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Mini Review

Roles of dental pulp fibroblasts in the recognition of bacterium-related factors and subsequent development of pulpitis

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Summary As caries-related bacteria invade deeply into dentin and come into close proximity to the pulp, inflammatory cells (such as lymphocytes, macrophages and neutrophils) infiltrate into the bacterium-invaded area and consequently pulpitis develops. Many types of cytokines and adhesion molecules are responsible for the initiation and progression of pulpitis. Dental pulp fibroblasts, a major cell type in the dental pulp, also have capacity to produce pro-inflammatory cytokines and express adhesion molecules in response to pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide. The innate immune system senses microbial infection using pattern recognition receptors, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD), for PAMPs. In this review, we summarize the roles of dental pulp fibroblasts in the recognition of invaded bacterium-related factors via TLR and NOD pathways, and the subsequent pulpal immune responses, leading to progressive pulpitis.

Introduction

Pulpitis is characterized as the immune response that is mainly triggered by the invasion of caries-related microorganisms into dentinal tubules and pulp (Fig. 1). In the innate immune response of dental pulp to shallow caries, pulpal dendritic cells (DCs) are considered important in immunosurveillance [1]. Pulpal DCs expressing class II major histocompatibility complex (MHC) molecules localize in the para-odontoblastic and perivascular regions, where these cells capture foreign antigens [2—4]. An increased accumulation of pulpal DCs in the para-odontoblastic area corresponding to the carious dentinal tubules is observed, even in the early stage of dentinal caries [5]. In addition to pulpal DCs, odontoblasts also play a pivotal role in the pulpal innate immune response against caries invasion. Normal odontoblasts express beta-defensin, which induces antimicrobial activity [6], and interleukin-8, which is a pro-inflammatory cytokine [7,8]. Transforming growth factor (TGF)-beta, which is important in anti-inflammatory activity

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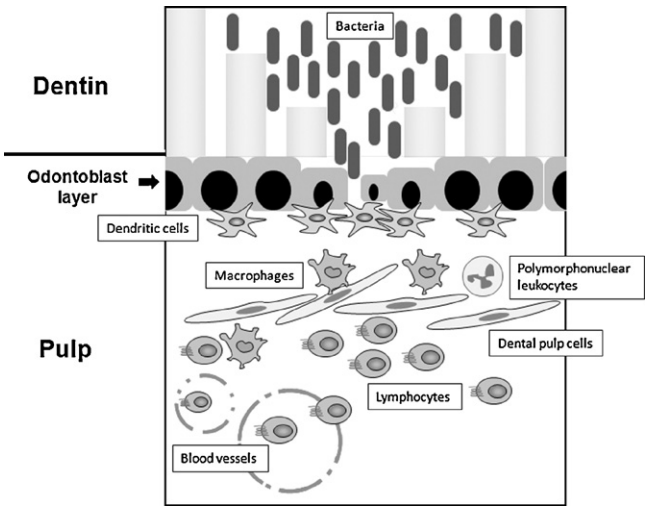

Fig. 1 A schematic illustration of dental pulp responses to dental caries.

as well as dentinogenesis and repair, is also secreted by odontoblasts [9,10]. These two cell types cooperatively contribute to pulpal responses against carious irritation [11].

As carious infection progresses to the pulp-dentin interface, a decrease in the proportion of Gram-positive aerobic bacteria and an increase of Gram-negative anaerobic bacteria occur [12], and marked infiltration of inflammatory cells is observed in the dental pulp [13—15]. In particular, significantly higher numbers of B cells and plasma cells are found in severe pulpitis together with an increased CD4/CD8 ratio of T cells [13,16]. Various pro-inflammatory mediators such as cytokines and prostaglandins (PGs) are also expressed in the inflamed pulp [7,14,17—25]. With the development of exposure to bacterial components, partial destruction of the odontoblast layer along with severe damage or death of odontoblasts can be observed, and the underlying dental pulp cells including fibroblasts and undifferentiated mesenchymal or stem cells in the cell-rich zone are activated to participate in the host response and initiate reparative dentin formation [26—28]. Thus, the dental pulp cells, a major cell type in the dental pulp, play a crucial role in maintaining the structural integrity of connective tissues, and they also have capacity to produce pro-inflammatory cytokines and express adhesion molecules in response to pathogen-associated molecular patterns (PAMPs), which are structures expressed by microorganisms [29—34]. Generally, the initial sensing of microbial pathogens is mediated by pattern recognition receptors (PRRs) for PAMPs. The PRRs, such as Toll-like receptor (TLR) and nucleotide-binding oligomerization domain (NOD), have been shown to recognize a number of PAMPs [35]. In this review, we describe the roles of odontoblasts and dental pulp cells in the recognition of invaded bacterium-related factors via TLR and NOD pathways, and the subsequent host responses of dental pulp, leading to progressive pulpitis.

