

Review Article

Potential role of odontoblasts in the innate immune response of the dental pulp

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ABSTRACT

Background: Odontoblasts are the cells lining of tooth's hard structure at the dentin-pulp border, which become the first cells encountered oral microorganisms entering dentin. However, they do not only form a physical barrier by producing dentin, but also provide an innate immune barrier for the tooth. **Purpose:** The aim of this review was to discuss the potential role of odontoblasts in the innate immune response of the dental pulp. **Reviews:** Recent studies have proven that odontoblasts express toll-like receptors, and capable of producing chemokines (i.e. IL-8, CCL2, CXCL2, and CXCL10), and cytokines (IL-1 β and TNF- α) following lipopolysaccharide exposure. Thereby odontoblasts are actively participating in the recruitment of immune cells in response to caries-derived bacterial products. Furthermore, odontoblasts also produce antimicrobial peptides (hBD-1, hBD-2, and hBD-3), and transform growth factor β that induce antimicrobial and anti-inflammatory activities. **Conclusion:** The presence of those innate immune molecules indicates that the nonspecific, natural, and rapidly acting defense may also be an important function of odontoblasts.

Key words: odontoblasts, dental pulp, innate immunity

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INTRODUCTION

Cariogenic bacteria are the major cause of pulpal inflammation and infection. Gram-positive bacteria (*Streptococcus* and *Lactobacillus* spp.) are common oral micro flora detected in shallow dental caries or the outer dentinal tubules of deep dental caries; while gram-negative bacteria (*Fusobacterium*, *Porphyromonas* and *Prevotella* spp.) are found in deep dental caries and infected root canals.¹

Enamel protects the underlying dentin and the connective tissue situated in the dental pulp. Enamel demineralization caused by oral microorganism makes the enamel barrier is disrupted. Oral bacteria and their substances might penetrate into the dentinal tubule and entering the pulp. The dental pulp injury caused by carious lesions is unusual, in that toxins reach the tissue well ahead of the bacteria that release them, eliciting the development of inflammatory and immune reactions in the dental pulp.²

Several cell types contributing to innate and adaptive immunity are present in the tooth pulp such as lymphocytes, macrophages and dendritic cells.³ Odontoblasts, the cells lining of tooth's hard structure at the dentin-pulp border, are the first pulpal cells encountered oral dental pathogens from entering dentin. Due to odontoblasts location is in the periphery of the dental pulp and their cellular extension into dentin, it is likely that odontoblasts also play an important role in innate immune responses of the dental pulp. This hypothesis is based on a paradigm which compares the dentin-pulp complex with the epidermis. Although mesenchymal in origin, the odontoblasts are noted to be epithelial-like morphologically and functionally. The columnar, palisading presentation of the odontoblast layer in the pulp is reminiscent of columnar epithelium, as is the secretory capacity of the cell. The observation that dendritic cells reside within the odontoblast layer of the pulp is analogous with the epidermis, in which Langerhans cells are known to reside in close association with keratinocytes.⁴

It has long been unclear how the immune systems of the dental pulp works, particularly the role of odontoblasts - the first pulpal cells encounter dental pathogens. This review discusses recent progress in understanding the potential role of odontoblasts in the innate immune response of the dental pulp.

Innate immune response

The immune system is composed by two major subdivisions which are the innate or nonspecific immune system and the adaptive or specific immune system. The innate immune system is a primary defense mechanism against invading organisms, while the adaptive immune system acts as a second line of defense. Both aspects of the immune system have humoral and cellular components by which they carry out their protective functions. In addition, there is interplay between these two systems, i.e. cells or components of the innate immune system influence the adaptive immune system and vice versa.^{5,6}

The innate immune system is activated upon the initial invasion of microbes and does not require a period of time for induction. The basic protective strategy of an innate immune system is for the organism to constitutively produce generic receptors that recognize conserved patterns on different classes of pathogens to trigger an inflammatory response that limit pathogen invasion. These receptors include toll-like receptors (TLRs), lipopolysaccharide (LPS)-binding proteins, peptidoglycan recognition proteins, nucleotide-binding oligomerization domains, CD14, scavenger receptors, and C-type lectin which enable mammalian cells to differentially recognize highly conserved microbial structures and consequently mediate innate host responses.⁷

