Relationships between Caries Bacteria, Host Responses, and Clinical Signs and Symptoms of Pulpitis

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Abstract

Knowledge of caries bacteria and the inflammatory responses they elicit in the dental pulp is prerequisite to our understanding of the pathogenesis of pulpitis. Recent advances in immunology and neurophysiology can now explain some of the clinical manifestations of pulpitis. The purpose of this review is twofold. The first purpose is to review the literature of the caries microflora, the host immune responses they elicit, and how they do so. The relationship between both proinflammatory and anti-inflammatory cytokines and pulpitis is discussed. The proinflammatory properties of lipoteichoic acid, which is a common virulence factor among Gram-positive bacteria such as those found among the caries bacteria, are reviewed. The second purpose is to review how bacteria and their metabolites, as well as pulpal immune and inflammatory reactions to them, modify the pain sensation in pulpitis. *(J Endod 2007;33: 213–219)*

Key Words

Caries bacteria, cytokines, pain, pulpitis

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Caries bacteria are the major cause of pulpal inflammation and infection. The outcome of pulpal insult is a dynamic process that depends on both the invading microorganisms and host responses to them, which include inflammation and immunity. Evidence from experimental pulpitis models has clearly demonstrated that bacterial antigens and/or metabolic by-products can diffuse through dentinal tubules to elicit immune responses in the dental pulp (1–5). Immune complexes and by-products from immune responses, such as extracellular proteolytic enzymes released by phagocytosis, can further aggravate pulpal inflammation (6, 7), making the problem worse. A case in point was a patient suffering from impaired cellular immunity (athymic dysplasia) with IgA deficiency in whom only a mild inflammatory response with little tissue destruction was observed in a caries-exposed pulp (8). Thus an immunopathological mechanism of pulpitis may be operational.

Pulpal pain is usually the first clinical sign of pathology if the insult is not removed to resolve the edema from inflammation. Persistent inflammation in a low-compliance environment such as the dental pulp elicits pain and eventually leads to total pulp destruction (9, 10) and periapical pathosis (11). Inflammation is the outcome of complex interactions among various cell types. Because of the limited scope of this review, the roles of odontoblasts, fibroblasts, nerve fibers, mast cells, and endothelial cells will be reviewed in a future paper. The goals of this review are: (1) to summarize our current knowledge of caries bacteria and their elicited host immune responses and (2) to discuss the correlation between caries bacteria, their role in pulpal immune responses and inflammation, and the symptoms of pulpitis.

Caries Pathogens and Caries Progression

The microflora in dental caries is highly complex and vary between individual lesions. The composition of the dominant groups may depend on diet, saliva, and the chronicity of the lesion. Mutans group streptococci, such as *Streptococcus mutans* and *Streptococcus sobrinus*, and lactobacilli are important in the initiation and progression of caries (12–14). These microorganisms are acidogenic (produce acid) by fermenting dietary carbohydrates, which results in the demineralization of enamel and dentin. They are also aciduric (acid tolerant), which gives them a competitive survival advantage. Among acidogenic bacteria, arginolytic strains of oral streptococci and lactobacilli (bacteria that can produce base from arginine and thus produce both a decline and increase in pH rather than a decline only) are considered less cariogenic. Their ratio to that of nonarginolytic bacteria may determine the level of caries activity (15, 16). Furthermore, oral streptococci and lactobacilli are capable of intratubular invasion by binding to collagen type I in dentin (17, 18). After demineralization the exposed collagen is further degraded by host-derived matrix metalloproteinases that promote the advance of the caries (19).

As the lesion progresses deeper into the dentin, a transition from predominantly facultative, Gram-positive bacteria in shallow caries to deep dentinal caries dominated with lactobacilli and/or anaerobic bacteria takes place (20–22). This transition is probably influenced by a change in the ecosystem (i.e., nutrients, O_2 etc.). The availability of serum-like nutrients diffusing from the pulp into deep caries favors the growth of proteolytic over saccharolytic bacteria (23, 24) as does blood hemin for Prevotella species (25). Streptococci that require salivary glycoproteins and carbohydrates as an energy source are unlikely to thrive without saliva in a deep carious lesion.

We identified two types of deep carious lesions: those with high Lactobacillus and those with low Lactobacillus counts (26). In the low Lactobacillus lesions, a great

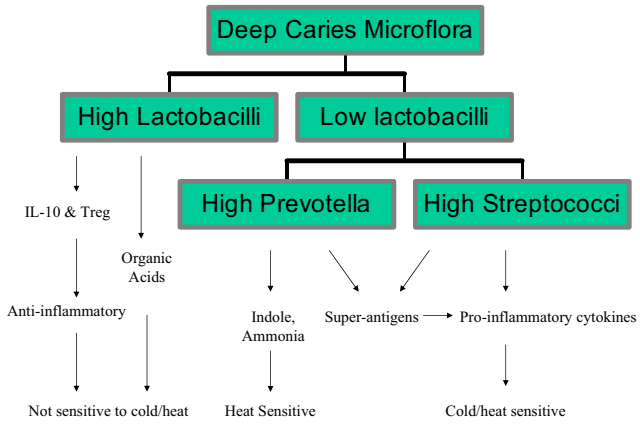

Figure 1. The metabolites of deep caries bacteria and their contributions to pulpal thermal sensitivity.

variation of the dominant microbes was noted and Gram-positive non-Lactobacilli rods, Gram-positive cocci, or *Prevotella intermedia* were dominant in certain samples. Results of recent molecular studies assessing the predominant flora in deep carious dentin support our findings (27, 28). Furthermore, Chhour et al. (29) used real-time polymerase chain reaction (PCR) to assess the total bacterial load of deep carious lesions and categorized the lesions into high-Lactobacillus, mid-Lactobacillus/Prevotella, high-Prevotella, and low-Lactobacillus/Prevotella lesions. However, neither Lactobacilli nor Prevotella were the dominant isolates reported by Hoshino (21). They observed predominantly anaerobic Gram-positive bacteria and Gram-negative rods in deep dentinal caries, among which *Pseudoramibacter alactolyticus* (was *Eubacterium alactolyticum*) was the predominant isolate.

When caries invade pulpally, the pulpal inflammation manifests itself by pain or hypersensitivity. In the following section, we will focus on the bacterial metabolites that can be the modifiers of pain symptoms associated with deep caries. The relationship between fermentation end products of various bacteria in deep caries and clinical manifestations to thermal tests is presented in Fig. 1.