TLRs and NODs in dental pulp

In mammals, the TLR family comprises more than 12 members [36,37]. The TLR family members can be conveniently divided into two subpopulations with regard to their cellular localization. TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 are expressed on the cell surface and recognize microbial membrane components, whereas TLR3, TLR7, TLR8 and TLR9 are expressed in intracellular vesicles such as the endosome and the endoplasmic reticulum and predominantly recognize microbial nucleic acid species. Of the cell-surface TLRs, TLR4 is essential for responses to lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria, which is a potent immunostimulatory molecule [38]. TLR2 recognizes a wide range of PAMPs derived from various pathogens; for example, triacyl lipopeptides from bacteria and mycobacteria, peptidoglycan and lipoteichoic acid (LTA) from Gram-positive bacteria and zymosan from fungi [39,40]. TLR5 recognizes flagellin, a protein component of bacterial flagella [41]. On the other hand, of the intracellular TLRs, TLR3 is implicated in triggering anti-viral immune response, upon recognition of RNA species, such as double-stranded RNA (dsRNA) of viruses and a synthetic analogue of dsRNA:polyinosinic-polycytidylic acid (poly I:C) [42,43]. TLR9 recognizes unmethylated CpG DNA motifs from bacteria and homozoin from *Plasmodium* [44,45].

In addition to TLRs, other cytosolic PRRs such as NOD-like receptors (NLRs) [46] and retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) for intracellular PAMPs exist [47]. NOD1 and NOD2 are well-characterized members of the NLR family, which recognize the monomeric structure of peptidoglycan [48]. NOD1 recognizes γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP), which is a motif found in peptidoglycan from Gram-negative bacteria. In contrast, NOD2 recognizes muramyl dipeptides (MDP), which are minimal motifs present in all peptidoglycans.

Odontoblasts

Immunohistochemical analysis demonstrated that TLR2 and TLR4 are mainly expressed on the odontoblast layer of normal pulp [49,50]. One of these reports shows that LPS-mediated TLR4 activation increased pro-inflammatory cytokines, IL-1β and TNF-α, in the odontoblasts using organotypic tooth crown odontoblast cultures, but TLR2 stimulation with TLR2 ligand (Pam3CSK4, a synthetic lipopeptide) decreased these pro-inflammatory markers, which suggest that pro-inflammatory cytokines and innate immune responses in decayed teeth may result from TLR4 signaling [50]. Moreover, cultured human odontoblast-like cells are highly responsive to Gram-negative bacteria, such as *Prevotella intermedia* and *Fusobacterium nucleatum*, compared with Gram-positive bacteria, such as *Streptococcus mutans* and *Lactobacillus casei*, despite heterogeneity of TLR2 and TLR4 cell-surface expression [51]. On the other hand, experimentally inflamed pulp in a murine model showed that the TLR2 mRNA level was 30-fold higher than the TLR4 mRNA level at 9 h after infection, and the TLR2-positive cells were observed in and around the odontoblast layer and the area infiltrated by inflammatory cells [52]. This report suggested that TLR2 may be mainly regulated during the early stage of pulp inflammation triggered by bacterial infection. Other *in vitro* studies with odontoblast-like cells in culture have also demonstrated that odontoblasts stimulated with LTA, a Gram-positive bacterium-derived component recognized at the cell surface through TLR2, initiate an immune response by triggering up-regulation of TLR2 and production of

