

Innate Immune Responses of the Dental Pulp to Caries

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Abstract

Various cells and inflammatory mediators are involved in the initial pulpal responses to caries. This review focuses on the cellular, neuronal, and vascular components of pulpal innate responses to caries. Discussion will include dentinal fluid, odontoblasts, neuropeptides, and neurogenic inflammation, which are not classic immune components but actively participate in the inflammatory response as the caries progress pulpally. Summaries of innate immune cells as well as their cytokines and chemokines in healthy and reversible pulpitis tissues are presented. (*J Endod* 2007;33:643–651)

Key Words

Caries, dental pulp, innate

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Innate immunity is activated upon the initial invasion of microbes. If the innate response is unable to abolish the insult, adaptive immunity is elicited with cellular (cell-mediated immunity) and specific antibody (humoral immunity) responses to enhance the protective mechanisms of innate immunity. In oral mucosa, the innate immune system consists of epithelial barriers, circulating cells, and proteins that block bacterial invasion and eliminate the invading microbes by recognizing microbes or substances produced in infection.

In general, innate immunity is not antigen specific but uses receptors to recognize molecular patterns common to microbes to initiate bacterial internalization and killing (phagocytosis). For example, mannose- and scavenger-receptors are classic receptors for phagocytosis expressed on neutrophils and macrophages. These phagocytes also exhibit certain opsonin receptors for C-reactive protein, fibronectin, and complement 3b (C3b) to facilitate internalization. Another group of receptors are G protein-coupled receptors (GPCRs) and toll-like receptors (TLRs), which do not participate in the ingestion of microbes but activate phagocytic functions (1). GPCRs bind to chemokines, lipid mediators [e.g., platelet activating factor (PAF), prostaglandin E₂, leukotriene B₄] or bacterial proteins, which results in the extravasation of leukocytes and the production of bactericidal substances. Binding of lipopolysaccharide (LPS) to TLR-4 or lipoteichoic acid (LTA) to TLR-2 leads to the induction of chemokines, cytokines, and up-regulation of T cell co-stimulatory molecules (CD86, CD80, CD40), which are important molecules in adaptive immunity. Because of the unique anatomic location of caries bacteria, classic phagocytic killing probably does not occur until the pulp is directly in contact with the caries front. Before actual carious exposure, the dental pulp beneath shallow caries is capable of mounting innate immune responses to slow down the caries invasion. A transition to an adaptive immune response will take place in the dental pulp as the caries front approaches the pulp.

The components of the innate response of the dentin/pulp complex to caries include at least the following six: (1) outward flow of dentinal fluid and the deposition of intratubular immunoglobulins; (2) odontoblasts; (3) neuropeptides and neurogenic inflammation; (4) innate immune cells, including immature dendritic cells (DCs), natural killer (NK) cells, and T cells, as well as (5) their cytokines and (6) chemokines (Table 1). Although the first two items are not classic components of innate immunity, they are uniquely involved in the initial inflammatory response to caries (see the following). 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 (2). Furthermore, sensory nerve fibers in inflamed pulps beneath deep caries express CD14 and TLR-4, which are known receptors for LPS (3). In contrast, pulpal healing is greatly reduced in denervated teeth (4).

The onset of the innate immune response in the dentin/pulp complex is difficult to specify because carious lesions usually progress slowly into the dental pulp. Evidence supports the notion that adaptive immune responses occur in irreversibly inflamed pulps separated by less than 2 mm from a deep carious front (5–8). The transition from an innate to an adaptive response probably occurs during the developing and/or progressing stages of reversible pulpitis under shallow caries. The caries-elicited adaptive immune responses in the dental pulp will be discussed in a separate review paper on adaptive immunity.

Dentinal Fluid and the Deposition of Intratubular Immunoglobulins

Increased outward flow of dentinal fluid as a result of the positive intrapulpal pressure is one of the initial protective responses to caries by the pulp that reduces the

TABLE 1. Components of pulpal innate immunity

Dentinal fluid and immunoglobulins
Odontoblasts
Neuropeptides and neurogenic inflammation
Innate immune cells (not Ag specific)
Lymphocytes: NK cells, T cells
Immature DCs, pulpal DCs
Monocytes and macrophages
Innate cytokines
Chemokines

diffusion of noxious stimuli through the dentinal tubules (9–11). The finding that nonvital teeth have a significantly higher bacterial invasion rate than vital human teeth further supports the protective role of dentinal fluid pressure (12).

