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Effects of inflammation in dental pulp cell differentiation and reparative response

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The responsiveness of the dentin–pulp complex is possible due to the stimulation of dental pulp cells, which begin to synthesize and secrete dentin matrix. The inflammatory process generated by harmful stimuli should be understood as a natural event of the immune response, resulting in the recruitment of hematopoietic cells, which cross the endothelial barrier and reach the site affected by the injury in order to eliminate the damage and provide an appropriate environment for the restoration of homeostasis. The repair process occurs in the presence of adequate blood supply, absence of infection, and with the participation of pro-inflammatory cytokines, growth factors, extracellular matrix components, and other biologically active molecules. Prostaglandins and leukotrienes are bioactive molecules derived from the metabolism of arachidonic acid, as a result of a variable range of cellular stimuli. The aim of this review is to describe the process of formation and biomineralization of the dentin–pulp complex and how pro-inflammatory events can modify this response, with emphasis on the lipid mediators prostaglandins and leukotrienes derived from arachidonic acid metabolism.

KEYWORDS

dental pulp, biomineralization, inflammatory mediators, prostaglandins, leukotrienes

Introduction

The responsiveness of the dentin–pulp complex to injury is possible by the stimulation of odontoblasts, which begin to synthesize and secrete reactionary dentin matrix, Dentin matrix secretion by primary odontoblasts that survived the tissue injury occurs via signaling molecules similar to those involved in the dentinogenesis phase (1–3). Odontoblasts therefore represent the first line of defense against bacterial invasion in this environment (4, 5).

The inflammatory process is important to fight infection and is essential to protect the tissue from injury and restore its physiological function. Cytokines and various signaling molecules are synthesized and secreted by the host cells of the dentin–pulpal complex prior to the recruitment and activation of immune system cells, which reveals that the dentin–pulpal complex generates a molecular immune response pattern prior to the cellular immune response (6).

Repair, therefore, occurs in the face of adequate blood supply and with the participation of pro-inflammatory cytokines, growth factors, components of the extracellular matrix, and other biologically active molecules (7, 8). The aim of this review is to describe the process of formation and biomineralization of the dentino-pulpal complex and how pro-inflammatory events can modify this response, with emphasis on the lipid mediators prostaglandins and leukotrienes derived from arachidonic acid metabolism.

Formation of the dentin–pulp complex

Deciduous teeth begin their formation between the 3rd and 8th week of intrauterine life, and their development involves a series of complex steps regulated by interactions between epithelial and mesenchymal tissues derived from the ectoderm (ameloblasts) and the neural crest (odontoblasts) (9, 10).

For tooth development, ectomesenchymal cells derived from the first branchial arch and neural crest migrate and form cell aggregations (11). The ectoderm thickens and generates sprouts that invade the neural crest-derived mesenchyme. The adjacent epithelium then starts sending signals to the mesenchyme that undergoes condensation around the epithelial band, which undergoes proliferation and surrounds the mesenchyme of the dental papilla (9). The epithelial cells that undergo the differentiation process start secreting enamel matrix and are called ameloblasts, while the differentiated mesenchymal cells, now called odontoblasts, secrete dentin (11).

After complete differentiation, odontoblasts are characterized by a tall columnar shape, polarized nuclear and cytoplasmic organelles, and are united to each other by junctional complexes (12). These are therefore post-mitotic cells, organized in the form of a peripheral cell layer, present along the dentin–pulp interface that have cellular processes extending within tubular structures surrounded by dentin, called dentinal tubules (8, 12, 13). After the cell differentiation process, odontoblasts synthesize organic matrix consisting of type I collagen, and play an important role in the mineralization of this matrix by secreting proteoglycans and non-collagen proteins that participate in nucleation and control of mineral phase growth (8, 14).

During dentin formation, odontoblasts secrete a matrix rich in type I collagen, which is called pre-dentin, thus constituting the organic phase, which in turn is mineralized through the incorporation of hydroxyapatite (HA) crystals through the biomineralization process, which involves mechanisms that control both the sites and the deposition rate of these crystals (14, 15).