Acid, Bacterial By-products, and Pain Modification

Bacterial metabolic by-products and cell wall components can diffuse through dentinal tubules to elicit pulpal inflammation (2, 5, 30–32). Although we have not identified all of the molecules that come through the dentinal tubules, the main metabolites in caries have been examined. Lactic acid is the predominant microbial by-product in active carious dentin (88%), which exhibits a low pH (mean 4.9) (33). Aciduric bacteria such as streptococci and lactobacilli produce more lactate under acidic conditions than at neutral pH (34, 35). In contrast, arrested dentinal caries exhibits an acetate-dominant profile with a higher pH (mean 5.7) (33). Organic acid from bacterial fermentation of carbohydrates, including lactic, acetic, and propionic acids (1 mM to 1 M), not only fail to excite the intratubular A-δ nerves but also reversibly suppress nerve impulses elicited by other stimuli (36). These findings contradict the action of these acids in other tissues, which is to cause severe pain. Two possible explanations have been proposed. First, the dental pulp may lack the chemosensitive pain fibers found in other tissues (37, 38). Second, increased concentrations of H^+ ions (39, 40) and/or Ca^{++} ions (41) liberated from dentin decalcified by the acids may decrease the excitability of the dentinal nerve fibers. The direct suppressive effect of acids on intratubular nerves could partially explain why carious teeth with high Lactobacillus counts usually are not sensitive to thermal stimuli (42). This finding also may explain why a higher neural density found beneath caries is not related to a worse pain experience (43).

Among the algogenic (pain producing) molecules produced by bacterial metabolism, ammonia is the most potent pain inducer, followed by urea and indole, which are by-products of amino acid fermentation (44). Many anaerobic bacteria are asaccharolytic (i.e., do not gain energy from conversion of sugars to acidic fermentation products) but are proteolytic, their growth depending on their ability to metabolize proteins or peptides. Asaccharolytic bacteria such as *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphyromonas gingivalis* can incorporate and ferment amino acids such as glutamic and aspartic acids into organic acids and ammonia (45, 46). Bacteria associated with deep caries and infected canals, such as Porphyromonas spp, *P. intermedia*, *F. nucleatum*, *Propionobacterium acnes*, and a few isolates of Actinomyces, Peptostreptococci, Bacteroides, and Eubacteria are indole-positive (a test used for bacterial taxonomy that determines the ability of the bacterium to split indole from the amino acid tryptophan) (47). Many studies have demonstrated a close association between pain and the recovery of *Prevotella*, *Porphyromonas*, and *Fusobacterium* from caries (26, 48) and infected canals (49, 50). The algogenic metabolites from anaerobic Gram-negative bacteria in deep caries could partially explain why Bacteroides spp, *P. intermedia*, and the amount of lipopolysaccharide (LPS) in caries are positively related to heat sensitivity or pain (42, 51). LPS activates the Hageman factor, leading to bradykinin production, a potent pain inducer (52, 53).

Lipoteichoic Acid

LTA is an amphiphilic molecule consisting of a polyglycerolphosphate with a complex glycolipid group attached (54, 55). It is anchored by hydrophobic forces to the cell membranes of Gram-positive bacteria, including most streptococcal strains (56). LTA is produced in large quantities by cariogenic bacteria when sucrose is available (57) and can be exported extracellularly when bacteria are grown at low pH (58). LTA released extracellularly by Gram-positive, acidogenic bacteria could diffuse pulpally and elicit immune responses.

LPS and LTA activate the innate immune system by similar mechanisms. Both LPS and LTA bind to CD14, activate signaling by Toll-like receptors (TLRs) (59, 60), and induce proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interlukin-8 (IL-8), interleukin-12 (IL-12), and anti-inflammatory cytokine interleukin-10 (IL-10) (61, 62). A recent study demonstrated that *Bacillus subtilis* LTA stimulates odontoblasts by TLR2 and induces the secretion of chemokines (CCL2 and CXCL2). CCL2 attracts immature dendritic cells (DCs) and CXCL2 is angiogenic (63). Lactobacillus LTA induces TNF-α production by TLR2 (64). Furthermore, LTA from *S. mutans* induced apoptosis of cultured pulp cells (mainly fibroblasts) in vitro, which could contribute to the initiation of and/or progression of pulpitis (65). A recent study demonstrated that TGF-β (transforming growth factor) gene expression was down-regulated when odontoblasts were challenged with LTA, which promotes immune defense rather than mineralization (63). A summary of the proinflammatory and anti-inflammatory effects of LTA is illustrated in Fig. 2.

Although LTA is much less potent than LPS in inducing proinflammatory cytokine production by macrophages (62), it exhibits a similar potency in the induction of macrophage vascular endothelial cell growth factor (VEGF) expression (66, 67). VEGF can also be produced by LTA-stimulated odontoblast-like cells and pulpal cells (67). VEGF is a potent inducer of angiogenesis and vascular permeability (68, 69). The ability of VEGF to enhance vascular permeability is estimated to be 50,000 times higher than that of histamine (70). Furthermore, VEGF is expressed in dentin matrix and the rate of its release from the matrix after injury closely relates to the healing capability of the pulpal tissue

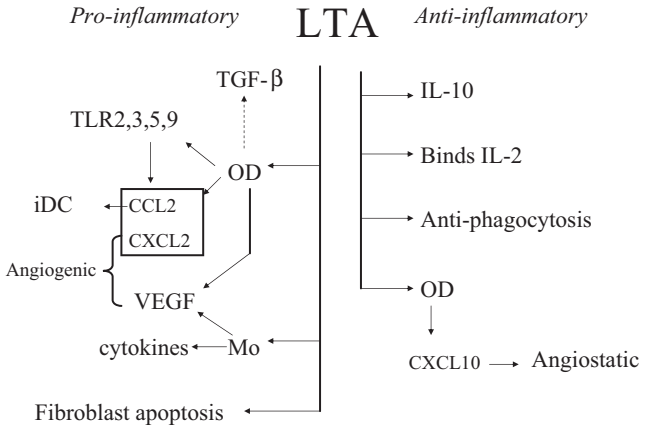

Figure 2. The pro-inflammatory and anti-inflammatory properties of LTA. Negative regulation is expressed by a dashed line (- - - - -). Abbreviations: iDC, immature dendritic cells; Mo, monocytes; OD, odontoblasts; VEGF, vascular endothelial cell growth factor.