The composition of dentinal fluid is not fully determined, but it is considered to be serum-derived tissue fluid containing serum proteins and immunoglobulins (Igs) (13). There is a dynamic change of localization and intensity of Igs deposited in uninfected dentin beneath caries that seems to follow the changes in vascular permeability during inflammation. In the normal pulp, IgG is detected in the interstitial fluid (14, 15) and is localized in the dentinal tubules near the predentin area (16, 17). Beneath shallow caries, IgG1, IgA1, and IgM, but not IgA2, were detected in uninfected dentinal tubules. In teeth with deep caries, IgG1, IgA1, IgA2, and IgM were localized in the uninfected dentinal tubules with high intensity (17). The possible protective functions of antibodies in dentinal fluids can be antigen specific or nonspecific. For example, natural antibodies against *Streptococcus mutans* in serum (18, 19) may diffuse extravascularly into dentinal tubules via the dentinal fluid to react with bacterial antigens in carious lesions (20–22). IgG along with serum-derived proteins such as albumin or fibrinogen can adhere to dentinal tubules and nonspecifically decrease the inward diffusion of antigens (17, 23, 24). On the other hand, products of the degradation of Igs in dentinal tubules might serve as a nutrient source for the caries pathogens (25). Future study of the role of intratubular Igs in the growth of caries bacteria is clearly needed.

The role of complement-mediated bacterial lysis in the initial pulpal response to caries appears to be limited. Although complement in the dentinal fluid may be activated by immune complexing of antibodies to bacterial antigens, Gram-positive bacteria, the predominant flora in shallow caries, are resistant to complement lysis (26, 27). In fact, the strongest association of caries bacteria and complement is found in plaque, and is much weaker in the hard portion of carious dentin (28). However, complement by-products such as C3a and C5a could participate in the initial response by recruiting and activating leukocytes.

Odontoblasts

Odontoblasts, with their cellular processes extending into dentinal tubules, are the first to encounter the caries bacterial antigens. The participation of odontoblasts in the innate immunity against caries is summarized in Fig. 1. They express low levels of interleukin-8 (IL-8), genes related to chemokines (CCL2, CCL26, CXCL4, CXCL12, CXCL14), and chemokine receptors (CXCR2, CCRL1, CCRL2) (29–31). Among these chemokines, CCL2, CXCL12, and CXCL14 are known to attract immature DCs, whereas CCL26 suppresses their recruitment (32–34). Odontoblasts cultured from normal pulps also constitutively express TLRs (TLR-1 through TLR-6 and TLR-9) to recognize various bacterial products (29). When odontoblasts were challenged with LTA, upregulation of TLR-2, TLR-3, TLR-5, and TLR-9 was observed, along with the secretion of chemokines (CCL2 and CXCL10), which attract immature DCs and leukocytes (29). Vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis and vascular permeability (35, 36), was induced when odontoblast-like cells and pulpal cells were challenged

with LTA (37). These findings further support the role of odontoblasts in the innate immune system of the dental pulp against caries invasion.

Domnich et al. (38) reported that normal odontoblasts constitutively express strong beta-defensin-1 (BD-1) and weak beta-defensin-2 (BD-2), similar to epithelial cells (39, 40). BD-2 stimulates odontoblast differentiation and is bacteriocidal against *S. mutans* and *Lactobacillus casei* as well as being a chemoattractant for NK cells, memory CD4+ T cells, and immature DCs (41). Upregulation of BD-2 in oral epithelial cells was induced by bacterial challenge (42). It is not known whether caries induces BD-2 secretion by odontoblasts. If it does, BD-2 may contribute to the accumulation of pulpal DCs beneath shallow caries (43) and to arresting the caries invasion by its bacteriocidal property. A phagocytic property of odontoblasts has also been suggested (20, 21, 44), and further studies are needed to verify this property.

In healthy pulps, transforming growth factor-beta (TGF-β) is secreted by odontoblasts, and its expression is increased in irreversible pulpitis (45, 46). TGF-β is important in dentinogenesis and repair because it promotes matrix metalloproteinase secretion and dentin mineralization (47, 48). TGF-β is proinflammatory during the initial stage of inflammation and recruits immune cells such as immature DCs (49). During later stages of inflammation, TGF-β exhibits anti-inflammatory effects through repression of lymphocyte proliferation, TLR signaling, and the activation of DCs and macrophages (50). Interestingly, TGF-β gene expression was downregulated when primary cultures of odontoblasts were challenged with *Bacillus subtilis* LTA in vitro (29), contrary to the results of other in vivo studies (45, 46). This discrepancy between gene and protein expression is not understood, but may be the result of differences in experimental conditions and/or unique properties of LTA from *B. subtilis* compared to multiple types of LTA derived from caries bacteria.

