 2, Shih-Pai Road, Taipei 11217, Taiwan. E-mail address: sfyang@vghtpe.gov.tw

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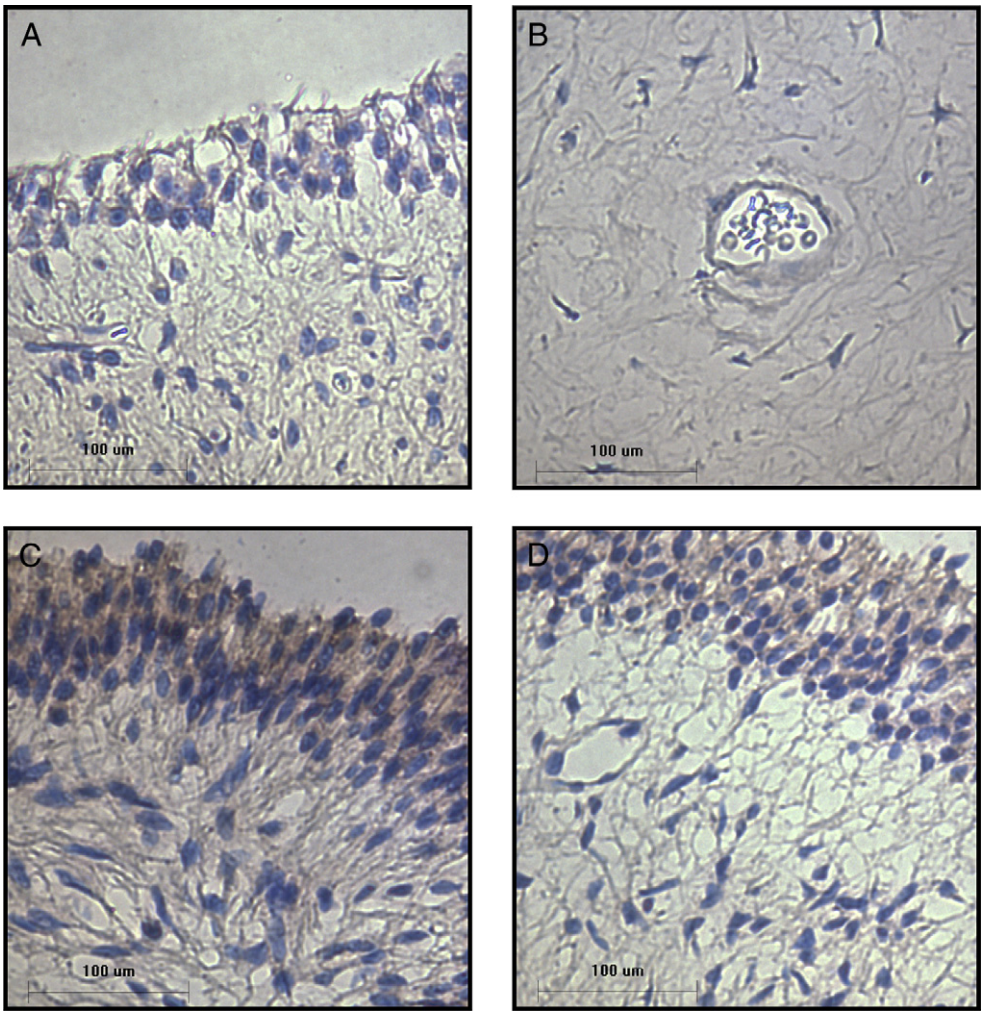

Figure 1. Immunolocalization of NOD-1 in the dental pulp. In healthy dental pulp, positive staining was detected in cytoplasm of odontoblasts (*A*) and vascular endothelial cells (*B*). In samples with carious lesion, NOD-1 staining was strongly enhanced in odontoblasts and cells of pulp connective tissues beneath the odontoblastic layer near the carious lesion (*C*). Distant from the coronal carious lesion, positive NOD-1 staining was observed in the radicular dental pulp (*D*). Original magnification for each photograph was ×400. Scale bars: 100 μm.

addition, the secretion of chemokines IL-8 and MCP-1 induced by a NOD-1 agonist and the related signaling pathways were determined in human dental pulp fibroblasts (HDPFs).

Materials and Methods

Human Dental Pulp Samples

The subjects in this study were recruited from the Department of Stomatology, Taipei Veterans General Hospital, Taiwan. Twenty-six human third molars, including 16 free from caries and 10 carious teeth, were collected for the preparation of dental pulp specimens. The teeth with caries were clinically asymptomatic, and the penetration of carious lesions was 1–2 mm into dentin on the radiographs. Written informed consent was obtained from each of the volunteers, and routine surgical procedures were used. All tooth extractions were conducted under the approval of the Institutional Review Board of the Taipei Veterans General Hospital.

Immunohistochemical Staining

Immediately after extraction, the root tips (apical 3–5 mm) were removed, and the tooth was fixed in 4% paraformaldehyde for 48 hours at 4°C. The tooth specimens were further demineralized in 10% ethylenediaminetetraacetic acid solution (pH 7.3) for approximately 10 weeks. After the specimens were fully demineralized, the samples were dehydrated in ethanol, immersed in xylene, and embedded in paraffin. Serial tissue sections (thickness, 6 μm) were collected on silane-coated glass slides (Dako Corp, Glostrup, Denmark) and deparaffinized in xylene, followed by immersion in alcohol. For antigen retrieval, sections were immersed in sodium citrate buffer (0.01 mol/L; pH 6.0) for 20 minutes at 95°C. To block the endogenous peroxidase activity, samples were treated with 3% hydrogen peroxide in ice-cold methanol for 30 minutes. Sections were blocked with blocking solution, Ultra V block (Lab Vision, Fremont, CA), at room temperature for 5 minutes. After being washed with Tris buffered saline Tween-20, the sections were incubated with the rabbit polyclonal anti-NOD-1 antibody (1:1000; Imegenex Inc, San Diego, CA) in UltrAb diluents (Lab Vision) at 4°C overnight. Control staining was performed with UltrAb diluents or purified rabbit immunoglobulin G (IgG). Subsequently, sections were washed for 30 minutes in phosphate-buffered saline−0.05% Tween 20 and incubated with biotinylated goat–anti-rabbit IgG secondary antibodies. Afterward, sections were stained with the streptavidin-biotin complex (Dako) according to the manufacturer's instructions. The