(71). A rapid increase in VEGF expression may result in an acute increase in interstitial tissue pressure in the noncompliant pulp space, leading to pulpal necrosis.

LTA also exhibits anti-inflammatory effects including anti-phagocytosis and direct binding to interleukin-2 (IL-2) (72, 73). LTA from hemolytic streptococci inhibits the uptake of streptococci by epithelial cells. LTA binding to IL-2 inhibits the function and measurability of IL-2 (73, 74). An increase of IL-2 titer in irreversible pulpitis was observed by Rauschenberger et al. (75) but not by Anderson et al. (76). This discrepancy could be the result of different amounts of LTA in their samples, which was not measured. Because IL-2 performs many functions critical to the successful elimination of pathogens, LTA release could significantly dampen the immune responses in general. Thus, LTA may not only provide a selective advantage to Gram-positive bacteria but also interfere with ongoing responses to other infectious agents.

Pro-inflammatory Cytokine Induction by Caries Bacteria

Bacterial antigens induce proinflammatory cytokines including IL-12, IL-1, TNF-α, and interferon-gamma (IFN-γ) (61, 77–79). IL-12 is mainly secreted by DCs and monocytes/macrophages. Its chief biologic function is to stimulate IFN-γ production by activated T cells and natural killer (NK) cells. IFN-γ in turn activates macrophages to kill phagocytosed microbes. IL-1 and TNF-α are rapidly produced by activated monocytes/macrophages to recruit neutrophils and monocytes to the site of infection. In general, Gram-positive and Gram-negative bacteria are comparable in their IL-1 induction but Gram-positive are more potent IL-12 and TNF-α inducers than Gram-negative bacteria (78, 79).

IL-6 is secreted by various cell types in response to microbes or cytokines such as IL-1 and TNF-α (80–83). IL-6 stimulates hepatocytes to synthesize two major acute-phase proteins: C-reactive protein (CRP), which increases the rate of bacterial phagocytosis, and serum amyloid A (SAA), which influences cell adhesion, migration, proliferation, and aggregation. These proteins also produce a systemic reaction that includes fever, increased erythrocyte sedimentation rate, increased secretion of glucocorticoids, activation of the complement, and clotting cascades (84, 85). IL-6 also stimulates the production of neutrophils from bone marrow in innate immunity (86, 87). IL-6 is involved in adaptive immunity by inducing the permanent differentiation of B-cells into plasma cells that produce antibodies and is therefore considered to be a type-2 cytokine (one that stimulates antibody production) by some researchers (88). IL-6, along with IL-1β, is secreted when pulpal cells are challenged with peptidoglycan preparations of Gram-positive bacteria (89, 90). Thus, IL-6 may be important in the later stage of pulpitis when the number of B cells increases.

Gram-positive cell walls stimulate the synthesis of TNF-α and IL-6 by human monocytes (91). Peptidoglycan is the major cell wall component of Gram-positive bacteria and a thin layer is also present in Gram-negative bacteria. It has been increasingly recognized as a potent proinflammatory molecule that plays an important role in rheumatoid arthritis and other autoimmune diseases (92–95). Because peptidoglycan is released upon lysis of the cell, its role in the progression of pulpitis needs further investigation.

The LPS of Gram-negative bacterial cells induces potent proinflammatory cytokines (96). Various preparations of Gram-negative bacteria induce dental pulp cells to secrete IL-6, IL-1β, and IL-8 (which recruits neutrophils to the site of inflammation, and thus is also known as the Neutrophil Chemotactic Factor) (81, 97–101). Although pulpal fibroblasts can produce pro-inflammatory cytokines and chemokines in vitro, the majority of IL-1 or IL-8 positive cells in inflamed pulps are immune cells and endothelial cells not associated with fibroblasts (102–104). The suppression of cytokine expression in vivo may arise from immunomodulation by other cytokines (105).

S. mutans induces peripheral blood mononuclear cells to produce high titers of proinflammatory cytokines including IFN-γ, IL-12 (77), TNF-α (106), and chemokines including IL-8 and MCP-1 (107). Cell wall molecules of *S. mutans*, such as proteins of the I/II family and the serotype f antigen (a polysaccharide rhamnose-glucose polysaccharide covalently bound to bacterial cell wall peptidoglycans that facilitates the adherence of *S. mutans* to hard surfaces) are responsible for the induction of these inflammatory cytokines (108). Cell-free supernatant fluids of viridans Streptococci induce IL-1, IL-6, IL-8, and TNF-α (109, 110). Extracellular products of *Streptococcus mitis* and *S. sanguinis* also induce proinflammatory cytokines (109, 111). The inflammatory mediators induced by these proinflammatory cytokines sensitize C fibers in the dental pulp (112–116). These findings may partially explain why some deep caries loaded with oral streptococci exhibit prolonged pain to heat testing (42).

Other bacterial components such as cell surface polysaccharides, heat shock proteins, and extracellular products (such as proteases and exotoxins) also induce pro-inflammatory cytokines (117). Superantigens, which are potent cytokine inducers that do not require antigen presentation, have been associated with certain strains of *Streptococcus mitis*, *P. intermedia*, and periodontal pathogens (109, 118–120). Proinflammatory cytokines play a crucial role in tissue destruction and the pathogenesis of many diseases (121–124). Anti-inflammatory cytokine treatment has proved beneficial in arthritis, inflammatory bowel disease, and periodontal disease (125–128). Similarly, proinflammatory cytokine induction by caries bacteria is an important virulence factor in pulpitis. Patients with compromised proinflammatory cytokine responses may react to caries invasion with little inflammation, whereas severe inflammation and pulpal tissue destruction may result in patients with a hyper-response of proinflammatory cytokines to bacterial challenge. Local application of anti-inflammatory cytokine reagents can therefore be therapeutic in early pulpitis by reducing tissue destruction as well as pain.

