# Inflammation and Regeneration in the Dentin-Pulp Complex: A Double-edged Sword

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## Abstract

Dental tissue infection and disease result in acute and chronic activation of the innate immune response, which is mediated by molecular and cellular signaling. Different cell types within the dentin-pulp complex are able to detect invading bacteria at all stages of the infection. Indeed, at relatively early disease stages, odontoblasts will respond to bacterial components, and as the disease progresses, core pulpal cells including fibroblasts, stems cells, endothelial cells, and immune cells will become involved. Pattern recognition receptors, such as Toll-like receptors expressed on these cell types, are responsible for detecting bacterial components, and their ligand binding leads to the activation of the nuclear factor-kappa B and p38 mitogen-activated protein (MAP) kinase intracellular signaling cascades. Subsequent nuclear translocation of the transcription factor subunits from these pathways will lead to proinflammatory mediator expression, including increases in cytokines and chemokines, which trigger host cellular defense mechanisms. The complex molecular signaling will result in the recruitment of immune system cells targeted at combating the invading microbes; however, the trafficking and antibacterial activity of these cells can lead to collateral tissue damage. Recent evidence suggests that if inflammation is resolved relatively low levels of proinflammatory mediators may promote tissue repair, whereas if chronic inflammation ensues repair mechanisms become inhibited. Thus, the effects of mediators are temporal context dependent. Although containment and removal of the infection are keys to enable dental tissue repair, it is feasible that the development of anti-inflammatory and immunomodulatory approaches, based on molecular, epigenetic, and photobiomodulatory technologies, may also be beneficial for future endodontic treatments. *(J Endod 2014;40:S46–S51)*

## Key Words

Dentin, enamel, epigenetic, extracellular matrix, histone deacetylase inhibitors, interleukins, low-level light therapy, migration, pulp, reactive oxygen species

Cellular and molecular responses occur in the pulp in response to dental caries and trauma, and these events can manifest into inflammatory and/or regenerative events at the tissue and cellular levels. The pulp, like any other injured tissue in the body, will initially mount a defense response in an attempt to remove the infection and enable the wound healing response to prevail (1, 2). Clearly, the tooth represents a specialized environment with low compliance with a limited tissue swelling capacity and has a relatively poor lymphatic drainage system. Subsequently, an infection can result in severe discomfort for the patient in the form of a "toothache." As part of the tooth's defense against invading microbes, cells within the pulp release molecular mediators, such as cytokines and chemokines, which lead to the recruitment of inflammatory and immune cells to the site of infection and injury. Subsequently, these cells attempt to eliminate the invading bacteria and remove any resulting host tissue debris (3). The source of the molecular mediators of the inflammatory response is somewhat dependent on the stage of infection because at a relatively early stage of disease it is the odontoblasts that will be involved in the initial environmental sensing and invoking of the innate immune response, whereas at later stages of the disease pulp fibroblasts, endothelial cells, pulp stem cells, and tissue resident immune cells will detect and respond to the bacteria (3–6). Notably, evidence now suggests that regenerative events may be enabled in relatively slowly progressing or arrested caries, whereas these events may be abrogated in rapidly progressing caries in which chronic inflammation may ensue (3).

## Infection and Inflammation in the Tooth

Carious bacterial biofilm composition evolves and adapts as disease progresses through the enamel, dentin, and pulp; in particular, as the environment becomes more anaerobic, the polymicrobial infections become increasingly complex and have a high bacterial diversity (7). Notably, pulp cell and tissue death occurs beneath rapidly progressing carious lesions as the bacteria release toxins and compete for nutrients within the microenvironment. The odontoblasts that are located at the periphery of the pulp are the first cells to encounter the invading bacteria. Several recent studies have shown that they are immunocompetent cells capable of orchestrating an inflammatory response. Indeed, they are able to detect infection within dentin at a relatively early stage because of the diffusion of bacterial components and metabolites within the tubules. In response, they are known to release autocrine and paracrine signaling factors such as chemokines and cytokines as well as antimicrobial peptides targeted at killing the invading microbes (5, 6). The odontoblasts and, subsequently, the pulp fibroblasts, endothelial cells, and stem cells detect bacterial components via pattern recognition receptors (PRRs). Arguably the best characterized family of PRRs is the Toll-like receptors (TLRs), which recognize a range of pathogen-associated molecular patterns. There are 11 members of the TLR family thus far identified, and they are

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This paper is based on a presentation from the International Association for Dental Research (IADR) Pulp Biology and Regeneration Group Satellite Meeting, which was held March 24–26, 2013 in San Francisco, California.

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0099-2399/$ - see front matter



http://dx.doi.org/10.1016/j.joen.2014.01.021

expressed on cell membranes and intracellularly on endosomes. Their expression is not limited to immune cells because they are also detected on structural cells from many tissues in the body. Dentally relevant examples of pathogen-associated molecular patterns that are detected by TLRs include lipoteichoic acids by TLR2, lipopolysaccharides (LPSs) by TLR4, flagellin by TLR5, and bacterial DNA/RNA by TLR9 (8, 9). TLRs 1–6 and 9 have been shown to be present in pulpal tissue and are expressed on odontoblasts, fibroblasts, pulp stem cells, and endothelial cells (4, 5, 10–13). Other PRRs have been shown to be present within the pulp and the nucleotide-binding oligomerization domain (NOD) 1 and 2 proteins are expressed intracellularly in pulp-derived cells. Both NOD 1 and 2 have been detected in odontoblasts, pulp fibroblasts, and endothelial cells, and NOD 1 has been shown to be up-regulated during pulpal inflammation. In addition, the Nod-like receptor family member pyrin domain containing 3, also known as the inflammasome, has recently been shown to be present on odontoblasts and pulp vascular endothelial cells (14–18).