Neuropeptides, Neurogenic Inflammation, and Caries

Sensory neuropeptides such as calcitonin gene-related peptide (CGRP), substance P (SP), and neurokinin A (NKA) are detected in normal human pulps, with a higher concentration of CGRP than SP or

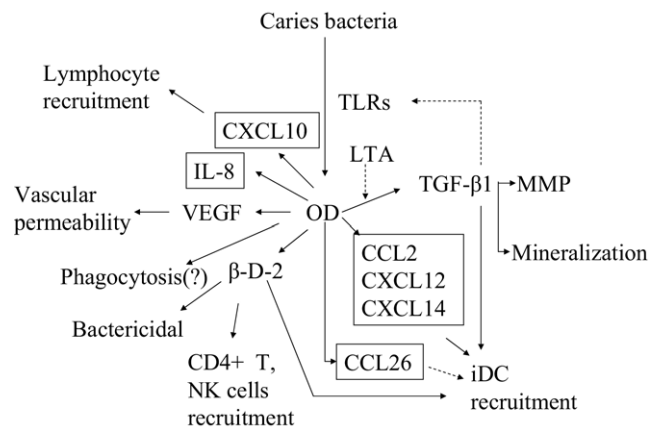


Figure 1. The innate immune defense of odontoblasts in responding to caries invasion. Chemokines secreted by odontoblasts are depicted in text boxes. The dashed lines represent negative regulation. TGF-β suppresses TLR antigen processing, and LTA inhibits TGF-β induction by odontoblasts. A phagocytic function of odontoblasts has been suggested but not confirmed. Abbreviations: β-D-2, beta-defensin-2; iDC, immature dendritic cells; LTA, lipoteichoic acid; OD, odontoblasts; MMP, matrix metalloproteinases; TGF-β, transforming growth factor-β; TLR, toll-like receptor; VEGF, vascular endothelial growth factor.

NKA (51). Arterioles in the dental pulp are heavily innervated by CGRP- and SP-containing fibers (52). Significant increases in innervation (nerve sprouting) and neuropeptide concentration were observed following pulpal injury (53–57). Increased concentrations of neuropeptides were detected in painful pulpitis samples (51, 58). In fact, SP expression was significantly greater in painful pulpitis specimens than in grossly carious asymptomatic specimens (59).

Neuropeptides from sensory nerves participate in neurogenic inflammation (60, 61). These vasoactive neuropeptides, released upon stimulation via an axon reflex mechanism, cause increased pulpal blood flow and vascular permeability (62). For example, CGRP is the prime mediator of neurogenic vasodilatation of arterioles. SP and NKA are the main messengers of postcapillary venular permeability (63). Although CGRP per se does not cause protein leakage, it is able to enhance the exudative response to SP and NKA (64). The end result of neurogenic inflammation is a transient increase of interstitial tissue pressure (61) and outward flow of dentinal fluid (65), which is considered protective as discussed above. If the pulp is healthy, excess interstitial fluid resulting from transient neurogenic inflammation will be absorbed into the circulatory or lymphatic system via edema-preventing mechanisms (66, 67). If pulp is not capable of resolving the increased tissue pressure, increased levels of neuropeptides and persistent edema could contribute to pain and local necrosis (68, 69). The unique intratubular localization of nerve fibers and their involvement in the outward flow of dentinal fluid from neurogenic inflammation make the neural component an important part of the initial vascular response to caries (70).

Neuropeptides and the Immune System

In the inflamed pulp, titers of CGRP and SP are elevated (51, 58, 71) and the number of nerve fibers immunoreactive to CGRP, SP, VIP (vasoactive intestinal polypeptide), and NPY (neuropeptide Y) in the pulp horn region significantly increases with the progression of caries (57). SP is chemotactic to T cells and augments antigen- and mitogen-induced production of interleukin-2 (IL-2) and interferon-gamma (IFN- γ) (72, 73) (Fig. 2). SP also upregulates interleukin-12 (IL-12) production by antigen-presenting cells (APCs) (74) and induces IL-8 production by pulpal cells (75). CGRP and VIP in the inflamed pulp can rapidly recruit immature DCs to sites

of acute inflammation and inhibit the migration of mature DCs to regional lymph nodes (76, 77). VIP is also capable of inducing the maturation of immature DCs, leading to an enhanced production of IL-12 and CD83 (78).

Inflammatory cytokines and chemokines [e.g., interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and IL-8] can induce powerful hyperalgesia (79–81). This is mediated indirectly through the release of prostanoid or nerve growth factor (NGF), upregulating bradykinin receptors, or by affecting sympathetic fibers (82, 83). Furthermore, IL-1 and other inflammatory mediators such as prostaglandins, histamine, and NGF can activate the release of neuropeptides and form a positive feedback loop in the inflammatory process (63, 84).

