Inflammation and Regeneration in the Dentin-pulp Complex: Net Gain or Net Loss?

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Abstract

The balance between the immune/inflammatory and regenerative responses in the diseased pulp is central to the clinical outcome, and this response is unique within the body because of its tissue site. Cariogenic bacteria invade the dentin and pulp tissues, triggering molecular and cellular events dependent on the disease stage. At the early onset, odontoblasts respond to bacterial components in an attempt to protect the tooth's hard and soft tissues and limit disease progression. However, as disease advances, the odontoblasts die, and cells central to the pulp core, including resident immune cells, pulpal fibroblasts, endothelial cells, and stem cells, respond to the bacterial challenge via their expression of a range of pattern recognition receptors that identify pathogen-associated molecular patterns. Subsequently, recruitment and activation occurs of a range of immune cell types, including neutrophils, macrophages, and T and B cells, which are attracted to the diseased site by cytokine/chemokine chemotactic gradients initially generated by resident pulpal cells. Although these cells aim to disinfect the tooth, their extravasation, migration, and antibacterial activity (eg, release of reactive oxygen species [ROS]) along with the bacterial toxins cause pulp damage and impede tissue regeneration processes. Recently, a novel bacterial killing mechanism termed neutrophil extracellular traps (NETs) has also been described that uses ROS signaling and results in cellular DNA extrusion. The NETs are decorated with antimicrobial peptides (AMPs), and their interaction with bacteria results in microbial entrapment and death. Recent data show that NETs can be stimulated by bacteria associated with endodontic infections, and they may be present in inflamed pulp tissue. Interestingly, some bacteria associated with pulpal infections express deoxyribonuclease enzymes, which may enable their evasion of NETs. Furthermore, although NETs aim to localize and kill invading bacteria using AMPs and histones, limiting the spread of the infection, data also indicate that NETs can exacerbate inflammation and their components are cytotoxic. This review considers the potential role of NETs within pulpal infections and how these structures may influence the pulp's vitality and regenerative responses. *(J Endod 2017;43:S87–S94)*

Key Words

Dentin, granulocytes, neutrophil extracellular traps, polymorphonuclear leukocytes, pulp, reactive oxygen species

Significance

Infection of the tooth's tissues elicits an inflammatory response, and until the infection and inflammation are resolved, dentin and pulp repair mechanisms are impeded. Neutrophils combat the infection within the pulp and release NETs, which encapsulate and kill bacteria. However, NET components can be proinflammatory and cytotoxic. Components of NETs could serve as new prognostic markers and provide novel therapeutic targets to aid in vital pulp therapy.

Previously, we have described how the pulp's response to infection and injury is similar to that of many other tissues in the body (1). Cells of the dentin-pulp complex detect invading bacteria by their expression of a range of pattern recognition receptors (PRRs), which identify pathogen-associated molecular patterns. The PRRs reported as being present in the pulp include Toll-like receptors; nucleotide-binding oligomerization domain proteins 1 and 2; and the Nod-like receptor family member pyrin domain containing 3 complex, also known as the inflammasome. The expression of many of these molecules has been shown on odontoblasts, pulp fibroblasts, pulp stem cells, neurones, and endothelial cells, and they are able to detect several components of the invading bacteria ranging from their DNA to outer membrane components, such as lipopolysaccharides (LPSs) (2–12). Once host cells have detected bacterial components, they induce the expression of antimicrobial peptides (AMPs) as well as invoke the inflammatory cascade with both processes aimed at containing, and ultimately eradicating, the infection (13–15). Initially, because of their location at the periphery of the pulp, it is the odontoblasts (16) that are the first responders; however, as the infection advances, cells deeper in the pulp core, including pulp fibroblasts, endothelial cells, and stem cells, also become involved in the defense reaction (4, 5). In addition, there are immune cells resident in healthy pulp tissue, such as dendritic cells and mast cells, that act as sentinels and also orchestrate the early local immune response (17–21).

At the molecular level, the detection of bacterial components via the PRRs results in the activation of intracellular signaling cascades, with the primary effects being mediated via the nuclear factor kappa B (NF-κB) proteins and p38 mitogen-activated protein kinases (6, 7, 22, 23). These pathways ultimately culminate in the translocation of

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master regulatory transcription factors, such as activator protein 1, signal transducer and activator of trascription, and NF-κB, from the cytoplasm to the nucleus where they activate the gene expression of proinflammatory cytokines and chemokines, such as interleukin (IL)-1α and IL-1β, tumor necrosis factor alpha, IL-4, IL-6, IL-8, and IL-10. Notably, this pool of inflammatory mediators can be added to by the cytokines released from the dentin by bacterial acids during the carious disease process (13–15, 24, 25). Subsequently, these molecules exert both autocrine and paracrine effects that can amplify the inflammatory and immune responses and, in particular, generate chemotactic gradients that lead to the recruitment of immune cells from the vasculature, including T and B lymphocytes, plasma cells, neutrophils, monocytes, and macrophages (17–19). Notably, the extravasation, migration, and antimicrobial defense responses of these immune cells can lead to significant collateral tissue damage. This response and the increasing infection significantly affect the vitality of the pulp tissue and result in extracellular matrix breakdown and the death of resident cells.

