

Pulpal irritants

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The dental pulp is characterized as a connective tissue and as such it is not considered an external tissue, yet its exposure to external stimuli is constant. This is due to a number of factors including the permeability of attrited or disrupted enamel as well as that of physiologic dentin and cementum. The pulp is extraordinarily sensitive to its external environment. Once thought to be a vestigial organ, it is now understood that the dental pulp is an important tissue whose role in the defense of the dentition may be as significant as its role in odontogenesis.

A variety of stimuli have been demonstrated to have an effect on the pulp. The reactions of the dental pulp to respective irritants are largely dictated by the character and duration of a stimulus. The resultant reaction is manifested by a continuum of disease bracketed by the normal pulp and the necrotic pulp and centrally composed of a gradient of inflammation that progresses clinically from reversible to irreversible (1). Pulpal irritants have been classified as mechanical, thermal, chemical and infective. While the latter has historically been discounted, modern science has proven it to be the most significant cause of pulpal morbidity and mortality. That said, iatrogenic irritants deserve attention particularly in view of the fact that they are most under the control of the clinician.

Mechanical irritants to the dental pulp

Mechanical irritants to the dental pulp can be broken down into two categories, mechanical and biomechanical. Mechanical irritants include orthodontic movement and tooth preparation while biomechanical irritation results from functional and parafunctional forces placed on the dentition during mastication, clenching or bruxing.

Mechanical irritants: operative procedures

Numerous classic studies have confirmed the effects of cavity preparation on the dental pulp (2–6). A

consistent finding of these early studies was that uncontrolled and extreme temperature changes produced during operative procedures were detrimental to the pulp (7) (Fig. 1). One animal study documented that a temperature rise of 5.5°C resulted in necrosis in 15% of monkey pulps (8). Several factors seem to contribute to excessive intraoperative heat generation, all of which can be controlled by the operator. Light pressure, sharp burs, high rotational speeds with proper water coolant and short intermittent cutting strokes prove to be the least irritating to the dental pulp (2).

The inherent insulating capacity of dentin is sufficient to protect the subjacent pulp tissue during restorative procedures unless the remaining dentin thickness (RDT) is small and ineffective water coolant is employed (8). Under these conditions the most consistent histological manifestation of pulpal trauma is the displacement of odontoblasts into the tubules (7) (Fig. 2). There are several theories as to the mechanism of this response. The normal tissue pressure of the dental pulp is between 5 and 20 mmHg, however, subsequent to cutting of dentin, it can exceed 60 mmHg in localized inflamed areas (9, 10). High tissue pressures beneath newly exposed dentinal tubules promote an outward fluid flow that may carry the odontoblast cell body with it into the tubule. While some have argued that the increased tissue pressure is due to inflammation, a more likely cause is a concomitant rise in pulpal blood flow in response to thermal stimulation (11). An alternate theory is that during dry tooth preparation the accompanying desiccation of dentin produces an outward fluid flow

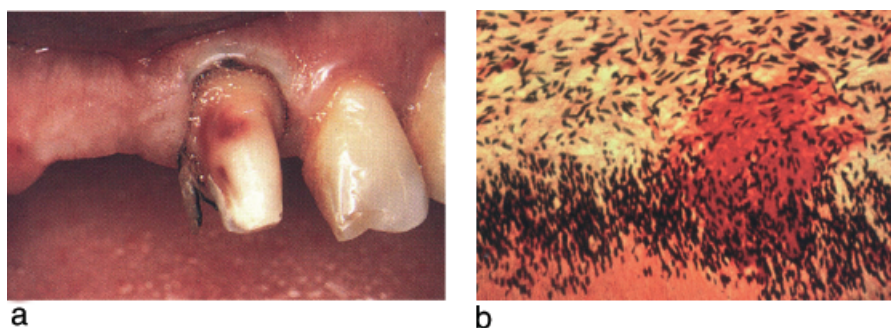


Fig. 1. Excessive heat generated during crown preparation is responsible for the phenomenon referred to as 'blushing'. The color change in the dentin (a) is due to vascular stasis and hemorrhage in the subodontoblastic vascular plexus (b).

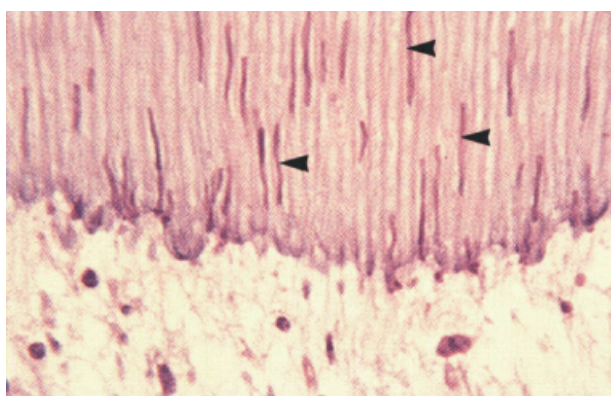


Fig. 2. The arrows depict aspirated odontoblasts in the dentinal tubules. Aspiration results in cell death and necessitates the differentiation of new replacement odontoblasts.

of a magnitude that will force the cellular components into the tubules. While the mechanism is debated, the net result of odontoblast displacement is a disruption of the cell layer that resolves after 20 days (6). During this interval tertiary dentin must be produced by progenitor cells that are initially more fibroblastic in nature and produce less organized fibrodentin (12). Alternately some tubules are not repopulated and become dead tracts (13). Both outcomes produce a more permeable dentin that can facilitate continued or future insult to the subjacent pulp.