Immune cells secrete somatostatin and β -endorphin to achieve homeostasis of neurogenic inflammation (57, 85). For example, CD3+ T cells produce more somatostatin and β -endorphin in inflamed than in uninfamed pulps (86). Somatostatin is also a neuropeptide but not vasoactive; it inhibits the antidromic release of SP (87). Somatostatin and CGRP generally suppress T cell proliferation and their cytokine production (IL-2 and IFN- γ) (88). β -Endorphin, an opioid peptide, negates the effect of vasoactive neuropeptides (89, 90). The participation of these molecules in inflammation and pain perception remains to be determined.

Bacteria or bacterial by-products in the necrotic root canal cause periapical inflammation (91, 92). The resulting percussion sensitivity can generally be explained by peripheral and central sensitization mechanisms (53, 93). Interestingly, percussion sensitivity sometimes is associated with vital pulps beneath deep caries. Diffusion of bacterial by-products through the radicular pulp into the periradicular tissues has been suggested as the cause of periapical inflammation, but evidence for this theory is lacking. Matsumoto et al. (94) demonstrated a rapid sensitization of mechanoreceptors in the periodontal ligament when vital pulp was irritated with mustard oil. Animal studies have shown that periapical inflammation begins before total pulpal necrosis (95, 96). Thus percussion sensitivity from teeth with reversible or irreversible pulpitis could result from central sensitization from a long-term pulpal inflammation and/or via an axon reflex in branching nociceptive nerve fibers.

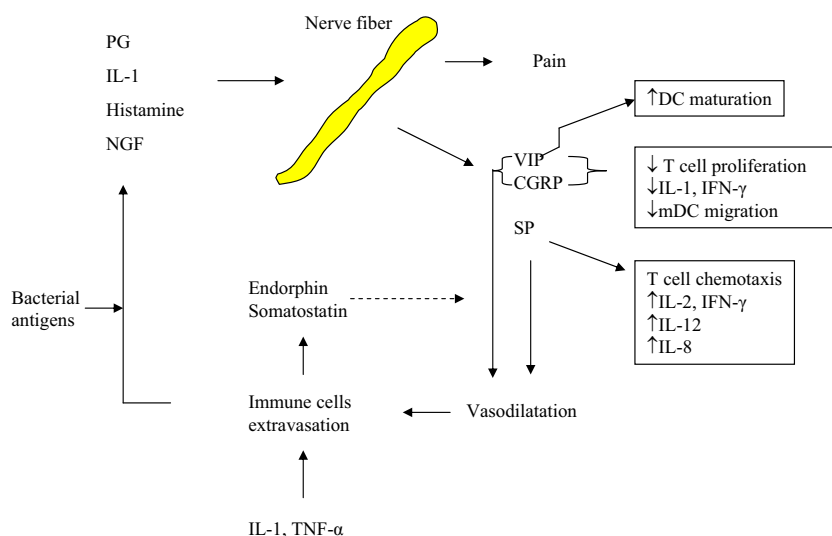


Figure 2. Feedback loop of inflammatory mediators and neuropeptides. Positive feedback loop is denoted with solid lines. Neuropeptides (VIP, CGRP, SP) cause vasodilatation and result in leukocyte extravasation. Inflammatory mediators (PG, IL-1, histamine, NGF) secreted by bacteria-stimulated leukocytes further stimulate the sensory nerves, leading to pain and further release of neuropeptides. Neuropeptides from sensory nerves produce various immunologic effects. Endorphin and somatostatin from immune cells suppress the release of neuropeptides (dashed line). Abbreviations: CGRP, calcitonin gene-related peptide; NGF, nerve growth factor; PG, prostaglandins; SP, substance P; VIP, vasoactive intestinal polypeptide.

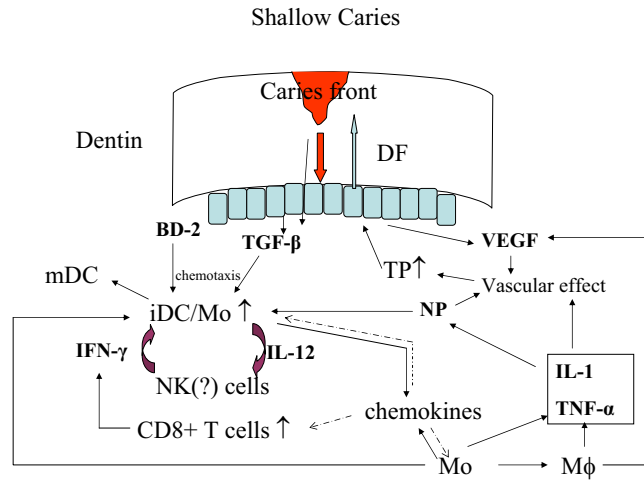


Figure 3. Cellular and molecular events in the dental pulp beneath shallow caries. Increasing pulpal tissue pressure from neurogenic inflammation results in an outward flow of dentinal fluid. TGF-β released from the demineralized dentin and odontoblasts along with BD-2 attracts immature DCs. VEGF, IL-1, and TNF-α secreted from activated macrophages and monocytes promote the vascular effect of neuropeptides. The presence of NK cells in the inflamed pulp is not determined yet. Chemokines for recruiting CD8+ T cells, macrophages, and monocytes in reversible pulpitis tissues are presently unknown (long dashed-dotted lines). Abbreviations: BD-2, beta defensin-2; DF, dentinal fluid; iDC, immature dendritic cell; Mo, monocyte; Mφ, macrophage; NP, neuropeptide; TGF-β, transforming growth factor-β; TP, pulpal tissue pressure; VEGF, vascular endothelial growth factor.

Innate Effector Immune Cells in the Dental Pulp

The principal innate effector cells in most tissues are neutrophils, mononuclear phagocytes (monocytes and macrophages), and innate lymphocytes including NK cells. In the dental pulp, both T cells and immature DCs are considered important in immunosurveillance as part of the innate response to caries. Possible interactions between innate immune cells, cytokines, odontoblasts, and neuropeptides in normal and reversible pulpitis pulps beneath shallow caries are proposed in Fig. 3. Neutrophils and macrophages are professional phagocytes in innate immune responses. Neutrophils may not be important in reversible pulpitis; only a few neutrophils were observed in pulpal tissues under shallow caries (6, 97, 98), and the physical barrier of the dentin prevents close contact between neutrophils and bacteria. Instead, an intriguing phagocytic role for odontoblasts has been suggested (20, 21, 44).

Tissue macrophages are generally derived from circulating monocytes and show a high degree of heterogeneity, which is influenced by their microenvironment. For instance, alveolar macrophages express high titers of pattern recognition receptors with high cytokine induction, whereas macrophages from the lamina propria of the gut exhibit high phagocytic and bactericidal activity but weak production of proinflammatory cytokines (99). Activated macrophages are effective killers that eliminate pathogens in both innate and adaptive immune responses (1), and are also important in tissue homeostasis, through the clearance of senescent cells, and in remodeling and repair of tissue after inflammation. Although the characteristics of macrophages in the healthy dental pulp have not been examined, VEGF, a potent inducer of angiogenesis and vascular permeability, is secreted when mouse macrophages are challenged with LTA (37). Furthermore, the number of macrophages increases with the progression of caries and is always higher than that of DCs at all stages of the caries invasion (100–102). Therefore, these monocyte-derived macrophages may be activated in

the early stage of pulpitis to protect the dental pulp by increasing vascular permeability, and to remove foreign antigens and damaged tissues from the insulated pulp.

NK cells are found in the bloodstream and can respond to inflammatory chemokines by extravasating into inflammatory sites (103). Because both NK cells and immature DCs express similar receptors for chemokines and have the potential to attract each other through their production of various chemokines (104), DCs and NK cells are likely to colocalize in inflamed tissues. DC–NK cell interactions can result in reciprocal activation and increased cytokine production by both DCs and NK cells (105). Activated NK cells promote DC maturation and cytokine production, which further enhances NK cell proliferation, IFN-γ production, and cytotoxicity (106). NK cells are an important source of early IFN-γ production, which not only activates macrophages to destroy phagocytosed microbes but also preferentially promotes type-1 T-cell responses in adaptive immunity (107). The presence of NK cells in the dental pulp may contribute to the high prevalence of IFN-γ mRNA in pulpal tissue beneath shallow caries lesions (Fig. 3) (108).

Because of the abundance of *S. mutans* in early carious lesions, their antigens could be among the early antigens processed by pulpal DCs or macrophages. We demonstrated that *S. mutans* rapidly induced peripheral blood mononuclear cells to yield high titers of IFN-γ and IL-12 (109, 110), and its IFN-γ induction was NK cell and IL-12 dependent (Fig. 4). It is plausible that NK cells and *S. mutans*-induced type-1 cytokines (IFN-γ, IL-12) set the stage for the initial pulpal inflammatory response to caries to be a cell-mediated type-1 immune response. Furthermore, *S. mutans* can rapidly transform monocytes into mature DCs within 24 hours in vitro (111), which may contribute to the local maturation of DCs in inflamed pulps.