During the early stages of disease or when the infection has been minimized, either by the host's immune response or clinical intervention, the tooth tissue may evoke tertiary dentinogenic responses. As has been described in detail elsewhere (26), these responses can be relatively simple in the form of reactionary dentinogenesis, which involves the direct activation of the existing primary odontoblasts, or the response may be relatively more complex in the case of reparative dentinogenesis, which involves orchestrated stem cell responses culminating in the generation of new odontoblastlike cells. We have also previously reviewed and described the links between the inflammatory and the tertiary dentinogenic responses and there is clear cross talk between the 2 processes (26–28). Indeed, it appears evident that many molecules that signal the inflammatory response, such as bacterial components, cytokines, complement, and reactive oxygen species (ROS), can also stimulate aspects of tertiary dentinogenic responses (29–35). Furthermore, signaling pathways, such as the p38 mitogen-activated protein kinase cascade, are also activated during both processes (36). Subsequently, it appears likely that the activity of the intracellular signaling cascades and associated cell responses are dose and context specific. Potentially, the relatively low doses of stimuli present during the early or resolving stages of disease stimulate regenerative responses, whereas more intense stimuli, which occur during active and chronic disease, inhibit regeneration. Interestingly, during incipient disease, when inflammatory levels are likely relatively low, the repair responses elicited may also serve in generating a physical barrier of dental hard tissue that "walls off" the invading bacteria. The dosage effects and responses discussed previously would appear to be somewhat intuitive because it would not be appropriate and potentially result in a waste of cellular resource to attempt to rebuild the damaged tissue while the infection and immune responses are both raging. Notably, the clotting and hemostatic responses will also ensue within the dentin-pulp complex to limit blood loss and provide a scaffold for later tissue repair. Interestingly, knowledge of this process is being exploited for the development of new scaffold materials that provide a framework to enable stem cell–based repair responses (37, 38).

Innate Immune Response to Dental Tissue Infection

Up to 700 bacterial species have been reported in the oral cavity, with individuals harboring up to 200 different species per individual (39). High-throughput nucleic acid sequencing approaches have shown that endodontic infections are highly complex and diverse and can contain well over 100 bacterial genera from several different phyla (40–43). Their polymicrobial nature is dominated by Gram-negative obligate anaerobic bacteria that form complex biofilms extending into dentinal tubules and the root canal network. Notably, likely because of environmental similarities (eg, anaerobic and nutrient availability), many of the bacteria present in deep endodontic infections are also present in periodontal infections (39). The composition and distribution of this biofilm within the tooth's root system make it clinically challenging to eliminate all invading microorganisms (44). As described previously, the dental tissue mounts its own innate immune response, which aims to eradicate the infection and restore inflammatory levels to those conducive for tissue repair (Fig. 1*A–D*). Similar to wound infections occurring at other sites in the human body, it is neutrophils (polymorphonuclear leukocytes) that are abundantly recruited and provide the first line of defense in the innate immune response in pulpal tissue (21, 45, 46). Neutrophils initially mature in the bone marrow, and it is estimated that even during health $\sim$1–2 $\times$ 10^{11} cells are generated per day (47). Because of their role and the increased demand placed on the immune system during infection, their levels released into the bloodstream increase; the cells also become primed, and their longevity increases (48). When circulating and surveying for microorganisms, neutrophils reportedly have an average life span of $\sim$5.4 days, following which point they subsequently undergo apoptosis and are removed by macrophages (49). Their priming, before reaching the site of infection, is important because it aids in their rapid response for pathogen clearance. This peripheral priming is achieved by activation by various cytokines, growth factors, complement, or bacterial components. As described earlier, during infection, a chemotactic gradient within the diseased pulp is generated by cytokines, such as IL-8, complement components, and bacterial peptides (N-formylmethionyl-leucyl-phenylalanine), which instruct the neutrophil to leave the circulation and traverse to the site of infection. The process of neutrophil recruitment involves the steps of tethering, rolling, adhesion, crawling, and, finally, transmigration. The process is initiated by changes on the surface of the vascular endothelium, and this is mediated by proinflammatory mediators released from tissue-resident cells or pathogen-associated molecular patterns. Notably, although neutrophils aim to combat the invading bacteria, it is known that they can also be one of the most significant mediators of local host tissue damage because of their release of ROS and proteolytic enzymes as they traverse the tissue and combat the infection (50).

Neutrophil Antibacterial Mechanisms

Once at the site of infection, neutrophils can use an antimicrobial armamentarium that exploits both intra- and extracellular killing mechanisms (Fig. 2), and they have at their disposal a range of antimicrobial proteins and molecules. After contact with bacteria, the neutrophil can undertake phagocytosis and encapsulation into phagosomes. The neutrophil then destroys the pathogens by intracellular release of ROS (via nicotinamide adenine dinucleotide phosphate oxygenase-dependent mechanisms) or AMPs, such as cathepsins, defensins, lactoferrin, and lysozyme. Notably, these AMPs are not only released by the neutrophil granules into phagosomes but also into the extracellular milieu. Hence, degranulation can provide an extracellular killing mechanism; however, it may also cause further host collateral tissue damage (51–53).

Human neutrophils consecutively form 3 types of granules packed with pro-AMPs and inflammatory proteins during their cellular maturation. Azurophilic (or primary) granules contain myeloperoxidase (MPO and azurocidin), specific (secondary)