TLRs are members of an evolutionary conserved interleukin-1 (IL-1) superfamily of transmembrane receptors that recognize pathogen-associated molecular patterns. TLRs constitute a major class of microbial recognition receptors. Their activation regulates the production of antimicrobial peptides, cytokines, and chemokines as well as their receptors, thus, consequently controls leukocyte trafficking, T-cell function, and also recruitment and maturation of dendritic cells; thereby, providing a bridge between innate and adaptive immunity.⁵

Recently 12 members of the TLR family have been identified in mammals.⁷ TLR2 alone or TLR2 heterodimerizing with either TLR1 or TLR6 is crucial for the recognition of Gram-positive bacterial cell wall components, including lipoteichoic acid, lipopeptides, and peptidoglycan. TLR3 recognizes viral double-stranded (ds) RNA, while TLR4 plays a major role in detecting LPS, a characteristic component of the cell wall of Gram-negative bacteria.^{5,7}

The components of the innate immune response of the dental pulp to caries include outward flow of dentinal fluid and the deposition of intratubular immunoglobulins, odontoblasts, neuropeptides, innate immune cells, cytokines and chemokines.⁴ The onset of innate immune response

in the dentin-pulp complex is difficult to specify because carious lesions usually progress slowly into the dental pulp. However, before actual carious exposure, the dental pulp beneath shallow caries is capable of evoking innate immune responses to slow down the bacterial invasion.⁸ The extremely rich innervation of the dental pulp can influence the immune response by either directly stimulating immunocompetent cells via neuropeptides or by increasing vascular permeability, which facilitates the delivery and accumulation of immune cells and macromolecules to the inflamed tissue.³

Odontoblast cells

Odontoblasts are the cells responsible for the formation of dentin, the collagen-based mineralized tissue that forms the bulk of teeth. They are derived from ectomesenchymal cells, exhibit a tall columnar shape, and establish a continuous single layer with a clear epithelioid appearance.⁹

The odontoblasts are unique cells. Whilst the cell body of other mineral forming cells is close to the cell process and stays within the calcified matrix, the cell processes of odontoblasts extend a considerable distance into the dentin matrix. In some cases, they may even extend all the way to the outer boundary of the dentin, while the cell body remains in the pulp at the inner boundary. In other words, the cell process extends some distance from its nutritional and controlling centre. The odontoblastic process is extremely fine and resides within dentinal tubules, which is like a capillary tube with a diameter that is much smaller than that of an erythrocyte.¹⁰

After dentinogenesis, odontoblasts are aligned along the periphery of the dental pulp, thus playing a role in the maintenance of tooth integrity owing to their capacity of depositing new layers of dentin throughout life. In addition, newly differentiated odontoblast-like cells may also form a layer of reparative dentin after some tissue injuries. Odontoblasts synthesize and secrete all the matrix constituents and therefore, they exhibit well-developed synthesis organelles. The odontoblast layer is separated from the mineralized dentin by a 10-40 µm-thick layer of unmineralized matrix, the predentin, which is similar to the osteoid that separates the osteoblasts from the bone's mineralized matrix.⁹

In addition to a role in forming dentin, odontoblasts may be involved in sensory transduction. The presence of tight, adhering and gap junctions may imply that these cells communicate with each other; and if one is affected, many others are also affected. Gap junction exists between and among odontoblasts and nerve fibres, and they provide a pathway of low electrical resistance between and among the odontoblasts and nerve fibres. The hydrodynamic effects of fluid displacement within the dentinal tubules or the odontoblasts may activate mechanoreceptors of sensory nerve axons.¹¹

Odontoblasts are also implicated in the regulation of pulp blood flow and in the development of pulp inflammation. The enzyme NADPH-diaphorase, involved

in the production of nitric oxide, is a potent vasodilator present in the odontoblasts. Their capacity to synthesize the inflammatory mediator PGI_2 has been demonstrated and this may excite nerves in the vicinity resulting in a brief hyperalgesia.¹²

The earliest signs of pulp reaction to insults (such as dental caries) are morphological changes and an overall reduction in the number and size of odontoblast cell bodies. The disruption in the underlying odontoblast cell layer occurs even before the appearance of inflammatory changes in the pulp. The nuclei of the cells may be aspirated into the dentinal tubules due to the outward flow of tubular fluid, or the cells may be irreversibly damaged which results in the release of tissue injury factors affecting neighbouring odontoblasts and underlying connective tissue. Cells may undergo vacuolization, ballooning degeneration of mitochondria, and reduction in the number and size of the endoplasmic reticulum.^{13,14}

DISCUSSION

The initial step of an innate immune response is the detection of pathogens through specialized pattern recognition receptors present in the cell membrane of immune and non-immune cells, among which TLRs are key participants.^{5,15} Previous studies have proven that odontoblasts constitutively express TLR1, 2, 3, 4, 5, 6, and 9.¹⁶ This large range of TLRs expressed by odontoblasts appears comparable to what has been reported for cultured epithelial cells, including keratinocytes, intestinal epithelial cells, and gingival epithelial cells. Thus, odontoblasts might be involved in the recognition of bacterial products such as triacetylated lipoprotein, lipoteichoic acid (LTA), diacylated lipoproteins, peptidoglycans, LPS, flagellin, and unmethylated CpG motif-containing DNA, and also of viral dsRNA.^{17,18}

Dental caries is caused by Gram-positive and Gram-negative bacteria. Odontoblasts express TLR2 and TLR4 on

the cellular processes and cell surfaces, suggesting a capacity of odontoblasts to receive signals from Gram-positive and Gram-negative bacteria in tooth decay (Figure 1).^{19–22} As mentioned earlier, TLR2 is crucial for the recognition of Gram-positive bacteria components, including LTA, lipopeptides, and peptidoglycan. Meanwhile, TLR4 is the predominant receptor for LPS, a characteristic component of the cell wall of Gram-negative bacteria.^{18,23}

TLR activation initiates the effectors phase of the innate immune response, mainly through the activation of the NF- κ B pathway.²⁴ This includes the production of pro-inflammatory cytokines and chemokines that recruit and activate blood-borne inflammatory cells.^{25,26} LPS-mediated TLR4 activation increases production of pro-inflammatory cytokines IL-1 β and TNF- α in odontoblasts. Those cytokines act on vascular endothelial cells at the site of infection to induce the expression of adhesion molecules that promote extravasations of phagocytes during inflammation.³

An *in vitro* study by Levin *et al.*²⁷ has revealed that odontoblasts are capable of producing IL-8 following exposure to bacterial LPS. IL-8 is a chemokine which has numerous functions and is considered to be the primary regulatory molecule in the acute inflammatory response. It has been shown to be chemotactic for neutrophils, T lymphocytes, and basophils, to stimulate neutrophil degranulation and oxidative burst activity, and to stimulate histamine release from mast cells. In addition, IL-8 has been shown to induce increased expression of the cell adhesion molecule MAC-1 on neutrophils, which enhances the adhesion of neutrophils to vascular endothelium.²⁸

In addition, unstimulated odontoblast cells also express several chemokine genes including CCL2, CXCL4, CXCL12, and CXCL14.²⁹ Following LTA stimulation, CCL2, CXCL2, and CXCL10 genes and two corresponding proteins (CCL2 and CXCL10) are clearly up-regulated.¹⁶ CCL2 is a key inflammatory chemokine produced during microbial infection that attracts immature DC and also monocytes, activated T cells, NK cells, and basophils

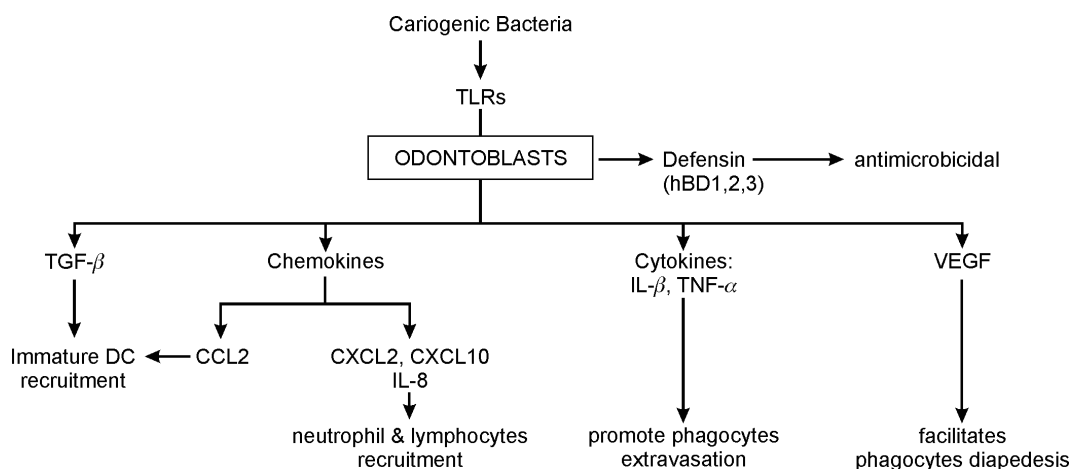


Figure 1. The innate immune response of odontoblasts in responding to cariogenic bacteria.

through CCR1 and CCR 2, thereby facilitating their interaction with invading bacteria. Furthermore, through up-regulation of CXCL2 and CXCL10 expression, odontoblasts are likely to contribute to the recruitment of neutrophils and lymphocytes, respectively, during infection.²⁶

Chemokines not only induce cell locomotion but also influence angiogenesis.³⁰ Among chemokines expressed by odontoblasts, CCL2, CXCL2, and CXCL12 are proangiogenic, whereas CXCL4, CXCL10, and CXCL14 are angiostatic.³¹ The production of angiostatic chemokines in the healthy dental pulp might be involved in the maintenance of blood vessels outside the odontoblast layer. During dental caries-induced inflammation, the number of capillaries is augmented in the pulp under the lesion, and some of them penetrate into the odontoblast layer.³² The expression of the proangiogenic chemokine, CXCL2, is strongly up-regulated in LTA-stimulated odontoblasts, suggesting that CXCL2 might thus contribute to the increased vascularization by binding to CXCR2 that is highly expressed on endothelial cells.³⁰

An *in vitro* study by Bofero *et al*³³ has revealed that odontoblast-like cells stimulated by LPS up-regulate vascular endothelial growth factor (VEGF) expression suggesting a novel role for odontoblasts in the regulation of pulpal angiogenesis. Up-regulated VEGF synthesis by odontoblasts stimulated with LPS might increase the permeability of existing pulp blood vessels, thus facilitating the process of diapedesis of neutrophils, lymphocytes, and monocytes. Furthermore, it might also recruit new blood vessels to the area closest to the carious lesion to enhance the access of the blood-derived antibodies and defense cells to protect the pulp tissue against bacterial challenge.

Previous studies^{21,34} have demonstrated that odontoblasts express human β -defensin (hBD)-1, hBD-2, and hBD-3. Defensins are a group of small (3-5kDa), cationic, cysteine-rich B-sheet peptides which have a broad spectrum of antimicrobial activity and are involved in the innate host defense.³⁵ Expression of defensins correlates with inhibition of bacterial RNA, DNA, and protein synthesis, as well as with reduced bacterial viability.³⁶ hBD-1 and hBD-3 display broad antimicrobial activities against Gram-positive and Gram-negative bacteria, fungi, and adenovirus. hBD-2 has antimicrobial activity against *Streptococcus mutans*³⁷ and has a high antimycotic potency as well as being a chemoattractant for NK cells, memory CD4⁺ T cells, and immature DC.³⁸ hBD-2 may initiate or enhance the cytokine-induced pro-inflammatory reaction of odontoblasts as well.³⁹

In healthy pulps, transforming growth factor beta (TGF- β) is secreted by odontoblasts,⁴⁰ and its expression is increased under carious lesions.⁴¹ Generally, TGF- β has a proinflammatory function during the initial stages of inflammation, while having anti-inflammatory effects during the later stages. The proinflammatory functions of TGF- β include immune cell recruitment and induction of matrix metalloproteinase secretion.⁴² TGF- β stimulates

accumulation of immature dendritic cells in odontoblast and subodontoblast layers of the pulp horn close to the lesion in locations strategic to encounter foreign antigens entering the dentinal tissue. After capture of foreign antigens at the dentin-pulp interface, dendritic cells migrate, while undergoing a process of maturation, via the afferent lymphatic to regional lymph nodes, to stimulate naïve T-lymphocytes; thus, initiating a primary immune response. During the later stage of inflammation, TGF- β exhibits anti-inflammatory effects through repression of lymphocyte proliferation, TLR signaling, and antigen-presenting dendritic cell and macrophage activation.⁴³

In conclusion, beside their function in forming and maintaining dentin, odontoblasts are also capable of recognizing and responding to microorganisms and thus, eliciting the immune cells. Odontoblasts express TLRs, and are capable of producing chemokines (i.e. IL-8, CCL2, CXCL2, and CXCL10), and cytokines (IL-1 β and TNF- α) following lipopolysaccharide exposure; thereby, actively participating in the recruitment of immune cells in the response to caries-derived bacterial products. Furthermore, they also produce antimicrobial peptides (hBD-1, hBD-2, and hBD-3) and TGF- β that induce antimicrobial and anti-inflammatory activities, respectively. The presence of these innate immune molecules indicates that the nonspecific, natural, and rapidly acting defense may also be important function of odontoblasts.

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