Bacterial ligand binding to TLRs, NODs, and the inflammasome results in the activation of key intracellular signaling pathways involving nuclear factor-kappa B (NF-$\kappa$B) and p38 mitogen-activated protein (MAP) kinase, which result in the elaboration of extracellular cytokine and chemokine secretion (10–13). These secreted molecules are relatively small but highly potent autocrine and paracrine mediators of inflammation and have been shown to be released by odontoblasts, pulpal fibroblasts, stem cells, and tissue-resident immune cells. They generate an intricate signaling network, and binding to their receptors present on several cell types can result in amplification of the inflammatory response within the tissue. Key and well-characterized cytokines and chemokines include interleukin (IL)-1$\alpha$, IL-1$\beta$, tumor necrosis factor alpha (TNF-$\alpha$), IL-4, IL-6, IL-8, and IL-10. In addition, there are many more inflammatory molecular mediators (eg, S100 proteins), many of which are shown as being up-regulated in diseased pulpal tissue (19–23). Indeed, our preliminary studies (Fig. 1*A–C*), which aim to develop an *in vitro* model system, are in good agreement with these previous reports. Our findings show that proinflammatory mediators involved in pulpal and cariogenic disease including *Streptococcus mutans*, TNF-$\alpha$, and IL-1$\beta$ along with the bacterial component LPS are able to stimulate activation of the NF-$\kappa$B pathway, likely via the expressed TLRs, in primary dental pulp cells (24) within 1 hour. This activated intracellular signaling cascade subsequently results in increased cytokine and chemokine gene expression at 4 hours, which also appears to be chronically stimulated at later 24-hour time points in cultures (data not shown). Clearly, such cellular and molecular activity will result in the generation of complex autocrine and paracrine signaling, which are unlikely to be resolved until the bacterial infection is removed.

In addition to the cellular expression of these molecules, the demineralization of dentin by bacterial acids during the carious disease process may also add to cytokine levels because these molecules are known to be present within the dentin matrix and released in an acidic environment (25, 26). The milieu of the signaling molecules released into the extracellular environment will also result in the generation of chemotactic gradients for focused recruitment and activation of immune system cells from the vasculature and surrounding tissue. Immune system cells are also known to be resident in healthy pulp where they are proposed to play a sentinel role; however, as a result of disease, T and B lymphocytes, plasma cells, neutrophils, and macrophages are all significantly increased in levels at the site of the diseased lesion (27–29). The migration of the immune cells through the pulpal tissue and their antimicrobial activity can cause significant collateral host tissue damage. Notably, during the process of immune cell chemotaxis, proteases, such as metalloproteinases, are released, and to combat bacteria, immune cells release reactive oxygen species (ROS) and other potent enzymes, which can cause significant pulp cell and tissue collateral damage. The damage signals released by the host's dying cells can lead to further exacerbation of the proinflammatory response (5, 30, 31).

## Molecular and Cellular Events Underpinning Dental Tissue Regeneration

Tertiary dentinogenic events can occur in response to tissue injury, and data indicate that infection and inflammation strongly impact on the repair processes within the dental tissue. Reactionary dentinogenesis occurs in response to a relatively mild dental tissue injury, such as during the earlier stages of dental caries, and odontoblasts lining the pulp chamber and root canal survive and up-regulate their synthetic and secretory activity. However, the process of reparative dentinogenesis is relatively more complex and occurs in response to a greater intensity of tissue injury, such as a more rapidly progressing carious injury, which initially results in odontoblast death and dentin loss beneath the lesion and may lead to pulp exposure. Subsequently, if conditions are conducive to repair, stem/progenitor cells are recruited to the site of injury where they differentiate to form new odontoblastlike cells that secrete tertiary dentin, resulting in dentin bridge formation above the exposed pulp (32). In addition to restoration of the hard physical structure of the tooth, the soft pulpal tissue architecture will also regenerate beneath the lesion, and angiogenic and neurogenic repair will occur (33–35). Key growth factors involved in signaling pulpal angiogenic and neurogenic events include vascular endothelial growth factor, fibroblast growth factor-2, nerve growth factor (NGF), brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor, and in addition several proinflammatory cytokines exhibit multifunctionality and can also contribute to the signaling of these repair processes (36–47). Clearly, the roles of these cytokines at any given point within the disease and repair process may be context and concentration dependent. Multiple sources, including local cell secretion and the hard and soft extracellular matrices (ECMs), can provide and deliver signaling molecules necessary for repair to the site of injury. Interestingly, many of these growth factors and cytokines in their free form have relatively short half-lives, usually in the order of minutes or seconds (48), and therefore their protected release from the ECM, where they have been sequestered, is likely essential to signaling repair. Notably, it is the odontoblasts that have secreted these signaling molecules into the dentin ECM, where they provide a "fossilized" reservoir of growth factors and cytokines, for future release when required (49–52). Several studies have now documented the importance of the "damage" signals released from dentin ECM not only by carious acids but also by restorative materials, including calcium hydroxide, mineral trioxide aggregate (MTA), and EDTA, which not only promote tertiary dentinogenic events but also the associated repair processes of angiogenesis and neurogenesis (53–56).

Clearly, the molecular signaling necessary is relatively simple in reactionary compared with reparative dentinogenesis (57). Indeed, during reactionary dentinogenesis, the locally released dentin ECM molecules, combined with bacterial components, and relatively low-level cytokines generated by resident cells will likely result in direct up-regulation of odontoblast dentin synthetic and secretory activities. However, in reparative dentinogenesis, after resolution or control of the infection, the signaling molecules derived from the tooth's ECM, such as transforming growth factor (TGF)-$\beta$1, NGF, adrenomedullin, and hepatocyte growth factor, will act