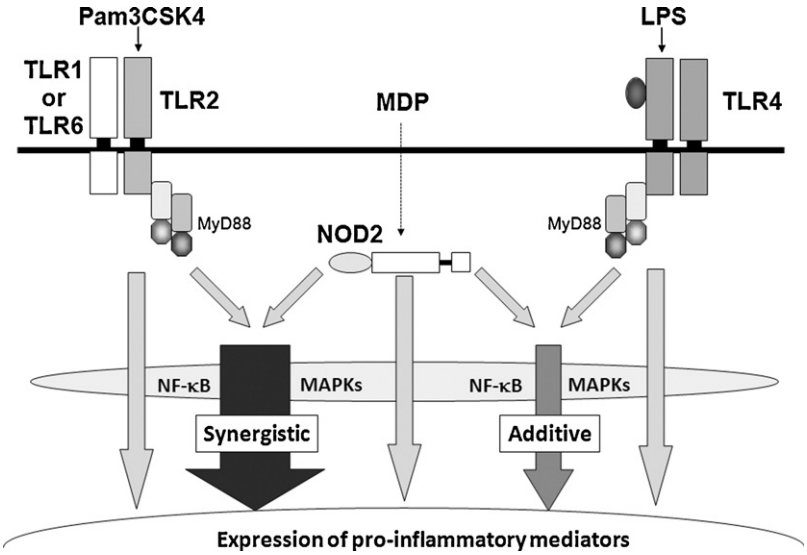

Fig. 2 A possible role of NOD2 in TLR2- and TLR4-mediated signal pathways. NOD2 against MDP acts synergistically with TLR2, not TLR4, agonist to stimulate the production of pro-inflammatory mediators in human dental pulp fibroblasts.

chemokines such as CCL2 and CXCL10 [53,54]. Conversely, LTA-dependent TLR2 activation in odontoblast-like cells did not lead to significant IL-1β and TNF-α production [55], similar to another report with engagement of TLR2 by Pam3CSK4 [50]. These findings suggest a possible role for TLR2-mediated innate immunotolerance to prevent an uncontrolled inflammatory reaction as well as immunostimulation to produce chemokines in odontoblasts.

Besides TLR2 and TLR4, *in vitro*-differentiated odontoblasts constitutively express *TLR1, 3, 5, 6* and *9* genes [53]. A recent study has reported that TLR9 is expressed in the mouse odontoblast-like cell line MDPC-23, a spontaneously immortalized cell line derived from fetal mouse molar dental papillae, and that CpG DNA induces potent pro-inflammatory cytokine expression via the activation of TLR9 [56]. Additionally, an immunohistochemical report revealed that the NOD2 protein expression was localized in odontoblasts and some vascular endothelial cells in normal human dental pulp 57]. However, more detailed studies on the functional role of these PRRs in the odontoblasts to recognize intradentinal irritation of caries-related bacteria are required in future.

Dental pulp cells

Dental pulp cells, especially dental pulp fibroblasts, are known to produce various inflammatory mediators, such as IL-8, IL-6 and vascular endothelial growth factor (VEGF), in response to the components of caries-related bacteria, prior to the discovery and establishment of the innate immune system feature that PRRs including TLRs recognize various PAMPs [29,31,32,58]. TLR2, TLR3, TLR4 and TLR5 expressions have been determined in dental pulp fibroblasts and their specific agonists can induce TLR-mediated inflammatory signals [54,55,59,60], although immunohistological detection of TLRs was not clear in the fibroblasts of dental pulp tissues [49,52]. A recent study has shown that the dental pulp stem cells as well as the dental pulp fibroblasts express TLR4, and that LPS-induced VEGF is dependent upon mitogen-activated protein kinase (MAPK) activation [61].

In our study, flow cytometric analysis showed that the expression level of TLR2 was higher than that of TLR4 in human dental pulp fibroblasts [59]. In accordance with this analysis, the levels of pro-inflammatory mediators, such as CXCL10, IL-8 and PGE_2, induced by LPS stimulation were much lower than those induced by Pam3CSK4. On the other hand, another group has shown that CXCL10 expression in dental pulp fibroblasts was up-regulated by LPS but not LTA, a TLR2-agonist [54]. These results suggest differences of reactivity between Pam3CSK4 and LTA to elicit chemokine production. In fact, our previous report indicates that the dental pulp fibroblasts can produce CXCL10 in response to peptidoglycan, but not LTA [24].