Anti-inflammatory Cytokine (IL-10) Induction by Caries Bacteria

Lactobacilli are the dominant flora in certain deep carious lesions and are negatively associated with thermal sensitivity (26). Clinically, we sometimes encounter teeth with vital pulps and deep caries but no obvious thermal sensitivity. This clinical finding may be explained partly by the direct suppressive effect of the organic acids produced by these

bacteria and partly by their induction of anti-inflammatory cytokine IL-10 and regulatory T cells (Treg) (129–131). *L. casei*, one of the Lactobacillus species most frequently recovered from deep caries, induced a significantly higher IL-10 titer than that of other caries bacteria at a low concentration (10^5 per mL) (132). Certain Lactobacilli such as *L. casei* and *L. paracasei* bind to DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) on immature DCs and induce Treg and IL-10 production (129–131). Furthermore, Lactobacilli appear to generate tolerogenic DCs, a phenotype characterized by increased co-stimulatory marker expression but low production of pro-inflammatory cytokines (133). Such tolerogenic DCs may contribute to the production of Treg in vivo (134, 135). This immune suppressive effect of *L. casei* helps explain the significantly reduced IFN-γ titer that we observed when peripheral blood mononuclear cells were challenged with *S. mutans* and *L. casei* together (unpublished data). *P. alactolyticus*, another representative isolate from caries, exhibits a strong type-2 cytokine profile, inducing more IL-10 than IFN-γ. The IL-10 induced by these bacteria and Treg can contribute to the significant elevation of IL-10 mRNA in pulps beneath deep caries (77).

Other Virulence Factors of Caries Pathogens

Gram-positive caries bacteria are capable of activating complement pathways. Based on experimental pulpitis studies, C3a and C5a generated from complement activation were thought to be important virulence factors of cariogenic bacteria that elicit neutrophil accumulation in the Arthus reaction (2, 3, 5, 30). Recent studies, however, showed that the presence of an intact complement cascade is neither necessary nor sufficient to trigger or propagate the Arthus reaction (136, 137). Thus, tissue damage from direct complement activation by Gram-positive bacteria in shallow caries may be minimal.

S. mutans and *L. casei* are cytotoxic and are capable of causing total pulpal necrosis of rat teeth (138, 139). *S. sanguinis* and *Enterococcus faecalis* were the most cytotoxic isolates of the commonly isolated endodontic pathogens to fibroblasts and macrophages (140, 141). The molecular mechanism of the cytotoxicity of *S. sanguinis* is not yet understood. Thorough reviews of the virulence factors of *E. faecalis* are available (142, 143). Virulence factors of other Gram-negative bacteria associated with infected canals are reviewed by Baumgartner (144).

Remaining Dentin Thickness and Tubular Permeability

Remaining dentin thickness and tubular permeability are the most important determining factors of the pulpal inflammatory response (145–147). Although bacteria or their cell-wall components such as LPS are capable of passing through tubules to induce inflammatory responses in the dental pulp (2, 4, 5, 30), the thickness of dentin can greatly reduce the concentration of bacterial proteins and the amount of LPS that reaches the pulp (31, 148, 149). We infiltrated commercial LTA preparations from *S. mutans* through dentinal blocks using fluid filtration and observed that the dentin permeability decreased with time (Fig. 3), probably as a result of LTA binding to the dentinal walls (150). An increase of inflammatory cells in pulps of teeth with pulpitis was observed only when the caries front was <1.5 mm from the pulp (151, 152). McLachlan et al. (153) observed less expression of PMN-associated S100 family genes (which code for S-100 proteins, calcium-binding proteins, some of which have been shown to have intracellular and extracellular functions associated with inflammation) in inflamed pulps when the remaining dentin thickness was >2 mm. It appears that 2 mm of sound dentin provides a safeguard for a speedy recovery of the dental pulp to health.

Future Directions

Caries are composed of complex and dynamic flora, all of which the dental pulp is exposed to at one time or another. Studies of individ-

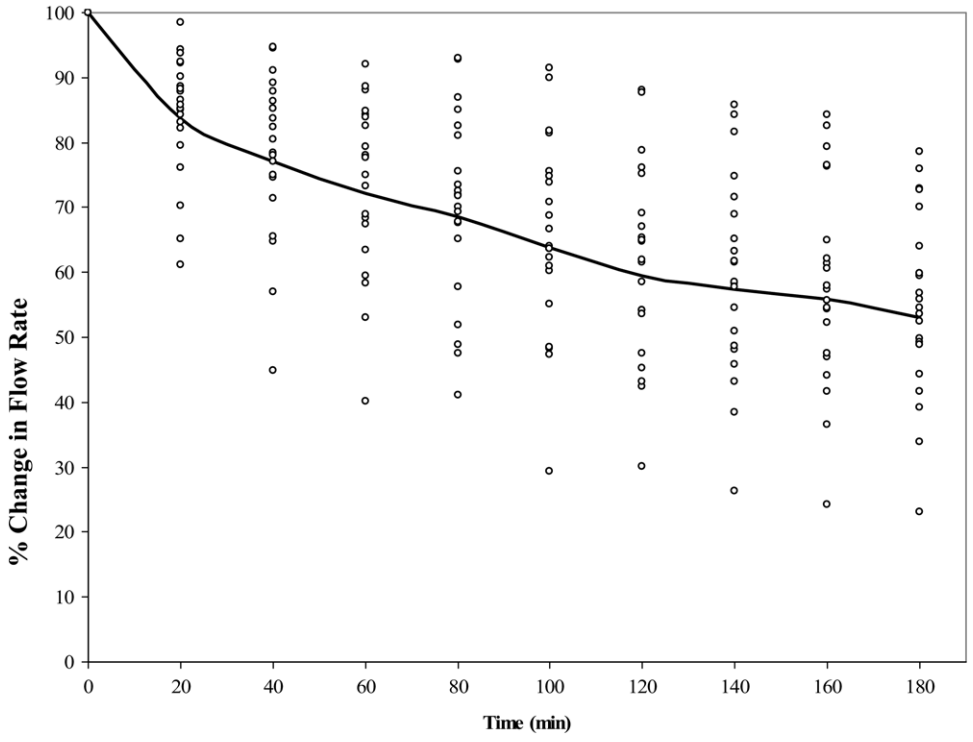

Figure 3. LTA reduces fluid flow. Human dentinal disks (2.6–1.3 mm in thickness) were infiltrated with LTA from *S. mutans* (50 μg/ml, Sigma) for 180 minutes according to the method described previously (157). Each dot represents one data point of one sample block at the specific time point. Percentage changes of flow rate of 23 blocks were plotted over time. There was a significant decrease of flow rate with time.

ual cultivable caries bacteria and their cell wall components have been undertaken to better understand the pathogenesis of pulpitis. Unfortunately, roughly 50% of caries bacteria are not cultivable (154), and we have not yet identified the molecules in shallow caries that diffuse through the dentinal tubules. Molecular approaches to characterize these chemicals are much needed. Analysis of a large sample population of carious dentin and the infecting microbes using a high-throughput gene expression technique such as microarrays would provide a more comprehensive understanding of the multitude of genes of importance in caries. Therefore, conclusions drawn from current studies may need to be modified or abandoned as more data are collected through future research.