T Cells in Innate Immunity in the Dental Pulp

The predominant T-cell type in the normal pulp is the memory CD8+ T cell (5, 6, 112), but its functions in the normal pulp remain undefined. The well-known functions of CD8+ T cells are to kill virus-infected or transformed host cells via induction of apoptosis or perforin production, and to produce IFN-γ to augment phagocytosis. The mech-

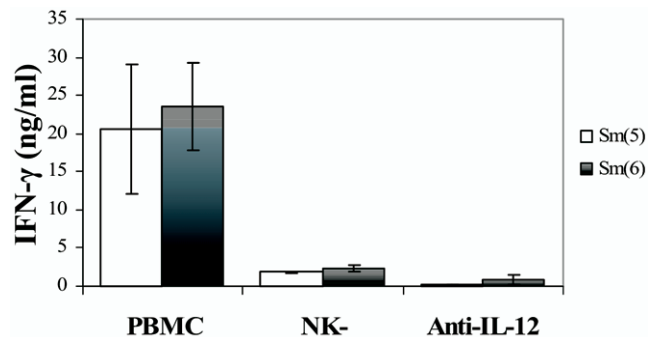


Figure 4. IFN-γ induction by *S. mutans* is NK- and IL-12-dependent. Human PBMCs were prepared according to our previous study (108). NK cells were depleted from the PBMC preparation by a standard microbead method (Miltenyi Biotec, Auburn, CA). PBMC or NK-depleted (NK-) preparations (10⁶/ml) were stimulated with *S. mutans* at 10⁵/ml or 10⁶/ml [Sm (5), Sm (6)] concentration in enriched RPMI medium for 20 hours (108). PBMC cultures (10⁶/ml) pretreated with anti-IL12 antibody (10 μg/ml, R & D) were also examined. The titers of IFN-γ in supernatant fluids were measured with ELISA. Removal of NK cells and anti-IL-12 treatment significantly suppressed IFN-γ induction (p < 0.05). The graph is representative of three experiments (162). Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ; IL-12, interleukin-12; NK, natural killer; PBMC, peripheral blood mononuclear cell; RPMI, Roswell Park Memorial Institute.

anism that preferentially attracts CD8+ T cells in normal healthy pulp was not understood until recently. Studies demonstrated that CD8+ T cells exhibited higher migratory capacity across endothelial cells than CD4+ T cells (113, 114), and memory CD8+ T cells can migrate into tissue sites distant from the site of their initial antigen challenge (115, 116). Therefore, an immunosurveillance role of CD8+ T cells was proposed (114, 117). The recruitment of T cells to noninflamed human skin is partially from a basal expression of E-selectin, chemokine CCL17, and intercellular adhesion molecule-1 (ICAM-1) in dermal vessels (118). Interestingly, endothelium in the healthy dental pulp exhibits low levels of E-selectin and P-selectin (119). Whether CD8+ T cells in normal pulp tissue are part of the immunosurveillance system remains to be determined.

Immature DCs and Pulpal DCs

DCs are a heterogeneous leukocyte population (120). A hallmark of DC physiology is the functional duality represented by two states of maturation, which are tightly linked to tissue homeostasis and inflammation. DCs in healthy peripheral tissues (steady state) are in an immature state characterized by potent microbe-sensing as well as antigen capture and processing capabilities. For example, resident Langerhan's cells in epidermis migrate continuously at a low rate to draining lymph nodes under steady-state conditions, presumably to induce or maintain tolerance to self or to innocuous antigens (121), and monocyte chemotactic proteins are thought to be responsible for the migration of DC precursors into healthy skin (122, 123). At the site of injury, rapid recruitment of immature DCs to acute inflammatory sites is observed in respiratory mucosa in response to chemotaxis by neuropeptides such as CGRP and VIP as well as PAF, CC-, and CXC-chemokines (76, 124). A similar rapid accumulation of pulpal DCs was also observed beneath cavity preparations (101, 125), and an increased number of DCs accumulated under caries (43, 100, 112). Immature DCs are therefore considered to be part of the innate phase of pulpal immune response.

Pulpal DCs expressing class II HLA-DR are dendritic in appearance and localize in paraodontoblastic and perivascular regions (Fig. 5), where they may perform immunosurveillance and capture incoming antigens. A recent study suggests possible functional and phenotypical differences of DCs in these two regions (126). The majority of pulpal DCs (81%) express coagulation factor 13a (FXIIIa+) phenotype, which is the hallmark of dermal DCs (127, 128). FXIIIa is expressed by immature DCs, monocytes, and macrophages (129, 130) but not by monocyte-derived mature DCs (131). The FXIIIa+ pulpal DCs are further divided into two groups: one large group (87%) with monocyte and macrophage markers (CD14+, CD68+), which is similar to a small subset of dermal DCs (FXIIIa+, CD14+, CD1a-); and a small group