Although odontoblast displacement is rarely seen with atraumatic technique, deep preparations using appropriate safeguards can still induce cell damage by transecting odontoblast processes. The extent of the cytoplasmic process of odontoblasts is still under debate but there is general agreement that at least the inner one-third of dentin is occupied by these

cytoplasmic extensions (14). Once transected during the cutting of dentin, the fate of the odontoblast is variable depending on the proximity to the cell body. If the odontoblast is destroyed the potential sequelae are the same as for displacement.

Recent studies have reported that deep cavity preparation not only affects the underlying odontoblasts but also induces an accumulation of HLA-DR-positive cells and protein gene product 9.5-positive nerve fibers (15). This effect appears to be inversely proportional to RDT. A 50% reduction in RDT changed the distribution of HLA-DR-positive dendritic cells in human teeth. A two-thirds decrease in RDT in non-carious teeth stimulated an influx of increased numbers of HLA-DR-positive cells that displaced odontoblasts and extended into the dentinal matrix and associated dentinal tubules beneath the cavities. The odontoblast displacement resolved 2 months postoperatively and dentin sialoprotein (DSP)-positive cells lined the dentin indicating that newly differentiated odontoblasts had repopulated the region. Interestingly this sequence was not observed under preparations in carious teeth. Despite the presence of increased numbers of HLA-positive dendritic cells under carious dentin, odontoblast displacement was not observed. Furthermore, subsequent to caries excavation and restoration, small aggregates of HLA-DR-positive cells, neuronal components and CD45-positive T-lymphocytes persisted implying continued irritation of the subjacent pulp.

It is important to consider that animal studies on the effects of restorative procedures on the dental pulp report the effects on *healthy* pulps. The clinical reality in dentistry is that extensive restorative procedures are frequently performed on teeth with histories of

repeated cycles of disease and intervention that leave them compromised. Hence the need to minimize the morbidity of restorative procedures is even greater and the application of our understanding of the pulpal reaction to dentin manipulation must be even more judicious.

Mechanical irritants: orthodontic movement

The most conspicuous pulpal change observed in response to orthodontic forces is hemodynamic. Both human and animal studies have confirmed that both lateral and intrusive forces result in an increase in pulpal blood flow (16–18). Furthermore, blood flow alterations are not confined to the tooth in active movement. Observed increases in blood flow are seen in teeth adjacent to the focus of movement forces implying that directed forces on one tooth can shunt blood to proximal vessels supplying other oral structures including teeth. If orthodontic forces are extreme, circulatory interruptions can occur resulting in pulpal necrosis (19).

Biomechanical irritation: parafunctional habits

Occlusal loading of teeth effects deformation to varying degrees (20). While enamel is largely resistant to flexure the underlying dentin demonstrates considerable elastic characteristics. As a result, defects in enamel secondary to cracks, decay and/or restorative preparation allow cuspal flexure with subsequent pulpal responses. These responses are mediated by induced dentinal fluid flow.

Multiple factors influence the degree of tooth deformation during occlusal loading. Investigators have noted that preparation geometry has a direct impact on cuspal flexure. The width of the occlusal isthmus relative to the faciolingual dimension of the tooth as well as the ablation of marginal ridges directly impact on the degree of cuspal flexure (21–23). MOD preparations have been shown to effect a 50% reduction in cuspal stiffness and resistance to fracture. Physical properties of the restorative material can also play a part in cuspal flexure. Studies have shown that polymerization shrinkage of certain resin composites can induce an inward deflection of cusps with resultant stresses on tooth structure (24).

Symptomology from cuspal flexure can result from two primary sources. It has been theorized that cuspal

flexure results in dentin deformation thus promoting dentinal fluid flow that activates nerve endings in the odontoblast layer of the tooth. This is supported in part by an *in vitro* study that found that dentinal fluid flow could be induced by occlusal loading of restored teeth (25). A second source of pulpal pain is bacterial microleakage created by a gap at the restoration/dentin interface that is repeatedly opened during cycles of occlusal loading. If repeated cuspal flexure gives rise to a crack, dentinal exposure to bacteria and their by-products is even greater.

Dentinal cracks expose tubules unoccluded by smear layer and, therefore, offer a direct portal to the subjacent pulp. When dentinal tubules are freely exposed there is an outward flow of dentinal fluid driven by relatively high pulpal tissue pressures. Dentinal fluid is composed of proteins such as fibrinogen and serum albumin that can coagulate and effectively block the tubule lumen thereby limiting fluid egress and resultant dentin hypersensitivity. This phenomenon can occur within 2 days. As this serves as a short-term protective mechanism for the pulp, dentinal sclerosis and tertiary dentin formation ultimately can provide greater protection for the pulp and ablation of symptoms.

Chemical irritants to the dental pulp

The effects of restorative materials on the dental pulp have been investigated and seem to relate directly to the permeability of the associated dentin. The degree of dentin permeability, however, is often variable and is governed by several factors including age and caries status (26). Perhaps the most important variable in dentin permeability is the thickness of dentin between the floor of the cavity preparation and the pulp (27).