Caries bacteria elicit various concentrations of both proinflammatory and anti-inflammatory cytokines. These bacteria have usually been grown and studied in a planktonic state. Gene and protein expressions, however, are clearly different when comparing bacteria grown in the planktonic state to those in a biofilm community (155–157). Bacteria grown in a biofilm environment, closely simulating the in vivo carious lesion, would provide valuable information about virulence factors involved in the caries invasion. Furthermore, dynamics of the polymicrobial caries infection and its evolution over time have not been addressed with these in vitro studies. Thus, a more realistic in vivo and/or in vitro caries model is needed to critically examine the virulence factors of caries bacteria.

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References

1. Bergenholtz G. Evidence for bacterial causation of adverse pulpal responses in resin-based dental restorations. Crit Rev Oral Biol Med 2000;11:467–80.
2. Bergenholtz G, Lindhe J. Effect of soluble plaque factors on inflammatory reactions in the dental pulp. Scand J Dent Res 1975;83:153–8.
3. Bergenholtz G, Warfvinge J. Migration of leukocytes in dental pulp in response to plaque bacteria. Scand J Dent Res 1982;90:354–62.
4. Warfvinge J. Dental pulp inflammation; experimental studies in human and monkey teeth. Swed Dent J Suppl 1986;39:1–36.
5. Warfvinge J, Dahlen G, Bergenholtz G. Dental pulp response to bacterial cell wall material. J Dent Res 1985;64:1046–50.
6. Adamkiewicz VW, Pekovic DD. Experimental pulpal Arthus allergy. Oral Surg Oral Med Oral Pathol 1980;50:450–6.
7. Bergenholtz G, Ahlstedt S, Lindhe J. Experimental pulpitis in immunized monkeys. Scand J Dent Res 1977;85:396–406.
8. Trowbridge H, Daniels T. Abnormal immune response to infection of the dental pulp. Report of a case. Oral Surg Oral Med Oral Pathol 1977;43:902–9.
9. Van Hassel HJ. Physiology of the human dental pulp. Oral Surg Oral Med Oral Pathol 1971;32:126–34.
10. Tonder KJ. Vascular reactions in the dental pulp during inflammation. Acta Odontol Scand 1983;41:247–56.
11. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germfree and conventional laboratory rats. J South Calif State Dent Assoc 1966;34:449–51.
12. van Houte J. Role of micro-organisms in caries etiology. J Dent Res 1994; 73:672–81.
13. Loesche WJ, Syed SA. The predominant cultivable flora of carious plaque and carious dentine. Caries Res 1973;7:201–16.
14. Bowden GHW, Ekstrand J, McNaughton B, Challacombe SJ. The association of selected bacteria with the lesions of root surface caries. Oral Microbiol Immunol 1990;5:346–51.
15. Kleinberg I. A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to Streptococcus mutans and the specific-plaque hypothesis. Crit Rev Oral Biol Med 2002;13:108–25.
16. Wijeyeweera RL, Kleinberg I. Arginolytic and ureolytic activities of pure cultures of human oral bacteria and their effects on the pH response of salivary sediment and dental plaque in vitro. Arch Oral Biol 1989;34:43–53.
17. Love RM, McMillan MD, Jenkinson HF. Invasion of dentinal tubules by oral streptococci is associated with collagen recognition mediated by the antigen I/II family of polypeptides. Infect Immun 1997;65:5157–64.
18. McGrady JA, Butcher WG, Beighton D, Switalski LM. Specific and charge interactions mediate collagen recognition by oral lactobacilli. J Dent Res 1995;74: 649–57.
19. Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998;77:1622–9.
20. Duchin S, van Houte J. Relationship of Streptococcus mutans and lactobacilli to incipient smooth surface dental caries in man. Arch Oral Biol 1978;23:779–86.
21. Hoshino E. Predominant obligate anaerobes in human carious dentin. J Dent Res 1985;64:1195–8.
22. Milknes AR, Bowden GH. The microflora associated with the developing lesions of nursing caries. Caries Res 1985;19:289–97.
23. Brown LR, Lefkowitz W. Influences of dentinal fluids on experimental caries. J Dent Res 1966;45:1493–8.
24. Steinman RR, Leonora J, Singh RJ. The effect of desalivation upon pulpal function and dental caries in rats. J Dent Res 1980;59:176–85.
25. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 1994;78:522–30.
26. Hahn CL, Falkler WA Jr, Minah GE. Microbiological studies of carious dentine from human teeth with irreversible pulpitis. Arch Oral Biol 1991;36:147–53.
27. Martin FE, Nadkarni MA, Jacques NA, Hunter N. Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis. J Clin Microbiol 2002;40: 1698–704.
28. Munson MA, Banerjee A, Watson TF, Wade WG. Molecular analysis of the microflora associated with dental caries. J Clin Microbiol 2004;42:3023–9.
29. Chhour KL, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N. Molecular analysis of microbial diversity in advanced caries. J Clin Microbiol 2005;43:843–9.
30. Bergenholtz G. Effect of bacterial products on inflammatory reactions in the dental pulp. Scand J Dent Res 1977;85:122–9.
31. Nissan R, Segal H, Pashley D, Stevens R, Trowbridge H. Ability of bacterial endotoxin to diffuse through human dentin. J Endod 1995;21:62–4.
32. Puapichartdumrong P, Ikeda H, Suda H. Outward fluid flow reduces inward diffusion of bacterial lipopolysaccharide across intact and demineralised dentine. Arch Oral Biol 2005;50:707–13.
33. Hojo S, Komatsu M, Okuda R, Takahashi N, Yamada T. Acid profiles and pH of carious dentin in active and arrested lesions. J Dent Res 1994;73:1853–7.
34. Rhee SK, Pack MY. Effect of environmental pH on fermentation balance of Lactobacillus bulgaricus. J Bacteriol 1980;144:217–21.
35. Iwami Y, Abbe K, Takahashi-Abbe S, Yamada T. Acid production by streptococci growing at low pH in a chemostat under anaerobic conditions. Oral Microbiol Immunol 1992;7:304–8.
36. Panopoulos P, Mejare B, Edwall L. Effects of ammonia and organic acids on the intradental sensory nerve activity. Acta Odontol Scand 1983;41:209–15.
37. Guzman F, Braun C, Lim RK. Visceral pain and the pseudaffective response to intra-arterial injection of bradykinin and other algesic agents. Arch Int Pharmacodyn Ther 1962;136:353–84.
38. Lim RK, Liu CN, Guzman F, Braun C. Visceral receptors concerned in visceral pain and the pseudaffective response to intra-arterial injection of bradykinin and other algesic agents. J Comp Neurol 1962;118:269–93.
39. Hille B. Charges and potentials at the nerve surface. Divalent ions and pH. J Gen Physiol 1968;51:221–36.
40. Orchardson R. The generation of nerve impulses in mammalian axons by changing the concentrations of the normal constituents of extracellular fluid. J Physiol 1978;275:177–89.
41. Olgart L, Haegerstam G, Edwall L. The effect of extracellular calcium on thermal excitability of the sensory units in the tooth of the cat. Acta Physiol Scand 1974;91:116–22.
42. Hahn CL, Falkler WA Jr. Correlation between thermal sensitivity and microorganisms isolated from deep caries. J Encoded 1993;19:26–30.
43. Rodd HD, Boissonade FM. Innervation of human tooth pulp in relation to caries and dentition type. J Dent Res 2001;80:389–93.
44. Panopoulos P. Factors influencing the occurrence of pain in carious teeth. Proc Finn Dent Soc 1992;88 Suppl 1:155–60.
45. Takahashi N, Saito K, Schachtele CF, Yamada T. Acid tolerance and acid-neutralizing activity of Porphyromonas gingivalis, Prevotella intermedia and Fusobacterium nucleatum. Oral Microbiol Immunol 1997;12:323–8.
46. Takahashi N. Acid-neutralizing activity during amino acid fermentation by Porphyromonas gingivalis, Prevotella intermedia and Fusobacterium nucleatum. Oral Microbiol Immunol 2003;18:109–13.
47. Isenberg H, ed. Essential procedures for clinical microbiology. Washington, DC: American Society for Microbiology, 1998.