Molecules involved in dentin biomineralization

Alkaline phosphatase (ALPL) is an indispensable enzyme for mineralization of the secreted matrix, as it provides phosphate ions that generate the precipitation of apatite minerals, and hydrolyzes inorganic pyrophosphate, a phosphate ester that inhibits mineralization (16). Alkaline phosphatase is found in four isoforms (isozymes), including Tissue Non-Specific Alkaline Phosphatase (TNAP) (17). The mineralization of the dentin matrix itself starts in plasma membrane-derived matrix vesicles composed of proteins and lipids, which allows the accumulation of high concentrations of calcium ions (Ca^{2+}) and phosphate (PO_4^{3-}), and the participation of Non-Specific Tissue Alkaline Phosphatase (TNAP). This enzyme encoded by the ALPL gene leads to modification of the extracellular matrix and expression of Phosphatase Orfan 1 (PHOSPHO 1); this being the initiating factor for HA deposition inside matrix vesicles (14). Collagen fibers also participate in the enucleation and growth of apatite crystals (18), as do non-collagen proteins characterized by acidity due to their high doses of aspartic and glutamic acids, and phosphorylated serine residues (19).

Among the proteins involved during the mineralization phase are Dentin Matrix Protein 1 (DMP-1) and Dentin Sialophosphoprotein (DSPP) (12, 20). DMP-1 is expressed in both pulp and odontoblastic cells and plays an important regulatory role in odontoblast differentiation, as well as participating essential in both the earliest and most advanced stages of odontogenesis (15). DMP-1-mediated mineral deposition begins when this protein binds to calcium ions, because the peptide arrangement of DMP-1 presents domains corresponding to the structure of HA crystals, which in turn reduces activation energy and favors the formation of structurally functional crystalline nuclei (21).

The DSPP once secreted is rapidly cleaved into COOH-terminal and NH2-terminal fragments. The latter is encoded by the 5' portion of the Dspp gene, resulting in Dentin Sialoprotein (DSP) and proteoglycans, while the 3' portion is responsible for encoding the NH2-terminal portion, which generates Dentin Phosphoprotein (DPP); these two being the most commonly found non-collagen proteins in the dentin matrix (22–24).

DSPP is a member of a family of proteins called SIBLINGs, an acronym for Small Integrin-Binding Ligand N-linked

Glycoproteins, which also include Bone Sialoprotein (BSP or IBSP), Dentin Matrix Protein-1 (DMP-1), Osteopontin (OPN), and Extracellular Matrix Phosphoglycoprotein (MEPE) (25, 26). These proteins play an important role in dentinogenesis and can be considered markers of differentiated odontoblasts (27).

DPP contains a large amount of aspartic acid and phosphoserines, which allows it to be characterized as a polyanionic molecule. The negative charge distributed along this protein increases its affinity for calcium ions and exposes them, in this way, to the collagen fibers present in front of the mineralized layer, allowing the growth of HA crystals, which gives it importance during the maturation phase of mineralized dentin (26, 28).

The increase in Dspp gene expression occurs through signaling pathways whose participation involves Bone Morphogenetic Protein-2 (BMP-2) and the Runt-related Transcription Factor (Runx2) (29).

Bone Morphogenetic Proteins (BMPs) are signaling molecules that are part of the Transforming Growth Factor β (TGF- β) superfamily (30). In addition to increasing Dspp gene expression, BMP-2 also plays a very important role in regulating the differentiation process of dental pulp cells into odontoblasts, and they are capable of both producing and cleaving this protein (31).

Runx2, in turn, is a transcription factor expressed in the papilla and dental sac and is involved in the differentiation of odontoblasts and osteoblasts. This transcription factor has the ability to increase DSPP expression in immature odontoblasts, as opposed to fully differentiated cells, which reveals that the effect of Runx2 is influenced by the differentiation state of the odontoblast cell (32). During tooth development, Runx2 is expressed in the dental mesenchyme until the cap stage and then has its expression is stopped in the dental papilla during the odontoblast differentiation stage, suggesting that this gene is important in tooth morphogenesis (32).

The process of terminal differentiation of odontogenic cells results from molecular interactions that occur between dental epithelium and ectomesenchymal cells, involving BMPs, fibroblast growth factors (FGF), and transcriptional factors such as Msx, which support the epithelium-mesenchymal interactions crucial for the onset of tooth development (33). The Msx gene is a member of the homeobox gene family expressed during the early stages of craniofacial formation, including the condensation of the ectomesenchymal tissue of the tooth germ (34). The MSX1 transcription factor induces mesenchymal cell proliferation and prevents odontoblast differentiation at the hood stage by inhibiting the expression of BMPs, including BMP-2 (35).

A non-collagenous protein present in the dentin matrix secreted by odontoblasts is integrin-bound sialoprotein (IBSP) (36). It is an acidic glycoprotein expressed by osteoblasts, odontoblasts, and cementoblasts during the early stages of mineralization and has the ability to bind to HA via sequential polyglutamine acidic bonds (37). IBSP is present in matrix vesicles and constitutes one of the initiating proteins of HA crystal deposition. This process is possible thanks to the synergistic action of the ALPL enzyme, because in the presence of IBSP, high levels of this enzyme are able to induce the initiation of mineral deposition (29).

Osteocalcin (BGLAP) is revealed as the main non-collagenous protein produced by odontoblasts and osteoclasts, whose role is to regulate the organization of the extracellular matrix through interaction with HA, protein matrix, and surface receptors (38).

In summary, odontoblasts secrete specific dentin matrix proteins, such as DSP and DPP derived from DSPP. Dentin also presents in its composition collagen proteins, IBSP, DMP-1, BGLAP, and ALPL that share common regulatory pathways through transcription factors RUNX-2 and MSX-1 (39, 40).

Immune system in the dentin–pulp complex

In addition to the important role of odontoblasts in the process of dentin matrix synthesis, as described earlier, these cells are also important sensory structures of the pulp organ, as they have the ability to detect bacterial invasion during the development of dental caries and sequentially initiate the immune response in the pulp (8).

The cells of the innate immune system possess receptors that recognize pathogen-associated molecular patterns (PAMPs), which include bacterial components such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA) (41, 42). Through pattern recognition receptors (PRRs), odontoblasts are able to respond to the invasion of pathogens Toll-like receptors (TLRs), specifically TLR2 and TLR4, and Leucine Rich Nucleotides (NLRs), the most prominent being the NOD2 receptors, capable of recognizing peptideglycans present in Gram-positive and Gram-negative bacteria and thus activating the MAPK and NF-κB signaling pathways, in order to produce pro-inflammatory cytokines (43). The interaction of TLR-2 with LTA, a structure present in the cell wall of Gram-positive bacteria, promotes nuclear translocation of the transcription factor NF-κB, and generates the production of chemokines, including CCL2, CXCL1, CXCL2, CXCL8, and CXCL10, recruits immature dendritic cells (4, 44, 45) and reduces the expression of type 1 collagen and DSPP, components of the dentinal matrix (44). The TLR4 receptor recognizes LPS present in Gram-negative bacteria and consequently increases the expression of important pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-8 (46). Furthermore, TLR4 receptors may play a crucial role in the immune response by activating and regulating pulp stem cell proliferation and migration processes (47).

The inflammatory process generated under harmful stimuli should be understood as a natural event of the immune response, resulting in the recruitment of hematopoietic cells, which cross the endothelial barrier and reach the site affected by the injury in order to eliminate the damage and provide a suitable environment for the restoration of homeostasis (48). This requires the activation of PRRs, the release of mediators, such as Leukotriene B_4 (LTB_4) and Prostaglandin E_2 (PGE_2), with LTB_4 being the main mediator in the recruitment of polymorphonuclear cells (49–52).

Inflammatory lipid mediators - prostaglandins and leukotrienes

PGs and LTs are bioactive molecules derived from the metabolism of arachidonic acid, a polyunsaturated fatty acid derived from cell membrane phospholipids, by action of the enzyme Phospholipase A2 (PLA2). PLA2 are a group of proteins that have the ability to hydrolyze the fatty acid at the sn-2 position of glycerophospholipids, especially the PLA2 IV group (cPLA2), as a result of a variable range of cellular stimuli (53–55). Because these are molecules generated from the oxidation of carbon 20 of the polyunsaturated fatty acid they are called eicosanoids (from the Greek eikosi = twenty) (54, 56, 57).

Free arachidonic acid can be metabolized via the Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) pathways and generate Prostaglandins (PGs) or Thromboxanes (TX), or it can be oxidized along the Lipoxygenase (LO) pathway, which includes the enzyme 5- Lipoxygenase (5-LO), to produce different classes of LTs and Lipoxins (54).

One of the pathways of AA metabolism is the 5-LO pathway. In the presence of FLAP, a membrane-associated nuclear protein, the 5-LO enzyme is activated and oxidizes AA, converting it to 5S-hydroxyperoeicosatetraenoic acid (5S-HpETE), which is further reduced by the enzyme peroxidase to 5S- hydroxyeicositetetraenoic acid (5S-HETE) or is converted to LTA4, which by action of LTA4 hydrolase, results in LTB_4 (58). These mediators are involved in chemotaxis of neutrophils, dendritic cells and T cells, increase vascular permeability and act directly on antigen presenting cells (59). Chemotaxis is correlated to the activation of the BLT1 receptor in addition to the BLT2 receptor, with the latter showing low affinity for LTB_4 (60).

PGs, in turn, are produced by a sequence of events involving the actions of the COX-1 and COX-2 enzymes and have their structural basis consisting of prostanoic acid, which is composed of a cyclopentane ring and two carbon chains, and are known as prostanoids (61).

Importantly, the enzyme COX-2 has its expression induced by a range of stimuli related to the inflammatory response, such as growth factors and cytokines, and is therefore considered the inducible isoform of COX. This enzyme is responsible for the synthesis of PGs involved in the inflammatory response, although they are expressed in organs such as the brain and kidneys under physiological conditions. PGs derived from COX-1 are involved in the maintenance of biological functions (62). The enzyme COX metabolizes AA and converts it into the intermediate isoform of PGE_2: PGH2, which is converted to PGE_2 by means of the microsomal Prostaglandin E synthases 1 and 2 (mPGE-1 and mPGE-2), and this mediator acts on 4 different types of membrane receptors (EP1, EP2, EP3, and EP4) coupled to G proteins (Gαs, Gi and Gq) and, depending on the type of receptor stimulated, different cellular pathways are activated (63).

Thus, the induction of the inflammatory process generates PG release (64), which causes increased local blood flow, increased vascular permeability (when associated with other soluble factors, such as leukotrienes), and sensitization of afferent nerve fibers, generating hyperalgesia by acting on peripheral sensory neurons, in sites of the spinal cord and brain. These mediators contribute to the amplification of the pattern of inflammatory response, in order to promote both the increase and prolongation of the effects and signals produced by pro-inflammatory agents (62).

It is known that experimentally the presence of LPS generates in the pulp an increase in AA metabolism, resulting in increased gene and protein expression of COX-2 and increased production of PGE_2 in the inflammatory environment, which is involved in the increased vascular permeability of the pulp (65–67). LTB_4 production and its receptor expression (BLT1 and BLT2) were shown to be almost parallel to neutrophil infiltration, which reveals involvement of this mediator in the infiltration of these cells in experimental pulp inflammation (66, 68).

In intense inflammatory pulp conditions, the deposition of reactive dentin by odontoblasts can be stopped (8, 69), and dentin repair can be performed from stem cells present in the dental pulp. By modifying the local environment, these cells have their behavior and differentiation potential affected, because when primary odontoblasts die, they are able to undergo differentiation into cells called odontoblast-like cells, which start to secrete dentin matrix and deposit it in the form of repair dentin (1, 2, 70, 71). It should be noted that mesenchymal stem cells are potentially immunoregulatory structures, endowed with anti-inflammatory function, with the capacity for self-renewal and multilineage differentiation, and capable of producing structures similar to the original ones in the dental pulp (72). Thus, the immune response directed to infectious processes involves complex molecular mechanisms with coordinated actions, the eicosanoids being lipid mediators derived from arachidonic acid capable of regulating homeostatic and inflammatory processes. However,

the amount of eicosanoids produced is in dependent on the activation state and the physiological condition of the tissue (73).

Eicosanoids regulate the innate immune response by presenting immunomodulatory properties, highlighting the production of PGE_2 (64). PGE_2 is able to bind to 4 different types of receptors (EP1, EP2, EP3, and EP4, also known as PTGR1, PTGER2, PTGER3, and PTGER4). The EP1 receptor is coupled to the Gq protein, and once activated, increases the intracellular calcium concentration. EP2 and EP4 receptors are coupled to the Gs protein and, upon activation, are able to increase intracellular cyclic AMP (cAMP). On the other hand, the EP3 receptor is coupled to the Gi protein and, unlike the EP2 and EP4 receptors, decreases cAMP formation. However, PGs generated from COX-2 and localized to the nuclear membrane can control nuclear pathways through interaction with peroxisome proliferator-activated receptor (PPAR), which regulates nuclear events of cell growth and survival (74, 75). This event occurs in some cases when PGs and their metabolites bind on these nuclear receptors, and PGE_2 can indirectly activate the PPARδ receptor (75).

Involvement of lipid inflammatory mediators in biomineralization

Not only the modulation of cell proliferation was affected by lipid inflammatory mediators but also the expression of important genes inducing dentinal matrix mineralization (76, 77). Previously, PGE_2 has already been shown to have an anabolic effect on osteoblast proliferation and differentiation and induce IBSP transcription (78).

Growth factors such as BMP-2 and bioactive molecules from the dentinal matrix are important signaling molecules for dental pulp stem cells, stimulating them to differentiate into odontoblast-like cells (79). BMP2 protein is essential for the control of dentinal matrix mineralization and is correlated to the differentiation of dental pulp cells (80).

PGE_2 induces BMP-2 production in culture of stem cells extracted from tendon, and consequently promotes differentiation into cells of the osteoblastic lineage (81). On the other hand, LTB_4 is known to favor the resorption process in bone tissue, as it recruits clastic cells and inhibits the blast differentiation process, even in the presence of stimulation with BMP-2 (82).

In addition to the expression of Ibsp and Bmp2, the transcriptional factor Runx2 also had gene expression stimulated by PGE_2 within the first 6 h of stimulation (76). Runx2 is the main gene controlling the differentiation process of odontoblasts and is expressed in odontoblast-like cells and in dental pulp stem cells in the region of reparative dentin deposition (83), which makes it the transcriptional factor promoting the differentiation of pulpal stem cells so that they are able to form reparative dentin (84).

The literature reports that this mediator has its production increased in cases of experimentally induced pulpal inflammation (65) and also was able to increase Runx2 gene expression (76). These data turn out to be a great finding, because during the inflammatory process PGE_2 has its production increased through pro-inflammatory cytokines (85) which reveals that this mediator is present in the inflammatory environment in the very first hours. This suggests that PGE_2 is an early inducer mediator of the mineralizing response, because by increasing the expression of Runx2 it tends to favor the deposition of mineralizing matrix, since it is an important transcriptional factor in the regulation of stem cell differentiation and formation of the dental organ (86). PGE_2, in fact, has already revealed a dual role by participating in both resorption and formation of this bone tissue (87). Similar to what was observed in undifferentiated dental pulp cells, human periodontal ligament cells cultured in osteogenic medium and stimulated with PGE_2 at different molarities had increased expression of RUNX2, which demonstrates that PGE_2 is able to modulate the expression of this gene involved in osteogenic regulation (76, 88).

Conclusion

It is well-established that pulp and dentin constitute a single unit, capable of responding to external stimuli, which makes the dentin–pulp complex an important strategic and dynamic barrier to the various injuries suffered by teeth. Given the above, odontoblasts are cells of fundamental importance for the initiation and amplification of innate immune response events in the search for protection of the pulp organ in the presence of pathogens, as well as undifferentiated cells present in the pulp and precursors of odontoblasts, both of which are able to respond to injury by modulating the immune response.

Author contributions

FL-S, GAC, and LSS wrote the first draft of the manuscript. GCCL, MFMA, LHF, and FWGP-S revised the manuscript. All authors approved the final version of this review.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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