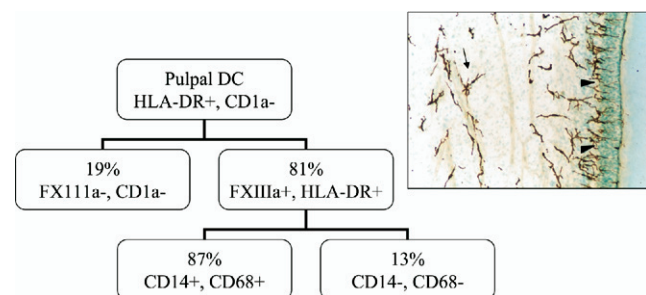


Figure 5. The heterogeneous phenotypes of pulpal DCs. All pulpal DCs are HLA-DR+ and CD1a-, and they are subtyped according to the expression of FXIIIa, CD68, and CD14 [modified from Okiji et al. (132)]. The insert shows the paraodontoblastic (*arrowheads*) and perivascular (*arrow*) distributions of FXIIIa+ human pulpal DCs [reprinted from Okiji et al. (134) with permission].

(13%) of true DCs (CD14-, CD68-, CD1a-) (132). It is believed that the true DC population is located mainly in the odontoblast/preodontin region (133) and that they are capable of migrating to regional lymph nodes to present antigens to naïve T cells (132, 134). Approximately 20% of the HLA-DR+ pulpal DCs express neither FXIIIa nor CD1a; their phenotype and immunologic function needs to be further characterized.

The origin of pulpal DCs has not been determined. Precursors from bone marrow give rise to immature DCs in most tissues, and to circulating monocytes. It is not clear at present if the variety of tissue-resident DCs are directly derived from a common myeloid DC precursor or from a variety of precursor subtypes with distinct tissue-selective homing properties. DCs can be generated in vitro from CD34+ hematopoietic progenitor cells, monocytes, or "committed" DC precursors in blood (120, 135–137). Monocytes exhibit the plasticity necessary to differentiate into macrophages and/or DCs (138, 139), and most pulpal DCs bear monocytic markers (CD14+, CD68+). It is plausible therefore that circulating monocytes could be the common precursors of immature DCs and macrophages in the dental pulp.

Upon recognition of microbial products through pattern recognition receptors (140, 141), resident immature DCs initiate a program of functional maturation, which includes their migration from peripheral tissues to secondary lymph nodes to present antigens to naïve T cells as mature DCs (142). Several factors can induce DC maturation and promote a proinflammatory phenotype: TLR stimulation (with LPS, LTA), CD40 ligand (CD40L), and inflammatory cytokines. Maturing DCs produce high concentrations of proinflammatory cytokines, such as IL-12, IL-1 β , and TNF- α (143), and chemokines (CCL2, 3, 5, and CXCL9, 10, 11) that sustain the recruitment of circulating immature DCs, DC precursors, and T cells to inflamed tissues (144).

Pulpal DCs are not only important in immunosurveillance and adaptive immune responses in pulpal defense but are also closely involved in odontoblast differentiation and regeneration (125). Studies have shown a close spatial proximity between major histocompatibility complex (MHC) class II+ cells, odontoblasts, and nerve fibers in the predentin and odontoblastic layers (133, 145) and a dynamic relationship between pulpal DCs and differentiation of odontoblast-like cells after injury (125). A significant reduction of immunocompetent cells is seen in denervated pulp beneath the dentinal cavity (146). Pulpal DCs and nerve fibers coincrease in number with increasing depth of caries (112). The synchronized accumulation of these two cell types could be explained by the chemotactic property of neuropeptides (76).

Cytokines in Innate Immunity

The cytokines secreted by innate immune cells include TNF- α , IL-1, IL-12, interleukin-18 (IL-18), IFN- γ , IL-6, and interleukin-10 (IL-10) (1). TNF- α and IL-1 act on vascular endothelial cells at the site of infection to induce the expression of adhesion molecules that promote extravasation of phagocytes during inflammation. Rapid induction of IFN- γ production from NK cells and resting T cells by IL-12 and IL-18 directs the subsequent adaptive cellular immune response toward type 1 (107, 147). IFN- γ activates not only phagocytes and APCs, but also potentiates many of the actions of TNF- α on endothelial cells, which include T-cell adhesion and extravasation to sites of infection (148). IFN- γ is also secreted by activated T cells and is important in the adaptive immune response (149).

IL-10, mainly produced by activated macrophages, inhibits functions of macrophages and DCs that control innate immune reactions and cell-mediated immunity. IL-6 is secreted by various cell types in response to microbes or cytokines, particularly IL-1 and TNF- α . IL-6 stimulates the synthesis of acute-phase proteins and neutrophils from

bone marrow progenitors. Small amounts of mRNA expression of IL-1 α , IL-1 β , IL-4, IL-6, IL-10, IL-18, and IFN- γ were detected in normal or asymptomatic pulps (108, 150). Future studies of comprehensive cytokine gene expression in pulps from normal and enamel caries teeth are warranted to understand their role in the initial immune response.

Chemokines in Leukocyte Trafficking

The name *chemokine* is a contraction of *chemotactic cytokine*. Chemokines, produced by the innate immune system (tissue macrophages, immature DCs), odontoblasts, and fibroblasts, recruit leukocytes to sites of infection by increasing the affinity of leukocyte integrins and stimulation of their migration extravascularly. Chemokines not only direct the migration of neutrophils and monocytes but also attract immature DCs and activate effector and memory lymphocytes during infection (151–153). This adhesion-dependent migration, rather than engagement of antigen receptors, ensures that the maximum possible number of previously activated T cells have the opportunity to locate infectious microbes and eradicate the infection. Once in the tissue, T cells encounter microbial antigens presented by APCs such as macrophages or pulpal DCs. T cells that specifically recognize antigens receive signals through their antigen receptors that increase the affinity of integrins for their ligands and bind them to extracellular matrices. T cells not specific for the antigen may return through lymphatic vessels to the circulation. Therefore, the process of migration results in the recruitment of circulating effector T cells to inflammatory sites regardless of their antigen specificity.

Chemokines were originally named after their functions, such as IL-8 and monocyte chemoattractant protein-1 (MCP-1). A standard nomenclature based in part on their chemical structure was later developed, with “L” (for ligand) and the number of the respective gene, such as CXCL8 for IL-8 and CCL2 for MCP-1. Chemokines are divided into four groups (CC, CXC, C, and CX3C), based on the number and arrangement of conserved cysteine motifs. The CC, CXC, and CX3C chemokines are distinguished by the presence between the first two cysteines of zero, one, or three amino acids, respectively. The C chemokine group, distinguished by the absence of the second and fourth cysteines, has only one known member, lymphotactin. For example, interferon- γ inducing protein (IP-10) has one amino acid between the first two cysteines. Therefore, its systemic name is CXCL10. Chemokine receptors are designated according to the type of chemokines they bind (CXC, CC, XC, and CX3C), followed by “R” (for receptor) and a number indicating the order of discovery. For example, CCR2 on immune cells is the receptor for CCL2/MCP-1, CCL8/MCP-2, CCL7/MCP-3, and CCL12/MCP-4.

Homeostatic chemokines expressed constitutively in nonlymphoid tissues such as the gastrointestinal tract, skin, and salivary gland are important in the immunosurveillance and homeostasis of the immune system (32, 153). For example, CCL1/I-309 and CCL17 present on dermal vessels are thought to contribute to the extravasation of the majority of T cells in healthy skin (118, 154). A similar homing mechanism for immature DCs in the healthy pulp was proposed by Durand et al. (29). They demonstrated that odontoblasts from normal pulp constitutively expressed 17 genes related to chemokine pathways, among which CCL2/MCP-1, CXCL12/SDF-1a/b (stromal cell-derived factor), and CXCL14/BRAK (breast and kidney chemokines) are known to recruit immature DCs and monocytes (32, 34, 155).

IL-8/CXCL8 is an inflammatory chemokine and is produced by activated leukocytes and tissue cells during inflammation to attract neutrophils. However, odontoblasts in steady state secrete a low level of IL-8 (30, 31), as do keratinocytes (30, 31, 156). Interestingly, IL-8 is important in wound healing because it stimulates angiogenesis as well as the migration and proliferation of keratinocytes (157–159). The bio-

logic significance of IL-8 secretion by odontoblasts in steady state needs to be further studied. Moreover, approximately two-thirds of the known chemokines exhibit antimicrobial properties (160, 161), and the importance of this antimicrobial activity in early pulpitis is yet to be determined.

Conclusion

The dental pulp is equipped to mount an innate response to invading caries bacteria, which can theoretically slow down bacterial invasion. However, the unique location of caries bacteria seems to prevent their being killed or eliminated by phagocytes. Instead, persistent infection leads to the activation of adaptive immunity and overwhelming inflammation and resultant increased edema and intrapulpal pressure, which becomes detrimental to the pulp encased in a low-compliance environment. On the other hand, clinical experience indicates that inflamed pulps can recover if the majority of the antigens are removed early enough. Knowledge gained of the pulpal inflammatory response may modify our future medicament of choice for moderate to deep caries cases.

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