Unbound components of resin materials and preparative agents such as acid etchants can affect the subjacent pulp by inducing an inflammatory response (28–30). (Fig. 3) This is mediated by the indirect effects of desiccation and/or demineralization of dentin as well as direct effects of the material itself when in contact with pulpal tissue. Studies have shown that the certain cytotoxic components of resin monomers (triethylene glycol dimethacrylate and 2-hydroxyethyl methacrylate) readily penetrate dentin (31). Similarly, eugenol and components (triamcinolone and demeclocycline) of Ledermix® (Wyeth-Pharma GmbH, Münster, Germany) have been shown to pass through dentin into the

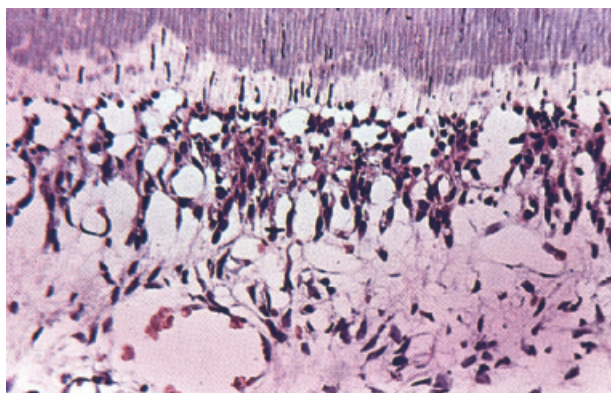


Fig. 3. Chemical irritants applied to dentin can result in damage and disorganization in the subjacent pulp.

subjacent pulp (32, 33). *In vivo* data show that these chemicals have an effect on the pulp, however, the effect seems to be short lived and in the absence of bacteria, reversible (34).

The mechanisms whereby restorative materials exert an injurious effect on the dental pulp are varied. Evidence exists that supports direct and in some instances, prolonged cytotoxicity, stimulation of hypersensitivity reactions or impairment of the host immune response to bacteria. Some of the components of resin restorations are released at cytotoxic levels after polymerization is completed leading to chronic stimulation and a resultant prolonged inflammatory response (35). Furthermore, even subtoxic concentrations of certain agents are capable of eliciting allergic reactions in humans (36). Primates hyperimmunized with BSA showed significant pulpal damage with repeated antigenic challenge in class V cavity preparations suggesting a role for antigen-antibody complex mediated hypersensitivity in tissue destruction (37). In a separate study, exposure to dentin primers elicited a delayed-type hypersensitivity reaction in guinea-pigs (38). These studies taken together present a compelling argument for immune-mediated pulpal tissue damage subsequent to exposure to restorative materials. Foreign body reactions have also been described in pulps containing extruded globules of resin material (39). Histological examination of such pulps shows macrophages and giant cells surrounding the resin particles. Lastly, resin monomers have been shown to decrease the activity of immunocompetent cells in a dose-dependent manner in *in vitro* functional assays (40). While all of these effects are documented, their extent and, therefore, morbidity on the dental pulp is

speculative and doubtless does not act solely to effect pulpal demise. As previously noted, most restorative materials are placed adjacent to pulps that are previously compromised by bacterial insult and that disease, debridement and restoration of the tooth have cumulative effects on the dental pulp.

Pulpal irritation is largely considered to be a negative sequellae, however, the irritant potential of certain restorative materials is central to their usefulness in restorative dentistry. Calcium hydroxide is one of the oldest and most widely used medicaments for stimulation of dentinal bridge formation subsequent to microscopic or gross pulpal exposure. The low-grade pulpal irritation that it induces is important for dentinal bridge formation (41, 42). The degree of inflammation is dependent on the preparation of calcium hydroxide used. Aqueous suspensions of calcium hydroxide applied to exposed pulps effect a superficial necrosis of pulpal tissue covering pulpal parenchyme displaying low-grade inflammation. Within 30 days the tissue subjacent to the necrotic zone has reorganized and resumed normal architecture. Hard setting calcium hydroxide preparations are effective in eliciting dentinal bridge formation with a much smaller to non-existent necrotic zone (43). This is preferable in vital pulp therapies such as the Cvek pulpotomy where maintenance of the maximum amount of vital pulp tissue is desirable and the extent of pulpal inflammation is minimal (44). The irritation potential of calcium hydroxide across intact dentin is dependent on factors such as the RDT and permeability. Application of calcium hydroxide to intact dentin appears to induce sclerosis by promoting crystal precipitation within the tubules accompanied by reductions in permeability (45).

In addition to the direct chemical effects of restorative materials, there are indirect factors that contribute to pulpal irritation. The technique sensitivity of certain materials predispose them to faulty bonds to tooth structure that can translate to dentin hypersensitivity, recurrent disease and pulpal inflammation or necrosis. Much attention has been given to the interface created between resin bonded materials and the dentin. During the etching process, the more highly mineralized peritubular dentin is preferentially dissolved leaving free collagen fibrils and opening lateral tubular branches (46, 47). Applied resin infiltrates the exposed collagen mesh creating a layer 5–10 μm thick referred to as the hybrid layer (48). This layer along with the resin permeating exposed tubules forms the bond between the resin and

dentin. If the preparation is too dry, the collagen fibrils collapse and the resin cannot effectively permeate the mesh, which results in a defective bond. As the optimal degree of hydration of the preparation surface can vary from material to material, resin restoration placement is technique sensitive. This same principle is applicable to the practice of bonding fractured tooth fragments where the segment has become dehydrated while outside of the mouth. Current protocols recommend rehydration of the segment prior to bonding thus increasing the mechanical and presumably the microbial seal (49). This is particularly important with a complicated crown fracture where the pulpal protection by intact dentin is absent.

Table 1. A variety of bacterial virulence factors and their effects are listed

Virulence Factors & Their Effects

Factor	Effect
Adherence	Promotes affinity for host tissues & invasion
Antiphagocytic factors	Host Immune Avoidance
Extracellular Enzymes	Promotes Host Invasion
Hemolysins	Decrease host resistance
Toxins	Host tissue damage
Exotoxins	Host cell death

Microbial irritants

Classic studies in the 1960s underscored the centrality of bacteria in the pathogenesis of the dental pulp (50). Subsequent studies have only strengthened the finding that by far the most noxious irritant to the dental pulp is persistent microbial exposure. Furthermore, the sub-jacent pulp is exquisitely sensitive to infection. Early studies of Brannstrom and Lind (51) illustrated that caries can exert its effects on the dental pulp even before in infection breaches the dentin enamel junction. Thereafter, the progression of infection exerts an increasing effect on the underlying pulp by eliciting defense and repair mechanisms chiefly aimed at decreasing dentin permeability and eradicating pathogens. During this process pulpal injury can occur both as the result of the direct effects of microbial products (Exogenous irritants) and as indirect consequences of microbial activation of non-specific host immune responses (endogenous irritants) (Tables 1 and 2).

‘Exogenous’ irritants

Characterization of the pulpal response to microbial infection has been based on studies of carious human teeth and experimentally induced caries in animal models (for a review see (52, 53)). These studies have confirmed that there are specific ‘fronts’ of attack during the progression of caries and that these fronts are comprised first of bacteria, then bacterial

Table 2. Products of the host innate and adaptive immune responses can act non-specifically to effect both pathogen and host cell damage and death

Endogenous Irritants & Their Effects

Factor	Effect
Complement factors C3a & C5a	Degranulation of mast cells and basophils
C5b-8 “Membrane Attack Complex”	Cell Lysis
Lysosomal Enzymes	Protein degradation
	Initiation of Kinin, Prostaglandin and Leukotriene pathways
	Cell Death
Cytokines	Chemotaxis of neutrophils
	Activation of effector cells
Oxygen-derived free radicals TNF- α	Cytotoxicity

metabolites and by-products of the proteolytic degradation of the dentin matrix that include previously sequestered growth factors (54). Bacterial metabolites and growth factors from digested matrix are the principle stimulatory molecules for the underlying vital pulp in initial to moderate lesions. Diffusion of soluble plaque factors placed on freshly cut dentin has been shown to elicit an influx of polymorphonuclear leukocytes and monocytes in the subjacent pulp (37). Identified microbial metabolites from the carious process include organic acids, such as propionic, butyric and isobutyric acids, polyamines, lipopolysaccharide (LPS), collagenase, extracellular vesicles as well as a variety of bacterially derived proteins that are antigenic to the host. Perhaps the most thoroughly studied virulence factor in the context of endodontic infections is the lipid A moiety of the gram negative cell wall constituent LPS, referred to as endotoxin. Endotoxin is a potent stimulator of the non-specific immune response and has been correlated with a variety of clinical symptoms. A particularly insidious characteristic of endotoxin is that it remains active after the organism is non-viable and is particularly tenacious. Other bacterial virulence factors have been characterized and contribute to the pathology of infectious diseases in the body. Their direct relevance to pulpal pathosis is unclear except as it pertains to cariogenic microorganisms and the pathogenesis of dental caries. Nevertheless, microbial virulence factors represent a category of exogenous irritants to the pulp.

Bacterial access to the vital pulp through sound dentin has been reported and appears to be a common feature of deep caries. Histological examination of deep dentinal lesions in human teeth has revealed variable invasion of a mixed flora, at low titers ($<10^3$ CFUs) into the subjacent pulp (55). The degree of invasion is directly related to dentin permeability, which is attenuated by dentinal sclerosis and reparative dentin formation and influenced by anatomic location and mechanism of exposure. It has been shown, for example, that dentinogenesis is more rapid following traumatic cavity preparations and exposed cervical dentin (56). Furthermore, axial dentin is more permeable than occlusal dentin and coronal dentin is more permeable than root dentin (57). The presence or absence of a smear layer adds another dimension to dentin permeability as the smear layer can decrease dentin permeability, yet its removal is necessitated by certain restorative material placement protocols.

Physiologic obstacles that inhibit the ingress of bacteria into exposed tubules exist. In their healthy state dentinal tubules are occupied by plasma proteins, odontoblastic cellular contents, collagen fibrils and mineral crystals. These structures allow the transport of immunoglobulins to the infection front, the dilution and removal of toxins, crystal formation and coagulation of proteins to occlude the tubules. The opposite situation is seen in rapidly progressing caries where the underlying cells may be destroyed leaving an open tubule or 'dead tract' that allows easy ingress of bacteria (Fig. 4).

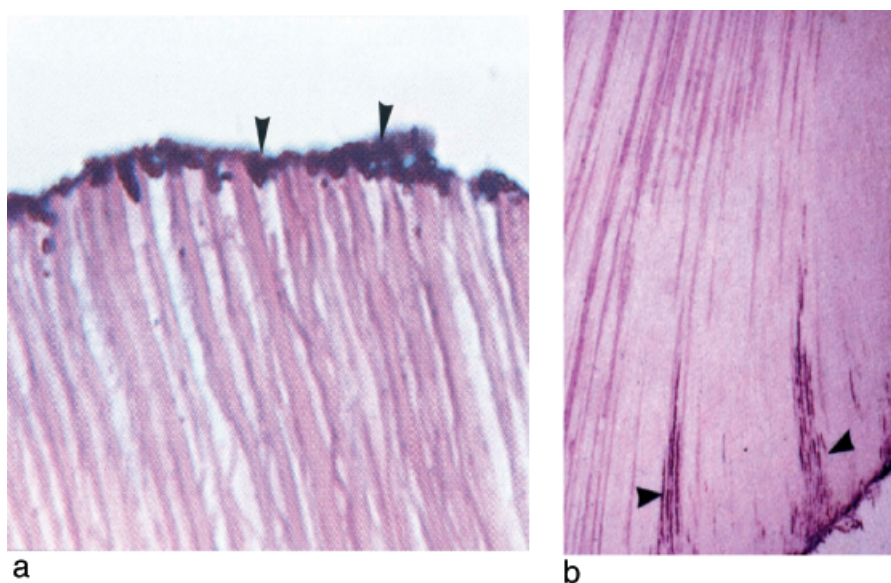


Fig. 4. (a) Bacteria colonize the cavity floor. (b) Arrows depict bacteria in the dentinal tubules.

The clinical significance of limited exposure of the pulp to bacteria through sound dentin is not known. It has been suggested that low titers of pathogens can be dealt with by the host immune system provided there is a thin layer of intervening sound dentin (58). By contrast, pulpal responses to *long-term* provocation by oral microbes across intact dentin have been documented. Freshly cut dentin left unrestored in human teeth for up to 240 days revealed an initial intense acute inflammatory response that slowly subsided within the first 2 weeks. This response was accompanied by an initial report of pain from experimental subjects that gradually subsided. This cessation of symptomology correlated with a regression of inflammation and resumption of normal tissue architecture and immune status within the pulp. Reparative dentin was also evident. Subsequent studies confirmed the recovery capacity of the pulp subsequent to bacterial challenge but they also indicated that recovery depends on the extent of the challenge (59). Full-coverage crown preparations represent the most extensive operative exposure of dentin. Teeth prepared for full coverage and left in provisional restorations for prolonged periods show an increased rate of pulpal necrosis (60).

‘Endogenous’ irritants

While certain bacterial virulence factors are directly damaging to the host tissue, others stimulate a prolonged non-specific host immune response that results in tissue damage. In the progressing carious lesion, the host immune response increases in intensity as the infection advances. Titers of T helper cells, B-lineage cells, neutrophils and macrophages are directly proportional to lesion depth in human teeth (61). In the most advanced phase of carious destruction, the humoral immunoreponse is accompanied by immuno-pathologic destruction of pulpal tissue. In animal studies where monkeys were hyperimmunized to BSA there was an observed increase in pulpal tissue destruction subsequent to antigenic challenge across freshly cut dentin (62). These findings support the contention that antigen-antibody complex formation, in addition to various products of the inflammatory cascade, give rise to a non-specific response that, while designed to rid the body of pathogens, effects destruction of parenchymal tissues as well (Fig. 5).

Neurogenic mediators are involved in the pulpal response to irritants and like immune components,

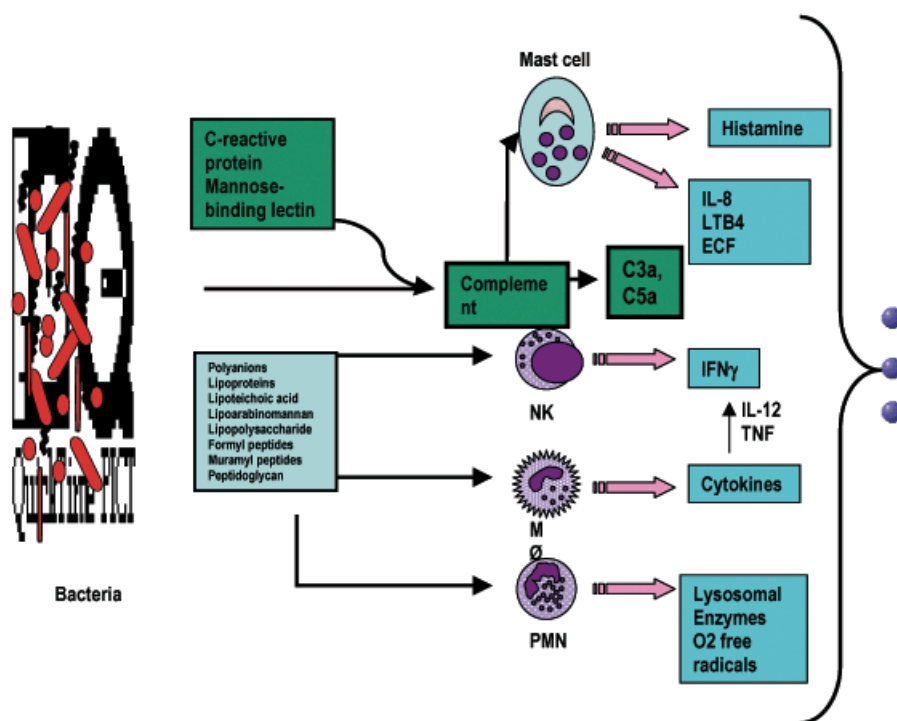


Fig. 5. Bacteria stimulate the innate and adaptive immune systems. The result is the elaboration of inflammatory mediators that effect chemotaxis and degranulation of neutrophils and activation of complement. Many of these mediators are non-specific in that they act on any cell with which they come in contact. This can be host or pathogen.

they can mediate pathology. External stimulation of dentin causes the release of pro-inflammatory neuropeptides from pulpal afferent nerves (63). Substance P (SP) and calcitonin gene-related peptide (CGRP) are released and effect vascular events such as vasodilatation and increased vascular permeability. This results in a net increase in tissue pressure that can progress to necrosis in extreme and persistent circumstances. Other pulpal elements such as fibroblasts, odontoblasts and Schwann cells react to irritants by elaborating growth factors and chemokines that are designed to counteract pathogens but secondarily can contribute to pulpal destruction. Odontoblasts exposed to bacteria and their by-products express IL-8 mRNA and protein (64, 65). IL-8 is a potent chemotactic factor for neutrophils, a predominant inflammatory effector cell observed in inflamed pulps. As previously mentioned, neutrophilic degranulation liberates lysosomal enzymes that digest host as well as microbial cells.

As caries progresses towards the pulp, the acid environment acts to dissolve mineral, liberate previously sequestered host growth factors and create a pH gradient from the lesion into dentin due to the buffering capacity of dentinal fluid. In essence soft carious dentin represents a 'poultice' of growth factors, enzymes, toxins and microbial metabolites whose stimulatory effect on the subjacent pulp is inversely proportional to the RDT. The documented pulpal response to these factors is varied and dependent on the mediator studied. Numerous investigators have shown that dentin matrix components can stimulate dentinogenesis (66, 67). Demineralized dentin matrix as well as dentin chips implanted at the site of pulpal exposure induce reparative dentin formation (68). Matrix component TGF- β 1 has been shown to be an inducer of tertiary dentin as well as a potent pulpal immunosuppressor (69–71). It has been theorized that during caries progression, TGF- β previously trapped during dentinogenesis, is released and is stimulatory to the pulp (72). This theory is supported by studies that demonstrated that transdentinal diffusion of TGF- β 1 induced an accumulation of dendritic cells in the odontoblast and subodontoblast layers (73). It is feasible that tertiary dentin formation is also stimulated transdentinally by this and other by-products of the carious degradation of dentin. *In vitro* studies employing the focal application of TGF- β 1 to dentin demonstrated that subjacent odontoblasts responded by TGF β -1 receptor expression as well as alpha 1(I)

collagen gene transcription (74). Insulin-like growth factors I and II and angiogenic growth factors are also components of the dentin matrix during tooth formation (72). Angiogenic factors are liberated during carious dissolution of dentin and it is likely that they resume bioactivity (75).

Summary

A variety of stimuli exert effects on the dental pulp. These effects are governed by the magnitude, duration and frequency of the stimulus, as well as intervening dentin permeability and thickness. Therapeutic interventions can diminish or ameliorate irritants provided they are administered in a timely manner.

References

1. Bergenholtz G. Pathogenic mechanisms in pulpal disease. *J Endod* 1990; **16**: 98–101.
2. Stanley HR. Traumatic capacity of high-speed and ultrasonic dental instrumentation. *J Am Dent Assoc* 1961; **63**: 749–766.
3. Stanley HR, Swerdlow H. Reaction of human pulp to cavity preparation: results produced by eight different operative techniques. *J Am Dent Assoc* 1959; **58**: 49.
4. Kramer IRH. Pulp changes of non-bacterial origin. *Int Dent J* 1959; **9**: 435.
5. Brannstrom M. Cavity preparation and the pulp. *Dent Prog* 1961; **2**: 4.
6. Langeland K, Langeland L. Pulp reactions to crown preparations, impression, temporary crown fixation, and permanent cementation. *J Prosthet Dent* 1965; **15**: 129.
7. Langeland K. Histologic evaluation of pulp reactions to operative procedures. *Oral Surg* 1959; **12**: 1235.
8. Zach I, Cohen G. Thermogenesis in operative techniques. Comparisons of four methods. *J Prosthet Dent* 1962; **12**: 977.
9. Stenvik A, Mjor IA. Tissue pressure and histology of normal and inflamed tooth pulps in Macaque monkeys. *Arch Oral Biol* 1972; **17**: 1501–1511.
10. Heyeras KJ, Kvinnsland I. Micropuncture measurements of interstitial fluid pressure in normal and inflamed dental pulps in cats. *J Endod* 1983; **9**: 105–109.
11. Olgart LM. Involvement of sensory nerves in hemodynamic reactions. *Proc Finn Dent Soc* 1992; **88**(Suppl): 403–410.
12. Baume IJ. The biology of pulp and dentine. A historic, terminologic-taxonomic, histologic-biochemical, embryonic, and clinical survey. *Monog Oral Sci* 1980; **8**: 1–220.
13. Fish EW. *An Experimental Investigation of Enamel, Dentine and the Dental Pulp*. London: John Bale Sons and Danielsson, 1932.
14. Yoshioka K, Yoshioka N, Ejiri S, Iwaku M, Ozawa H. Odontoblast processes in human dentin revealed by