48. Massey WL, Romberg DM, Hunter N, Hume WR. The association of carious dentin microflora with tissue changes in human pulpitis. Oral Microbiol Immunol 1993;8:30–5.
49. Gomes BP, Pinheiro ET, Gade-Neto CR, et al. Microbiological examination of infected dental root canals. Oral Microbiol Immunol 2004;19:71–6.
50. Sundqvist G. Bacteriological studies of necrotic dental pulps [Thesis]. Umeå, Sweden: University of Umeå, 1976.
51. Khabbaz MG, Anastasiadis PL, Sykaras SN. Determination of endotoxins in caries: association with pulpal pain. Int Endod J 2000;33:132–7.
52. Bjornson HS. Activation of Hageman factor by lipopolysaccharides of Bacteroides fragilis, Bacteroides vulgatus, and Fusobacterium mortiferum. Rev Infect Dis 1984;6 Suppl 1:S30–3.
53. Miller RL, Webster ME, Melmon KL. Interaction of leukocytes and endotoxin with the plasmin and kinin systems. Eur J Pharmacol 1975;33:53–60.
54. Doyle RJ, Sonnenfeld EM. Properties of the cell surfaces of pathogenic bacteria. Int Rev Cytol 1989;118:33–92.
55. Wicken AJ, Knox KW. Lipoteichoic acids: a new class of bacterial antigen. Science 1975;187:1161–7.
56. Hogg SD, Whiley RA, De Soet JJ. Occurrence of lipoteichoic acid in oral streptococci. Int J Syst Bacteriol 1997;47:62–6.
57. Rolla G, Oppermann RV, Bowen WH, Ciardi JE, Knox KW. High amounts of lipoteichoic acid in sucrose-induced plaque in vivo. Caries Res 1980;14:235–8.
58. Jacques NA, Hardy L, Knox KW, Wicken AJ. Effect of growth conditions on the formation of extracellular lipoteichoic acid by Streptococcus mutans BHT. Infect Immun 1979;25:75–84.
59. Yoshimura A, Lien E, Ingalls RR, Tuomanen E, Dziarski R, Golenbock D. Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. J Immunol 1999;163:1–5.
60. Dziarski R, Tapping RI, Tobias PS. Binding of bacterial peptidoglycan to CD14. J Biol Chem 1998;273:8680–90.
61. Henderson B, Wilson M. Cytokine induction by bacteria: beyond lipopolysaccharide. Cytokine 1996;8:269–82.
62. Ginsburg I. Role of lipoteichoic acid in infection and inflammation. Lancet Infect Dis 2002;2:171–9.
63. Durand SH, Flacher V, Romeas A, et al. Lipoteichoic acid increases TLR and functional chemokine expression while reducing dentin formation in in vitro differentiated human odontoblasts. J Immunol 2006;176:2880–7.
64. Matsuguchi T, Takagi A, Matsuzaki T, et al. Lipoteichoic acids from Lactobacillus strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. Clin Diagn Lab Immunol 2003;10:259–66.
65. Wang PL, Shirasu S, Daito M, Ohura K. Streptococcus mutans lipoteichoic acid-induced apoptosis in cultured dental pulp cells from human deciduous teeth. Biochem Biophys Res Commun 2001;281:957–61.
66. Botero TM, Mantellini MG, Song W, Hanks CT, Nor JE. Effect of lipopolysaccharides on vascular endothelial growth factor expression in mouse pulp cells and macrophages. Eur J Oral Sci 2003;111:228–34.
67. Telles PD, Hanks CT, Machado MA, Nor JE. Lipoteichoic acid up-regulates VEGF expression in macrophages and pulp cells. J Dent Res 2003;82:466–70.
68. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983–5.
69. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004;25:581–611.
70. Shulman K, Rosen S, Tognazzi K, Manseau EJ, Brown LF. Expression of vascular permeability factor (VPF/VEGF) is altered in many glomerular diseases. J Am Soc Nephrol 1996;7:661–6.
71. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. Arch Oral Biol 2000;45:1013–6.
72. Sela S, Marouni MJ, Perry R, Barzilai A. Effect of lipoteichoic acid on the uptake of Streptococcus pyogenes by HEp-2 cells. FEMS Microbiol Lett 2000;193:187–93.
73. Plitnick LM, Jordan RA, Banas JA, et al. Lipoteichoic acid inhibits interleukin-2 (IL-2) function by direct binding to IL-2. Clin Diagn Lab Immunol 2001;8:972–9.
74. Plitnick LM, Banas JA, Jelley-Gibbs DM, et al. Inhibition of interleukin-2 by a Gram-positive bacterium, Streptococcus mutans. Immunology 1998;95:522–8.
75. Rauschenberger CR, Bailey JC, Cootauco CJ. Detection of human IL-2 in normal and inflamed dental pulps. J Endod 1997;23:366–70.
76. Anderson LM, Dumsha TC, McDonald NJ, Spitznagel JK Jr. Evaluating IL-2 levels in human pulp tissue. J Endod 2002;28:651–5.
77. Hahn CL, Best AM, Tew JG. Cytokine induction by Streptococcus mutans and pulpal pathogenesis. Infect Immun 2000;68:6785–9.
78. Hessle C, Andersson B, Wold AE. Gram-positive bacteria are potent inducers of monocytic interleukin-12 (IL-12) while gram-negative bacteria preferentially stimulate IL-10 production. Infect Immun 2000;68:3581–6.
79. Hessle CC, Andersson B, Wold AE. Gram-positive and Gram-negative bacteria elicit different patterns of pro-inflammatory cytokines in human monocytes. Cytokine 2005;30:311–8.
80. Mostefaoui Y, Bart C, Frenette M, Rouabhia M. Candida albicans and Streptococcus salivarius modulate IL-6, IL-8, and TNF-alpha expression and secretion by engineered human oral mucosa cells. Cell Microbiol 2004;6:1085–96.
81. Yang LC, Tsai CH, Huang FM, Liu CM, Lai CC, Chang YC. Induction of interleukin-6 gene expression by pro-inflammatory cytokines and black-pigmented Bacteroides in human pulp cell cultures. Int Endod J 2003;36:352–7.
82. Sanceau J, Kaisho T, Hirano T, Wietzerbin J. Triggering of the human interleukin-6 gene by interferon-gamma and tumor necrosis factor-alpha in monocytic cells involves cooperation between interferon regulatory factor-1, NF kappa B, and Sp1 transcription factors. J Biol Chem 1995;270:27920–31.
83. Sizemore N, Leung S, Stark GR. Activation of phosphatidylinositol 3-kinase in response to interleukin-1 leads to phosphorylation and activation of the NF-kappaB p65/RelA subunit. Mol Cell Biol 1999;19:4798–805.
84. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon β 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA 1987;84:7251–5.
85. Koj A. Initiation of acute phase response and synthesis of cytokines. Biochim Biophys Acta Mol Basis Dis 1996;1317:84–94.
86. Suwa T, Hogg JC, English D, Van Eeden SF. Interleukin-6 induces demargination of intravascular neutrophils and shortens their transit in marrow. Am J Physiol Heart Circ Physiol 2000;279:H2954–60.
87. Ulich TR, del Castillo J, Guo KZ. In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. Blood 1989;73:108–10.
88. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 1996;9:532–62.
89. Matsushima T, Ohbayashi E, Hosoda S, Shibata Y, Yamazaki M, Abiko Y. Stimulation of IL1b and IL6 production in human dental pulp cells by peptidoglycans from carious lesion microorganisms. In: Shimono M, Maeda T, Suda H, Takahashi K, eds. International Conference on Dentin/Pulp Complex 1995; 1995, Chiba, Japan: Quintessence Publishing; 1995:310–2.
90. Matsushima K, Ohbayashi E, Takeuchi H, Hosoya S, Abiko Y, Yamazaki M. Stimulation of interleukin-6 production in human dental pulp cells by peptidoglycans from Lactobacillus casei. J Endod 1998;24:252–5.
91. Heumann D, Barras C, Severin A, Glauser MP, Tomasz A. Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes. Infect Immun 1994;62:2715–21.
92. Myhre AE, Aasen AO, Thiemermann C, Wang JE. Peptidoglycan—an endotoxin in its own right? Shock 2006;25:227–35.
93. Wang JE, Dahle MK, McDonald M, Foster SJ, Aasen AO, Thiemermann C. Peptidoglycan and lipoteichoic acid in gram-positive bacterial sepsis: receptors, signal transduction, biological effects, and synergism. Shock 2003;20:402–14.
94. Schrijver IA, van Meurs M, Melief MJ, et al. Bacterial peptidoglycan and immune reactivity in the central nervous system in multiple sclerosis. Brain 2001;124:1544–54.
95. Schrijver IA, Melief MJ, Tak PP, Hazenberg MP, Laman JD. Antigen-presenting cells containing bacterial peptidoglycan in synovial tissues of rheumatoid arthritis patients coexpress costimulatory molecules and cytokines. Arthritis Rheum 2000;43:2160–8.
96. Henderson B, Poole S, Wilson M. Bacterial modulins: a novel class of virulence factors which cause host tissue pathology by inducing cytokine synthesis. Microbiol Rev 1996;60:316–41.
97. Coil J, Tam E, Waterfield JD. Proinflammatory cytokine profiles in pulp fibroblasts stimulated with lipopolysaccharide and methyl mercaptan. J Endod 2004;30:88–91.
98. Hosoya S, Matsushima K. Stimulation of interleukin-1 beta production of human dental pulp cells by Porphyromonas endodontalis lipopolysaccharide. J Endod 1997;23:39–42.
99. Tokuda M, Sakuta T, Fushuku A, Torii M, Nagaoka S. Regulation of interleukin-6 expression in human dental pulp cell cultures stimulated with Prevotella intermedia lipopolysaccharide. J Endod 2001;27:273–7.
100. Yang LC, Huang FM, Lin CS, Liu CM, Lai CC, Chang YC. Induction of interleukin-8 gene expression by black-pigmented Bacteroides in human pulp fibroblasts and osteoblasts. Int Endod J 2003;36:774–9.
101. Nagaoka S, Tokuda M, Sakuta T, et al. Interleukin-8 gene expression by human dental pulp fibroblast in cultures stimulated with Prevotella intermedia lipopolysaccharide. J Endod 1996;22:9–12.
102. 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;88:214–20.