MAPKs have been implicated in many physiologic processes, including cell proliferation, differentiation and death [62—64]. Three major types of MAPKs in mammalian cells are extracellular signal regulated kinase (ERK) 1/2, p38 MAPK and c-Jun NH_2-terminal kinases (SAP/JNK). NF-κB is an oxidation-sensitive transcription factor that plays a critical role in the regulation of various genes that are important in cellular responses, including inflammation, innate immunity, growth and cell death [65]. In TLR2 ligand-stimulated human dental pulp fibroblasts, the phosphorylations of ERK 1/2, p38 MAPK, SAP/JNK and NF-κB were increased, and specific inhibitors of MAPKs or NF-κB markedly reduced the level of pro-inflammatory mediators [66].

We also hypothesized in the previous study that the differences in capacity between peptidoglycan and LTA might be due to NOD proteins, which are intracellular PAMPs, as well as TLR2, and then examined NOD1 and NOD2 expression in human dental pulp tissues and cultured dental pulp fibroblasts. We first examined whether the human cultured dental pulp fibroblasts expressed NOD1 and NOD2, and consequently clear expressions of NOD1 and NOD2 in human dental pulp fibroblasts were found by RT-PCR and flow cytometry [59]. Moreover, both of them constitutively expressed in the dental pulp fibroblasts actually functioned to produce IL-8, IL-6 and monocyte chemoattractant protein-1 (MCP-1). Next, we investigated whether healthy human dental pulp tissues

expressed NOD1 and NOD2 at the mRNA level. As a consequence, NOD2 expression was clearly detected, but NOD1 expression was non-detectable or hardly detected at the mRNA level in the dental pulp tissues; thereafter, using immunohistochemistry, we confirmed whether health dental pulp tissues expressed NOD2 at the protein level. In healthy dental pulp, NOD2 expression was observed in the area just under the odontoblast layer, unlike in the other study described above [57]. Further investigation to resolve this discrepancy remains to be carried out.

Several reports indicated that NOD2 is specifically responsible for the cooperative effect with agonists for selective TLRs, including TLR2 and TLR4 [67—69]. Interestingly, we demonstrated that both TLR2- and NOD2-mediated signals synergize at the production level of pro-inflammatory mediators such as CXCL10, IL-8 and PGE_2 [59]. In contrast, we observed that the additive effect was mediated by interactions between TLR4 and NOD2 intracellular pathways (Fig. 2). This difference between the cooperative effects of TLR2 and TLR4 with NOD2 might be due to their different expression levels in the dental pulp fibroblasts. Since pulpitis is characterized as the immune response triggered mainly by the invasion of caries-related bacteria into dentinal tubules and pulp, pathogen recognition by multiple PRR engagement, including TLR-NOD as shown here, might constitute a key event for the onset of resulting exacerbated pulpal inflammatory response. In particular, peptidoglycan from both Gram-positive and Gram-negative bacteria is recognized by both TLR2 and NOD2. Our findings may lead to the identification of cooperative mechanisms by these multiple PRRs and the selective blocking or inhibition of pulpal inflammation caused by caries-related bacteria.

Conclusion

This review article describes recent findings about the immune system of dental pulp for the recognition of bacterium-related factors. TLRs and cytosolic sensing systems such as NLRs play a crucial role in defending against pathogenic microbial infection through the induction of inflammatory cytokines. Furthermore, the responses of the innate immune system are important not only to eliminate pathogens but also to develop pathogen-specific adaptive immunity. Generally, immune cells such as dendritic cells, macrophages and polymorphonuclear leukocytes are regarded as key players in the innate immune responses. In the dental pulp, odontoblasts and dental pulp fibroblasts are also shown to express several TLRs and NODs. The role of these cells in the progression of pulpitis has not been clarified in detail, but they might act as modulators of both innate and adaptive immune responses. A recent report reveals that TGF-β1 inhibited TLR2 and TLR4 expression and attenuated odontoblast responses, and thus suggests the potential use of TGF-β1 in the clinical treatment of pulpal inflammation [51]. Our recent research demonstrates the anti-inflammatory effect of catechin (bioactive polyphenols in green tea) on human dental pulp cells affected by bacterium-derived factors, especially TLR2 ligand [66,70]. Therefore, understanding of the mechanism underlying PAMP-induced pro-inflammatory reactions in the dental pulp is important for the development of future therapeutic strategies and treatments for pulpitis.

Conflict of interest statement

The authors declare no conflict of interest.

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