- fluorescence labeling and transmission electron microscopy. *Histochem Cell Biol* 2002; **118**: 205–212.
15. Yoshida K, Yoshida N, Iwaku M. Class II antigen-presenting dendritic cell and nerve fiber responses to cavities, caries, or caries treatment in human teeth. *J Dent Res* 2003; **82**: 422–427.
16. Kvinnsland S, Heyeraas K, Øfjord ES. Effect of experimental tooth movement on periodontal and pulpal blood flow. *Eur J Orthodont* 1989; **11**: 200–205.
17. Nixon CE, Saviano JA, King GJ, Keeling SD. Histomorphometric study of dental pulp during orthodontic movement. *J Endod* 1993; **19**: 13–16.
18. Olgaard L, Gazelius B, Sundström F. Intradental nerve activity and jaw opening reflex in response to mechanical deformation of cat teeth. *Acta Physiol Scand* 1988; **133**: 399–406.
19. Butcher EO, Taylor AC. The effects of denervation and ischemia upon the teeth of the monkey. *J Dent Res* 1951; **30**: 265–275.
20. Messer HM. Permanent restorations and the dental pulp. In: Hargreaves KM, Goodis HE, eds. *Seltzer and Bender's Dental Pulp*. Carol Stream, IL: Quintessence International, 2002.
21. Vale WA. Cavity preparation. *Irish Dent Rev* 1956; **2**: 33–41.
22. Reeh ES, Messer HH, Douglas WH. Reduction in tooth stiffness as a result of endodontic and restorative procedures. *J Endod* 1989; **15**: 512–516.
23. Panitvisai P, Messer HH. Cuspal deflection in relation to restorative and endodontic procedures. *J Endod* 1995; **21**: 57–61.
24. Versluis A, Douglas WH, Cross M, Sakaguchi RI. Does an incremental technique reduce polymerization shrinkage stresses? *J Dent Res* 1996; **75**: 871–878.
25. Hirata K, Nakashima M, Sekine I, Mukouyama Y, Kimura K. Dentinal fluid movement associated with loading of restorations. *J Dent Res* 1991; **70**: 975–978.
26. Tagami J, Hosoda H, Burrow MF, Nakajima M. Effect of aging and caries on dentin permeability. *Proc Finn Dent Soc* 1992; **88**(Suppl): 149–154.
27. About I, Murray PE, Franquin JC, Remusat M, Smith AJ. Pulpal inflammatory responses following non-carious class V restorations. *Oper Dentistry* 2001; **26**: 336–342.
28. Qvist V, Stoltze K, Qvist J. Human pulp reactions to resin restorations performed with different acid etch restorative procedures. *Acta Odont Scand* 1989; **47**: 253–263.
29. Fujitani M, Inokoshi S, Hosoda H. Effect of acid etching on the dental pulp in adhesive composite restorations. *Int Dent J* 1992; **42**: 3–11.
30. Gwinnett AJ, Tay FR. Early and intermediate time response of the dental pulp to an acid etch technique *in vivo*. *Am J Dent* 1998; **11**(Special issue): S35–S44.
31. Fitzgerald M, Hanks CT. *In vivo* study of resin diffusion through intact vital human dentin (abstract). *J Dent Res* 1997; **76**(Special issue): 305.
32. Hume R. An analysis of the release and the diffusion through dentin of eugenol from zinc-oxide eugenol mixtures. *J Dent Res* 1984; **63**: 881–884.
33. Hume WR, Testa AE. Release of ^3H -triamcinolone from a steroid antibiotic mixture. *J Endod* 1980; **7**: 509–514.
34. Bergenholtz G, Cox CF, Loesche WI, Syed SA. Bacterial leakage around dental restorations: its effect on the dental pulp. A microbial and histopathological study. *J Oral Pathol* 1982; **11**: 439–450.
35. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. *Dent Mater* 1990; **6**: 282–287.
36. Hume WR, Gerzina TM. Bioavailability of components of resin-based materials which are applied to teeth. *Crit Rev Oral Biol Med* 1996; **7**: 172–179.
37. Bergenholtz G, Warfvinge J. Migration of leukocytes in the dental pulp in response to plaque bacteria. *Scand J Dent Res* 1982; **90**: 354–362.
38. Katsuno KA et al. A delayed type hypersensitivity reaction to dentine primer in the guinea pig. *J Dent* 1995; **23**: 295–299.
39. Inoue T, Miyakoshi S, Shimono M. Dentin/pulp adhesive resin interface. Biological view from basic science to clinic. In: Shimono M, Maeda T, Suda H, Takahashi K, eds. *Proceedings of the International Conference on Dentin/Pulp Complex 1995, Chiba, Japan*. Chicago: Quintessence Publishing Co., pp. 217–220, 1996.
40. Jontell M, Hanks CT, Bratel J, Bergenholtz G. Effect of unpolymerised resin components on the function of accessory cells derived from the rat incisor pulp. *J Dent Res* 1995; **74**: 1162–1167.
41. Schroder U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation, and differentiation. *J Dent Res* 1985; **64**(Special issue): 541–548.
42. Cvek M, Granath L, Cleaton-Jones P, Austin J. Hard tissue barrier formation in pulpotomized monkey teeth capped with cyanoacrylate or calcium hydroxide for 10 and 60 min. *J Dent Res* 1987; **66**: 1166–1174.
43. Heys DR, Cox CF, Heys RJ, Avery JK. Histological considerations of direct pulp capping agents. *J Dent Res* 1981; **60**: 1371–1379.
44. Cvek M. A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. *J Endod* 1978; **4**: 232–237.
45. Mjör IA, Finn SB, Quigley MB. The effect of calcium hydroxide and amalgam on non-carious dentine. *Arch Oral Biol* 1961; **3**: 283–291.
46. Gwinnett AJ, Tay FR, Pang KM, Wei SHY. Quantitative contribution of the collagen network in dentin hybridization. *Am J Dent* 1996; **9**: 140–144.
47. Mjör IA. *Initial Reactions to Tooth Preparations, in Pulp-Dentin biology in restorative dentistry*. Chicago: Quintessence Publishing, 2002.
48. Nakabayashi N. Resin reinforced dentine due to infiltration of monomers into dentine at the adhesive interface. *J Jpn Dent Mater* 1982; **1**: 78–181.
49. Farik B, Munksgaard EC, Andreasen JO, Kreiborg S. Drying and rewetting anterior crown fragments prior to bonding. *Endod Dent Traumatol* 1999; **15**: 113–116.

50. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposure of dental pulps in germ-free and conventional laboratory rats. *Oral Surg* 1965; **20**: 340–349.
51. Brannstrom M, Lind PO. Pulpal response to early dentinal caries. *J Dent Res* 1965; **44**: 1045–1050.
52. About I, Mitsiadis TA. Molecular aspects of tooth pathogenesis and repair: *in vivo* and *in vitro* models. *Adv Dent Res* 2001; **15**: 59–62.
53. Bergenholtz G. Factors in pulpal repair after oral exposure. *Adv Dent Res* 2001; **15**: 84.
54. Magloire H, Bouvier M, Joffe A. Odontoblast response under carious lesions. *Proc Finn Dent Soc* 1992; **88**(Suppl 1): 257–274.
55. Hoshino E, Ando N, Sato M, Kota K. Bacterial invasion of non-exposed dental pulp. *Int Endod J* 1992; **25**: 2–5.
56. Cox CE, White KC, Ramus DL, Farmer JB, Snuggs HM. Reparative dentin: factors affecting its deposition. *Quintessence Int* 1992; **23**: 257–270.
57. Pashley DH. Considerations of dentin permeability in cytotoxicity testing. *Int Endod J* 1988; **21**: 143–154.
58. Lundy T, Stanley HR. Correlation of pulp histopathology and clinical symptoms in human teeth subjected to experimental irritation. *Oral Surg* 1969; **27**: 187–201.
59. Lervik T, Mjor IA. Evaluation of techniques for the induction of pulpitis. *J Biol Buccale* 1977; **5**: 137–148.
60. Felton D, Madison S, Kanoy E, Kantor M, Maryniuk G. Long-term effects of crown preparation on pulp vitality (abstract). *J Dent Res* 1989; **68**(Special issue): 1004.
61. Izumi T, Kobayashi I, Okamura K, Sakai H. Immunohistochemical study on the immunocompetent cells of the pulp in human non-carious and carious teeth. *Arch Oral Biol* 1995; **40**: 609–614.
62. Bergenholtz G, Ahlstedt S, Lindhe J. Experimental pulpitis in immunized monkeys. *Scand J Dent Res* 1977; **85**: 396–406.
63. Byers MR, Narhi MV. Dental injury models: experimental tools for understanding neuroinflammatory interactions and polymodal nociceptor functions. *Crit Rev Oral Biol Med* 1999; **10**: 4–39.
64. Levin LG, Rudd A, Bletsa A, Reisner H. Expression of IL-8 by cells of the odontoblast layer *in vitro*. *Eur J Oral Sci* 1999; **107**: 131–137.
65. Huang GT, Potente AP, Kim JW, Chugal N, Zhang X. Increased interleukin-8 expression in inflamed human dental pulps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **88**: 214–220.
66. Tziafas D et al. Induction of odontoblast-like cell differentiation in dog dental pulps after *in vivo* implantation of dentine matrix components. *Arch Oral Biol* 1995; **40**: 883–893.
67. Smith AJ et al. Odontoblast stimulation in ferrets by dentine matrix components. *Arch Oral Biol* 1994; **39**: 13–22.
68. Smith AJ. Pulpal responses to caries and dental repair [Review] [93 refs]. *Caries Res* 2002; **36**: 223–232.
69. Tziafas D, Papadimitriou S. Role of exogenous TGF-beta in induction of reparative dentinogenesis *in vivo*. *Eur J Oral Sci* 1998; **106**(Suppl 1): 192–196.
70. Kulkarni AB et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 1993; **90**: 770–774.
71. D'Souza RN, Cavender A, Dickinson D, Roberts A, Letterio J. TGF-beta1 is essential for the homeostasis of the dentin-pulp complex. *Eur J Oral Sci* 1998; **106**(Suppl 1): 185–191.
72. Finkelman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ. Quantitation of growth factors IGF-I, SGF/IGF-II, and TGF-beta in human dentin. *J Bone Miner Res* 1990; **5**: 717–723.
73. Farges JC et al. TGF-beta1 induces accumulation of dendritic cells in the odontoblast layer. *J Dent Res* 2003; **82**: 652–656.
74. Magloire H, Romeas A, Melin M, Couble ML, Bleicher F, Farges JC. Molecular regulation of odontoblast activity under dentin injury. *Adv Dent Res* 2001; **15**: 46–50.
75. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. *Arch Oral Biol* 2000; **45**: 1013–1016.