103. Nakanishi T, Takahashi K, Ozaki K, Nakae H, Matsuo T. An immunohistologic study on the localization of selected bacteria and chemokines in human deep-carious teeth. In: Ishikawa T, Takahashi K, Maeda T, Suda H, Shimono M, Inoue T, eds. International Conference on Dentin/Pulp Complex; 2001; Chiba, Japan: Quintessence Publishing; 2001:143–5.
104. D'Souza R, Brown L, Newland J, Levy B, Lachman L. Detection and characterization of interleukin-1 in human dental pulps. Arch Oral Biol 1989;34:307–13.
105. Tokuda M, Nagaoka S, Torii M. Interleukin-10 inhibits expression of interleukin-6 and -8 mRNA in human dental pulp cell cultures via nuclear factor-kappaB deactivation. J Endod 2002;28:177–80.
106. Soell M, Holveck F, Scholler M, Wachsmann RD, Klein JP. Binding of Streptococcus mutans SR protein to human monocytes: production of tumor necrosis factor, interleukin 1, and interleukin 6. Infect Immun 1994;62:1805–12.
107. Jiang Y, Russell TR, Schilder H, Graves DT. Endodontic pathogens stimulate monocyte chemoattractant protein-1 and interleukin-8 in mononuclear cells. J Endod 1998;24:86–90.
108. Engels-Deutsch M, Pini A, Yamashita Y, et al. Insertional inactivation of pac and rmlB genes reduces the release of tumor necrosis factor alpha, interleukin-6, and interleukin-8 induced by Streptococcus mutans in monocytic, dental pulp, and periodontal ligament cells. Infect Immun 2003;71:5169–77.
109. Takada H, Kawabata Y, Tamura M, et al. Cytokine induction by extracellular products of oral viridans group streptococci. Infect Immun 1993;61:5252–60.
110. Soto A, Evans TJ, Cohen J. Proinflammatory cytokine production by human peripheral blood mononuclear cells stimulated with cell-free supernatants of Viridans streptococci. Cytokine 1996;8:300–4.
111. Banks J, Poole S, Nair SP, et al. Streptococcus sanguis secretes CD14-binding proteins that stimulate cytokine synthesis: a clue to the pathogenesis of infective (bacterial) endocarditis? Microb Pathog 2002;32:105–16.
112. Jyvasjarvi E, Kniffki KD. Studies on the presence and functional properties of afferent C-fibers in the cat's dental pulp. Proc Finn Dent Soc 1992;88 Suppl 1:533–42.
113. Narhi MV. The characteristics of intradental sensory units and their responses to stimulation. J Dent Res 1985;64 Spec No:564–71.
114. Hargreaves KM, Swift JQ, Roszkowski MT, Bowles W, Garry MG, Jackson DL. Pharmacology of peripheral neuropeptide and inflammatory mediator release. Oral Surg Oral Med Oral Pathol 1994;78:503–10.
115. Fu LW, Longhurst JC. Interleukin-1beta sensitizes abdominal visceral afferents of cats to ischaemia and histamine. J Physiol 1999;521:249–60.
116. Martin HA. Bradykinin potentiates the chemoresponsiveness of rat cutaneous C-fibre polymodal nociceptors to interleukin-2. Arch Physiol Biochem 1996; 104:229–38.
117. Wilson M, Seymour R, Henderson B. Bacterial perturbation of cytokine networks. Infect Immun 1998;66:2401–9.
118. Leung KP, Torres BA. Prevotella intermedia stimulates expansion of Vbeta-specific CD4(+) T cells. Infect Immun 2000;68:5420–4.
119. Matsushita K, Fujimaki W, Kato H, et al. Immunopathological activities of extracellular products of Streptococcus mitis, particularly a superantigenic fraction. Infect Immun 1995;63:785–93.
120. Zadeh HH, Kreutzer DL. Evidence for involvement of superantigens in human periodontal diseases: skewed expression of T cell receptor variable regions by gingival T cells. Oral Microbiol Immunol 1996;11:88–95.
121. Griffin WS. Inflammation and neurodegenerative diseases. Am J Clin Nutr 2006;83:470S–4S.
122. Bartold PM, Marshall RI, Haynes DR. Periodontitis and rheumatoid arthritis: a review. J Periodontol 2005;76(11 Suppl):2066–74.
123. Liuzzo G, Giubilato G, Pinnelli M. T cells and cytokines in atherogenesis. Lupus 2005;14:732–5.
124. Smolen JS, Redlich K, Zwerina J, Aletaha D, Steiner G, Schett G. Pro-inflammatory cytokines in rheumatoid arthritis: pathogenetic and therapeutic aspects. Clin Rev Allergy Immunol 2005;28:239–48.
125. Ardizzone S, Bianchi Porro G. Biologic therapy for inflammatory bowel disease. Drugs 2005;65:2253–86.
126. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. Ann Periodontol 2003;8:12–37.
127. De Keyser F, Van den Bosch F, Mielants H. Anti-TNF-alpha therapy in ankylosing spondylitis. Cytokine 2006;33:294–8.
128. Koller MD. Targeted therapy in rheumatoid arthritis. Wien Med Wochenschr 2006;156:53–60.
129. Chapat L, Chemin K, Dubois B, Bourdet-Sicard R, Kaiserlian D. Lactobacillus casei reduces CD8+ T cell-mediated skin inflammation. Eur J Immunol 2004;34: 2520–8.
130. Smits HH, Engering A, van der Kleij D, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 2005;115:1260–7.
131. von der Weid T, Bulliard C, Schiffrin EJ. Induction by a lactic acid bacterium of a population of CD4(+) T cells with low proliferative capacity that produce transforming growth factor beta and interleukin-10. Clin Diagn Lab Immunol 2001;8:695–701.
132. Hahn CL, Best AM, Tew JG. Comparison of type 1 and type 2 cytokine production by mononuclear cells cultured with streptococcus mutans and selected other caries bacteria. J Endod 2004;30:333–8.
133. Hart AL, Lammers K, Brigidi P, et al. Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 2004;53:1602–9.
134. Lutz MB, Schuler G. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? Trends Immunol 2002;23:445–9.
135. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol 2003;21:685–711.
136. Sylvestre DL, Ravetch JV. A dominant role for mast cell Fc receptors in the Arthus reaction. Immunity 1996;5:387–90.
137. Hazenbos WL, Gessner JE, Hofhuis FM, et al. Impaired IgG-dependent anaphylaxis and Arthus reaction in Fc gamma RIII (CD16) deficient mice. Immunity 1996;5:181–8.
138. Paterson RC, Pountney SK. Pulp response to Streptococcus mutans. Oral Surg Oral Med Oral Pathol 1987;64:339–47.
139. Paterson R, Pountney S. Pulp response to and cariogenicity of Lactobacillus casei in monoinfected gnotobiotic rats. Oral Surg Oral Med Oral Pathol 1987;64:611–24.
140. