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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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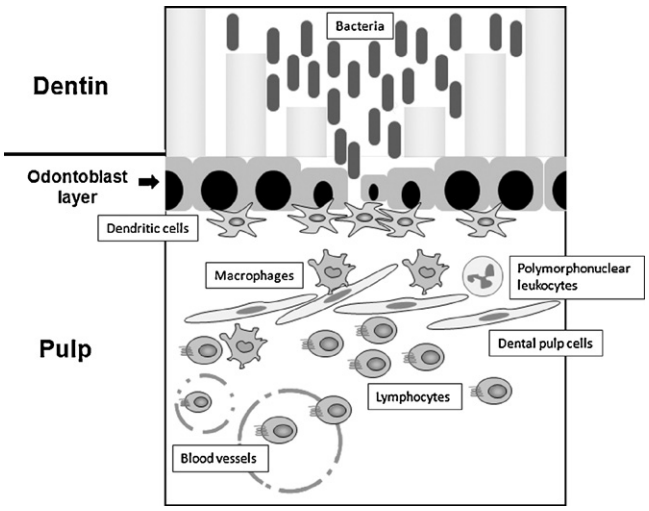

Fig. 1 A schematic illustration of dental pulp responses to dental caries.

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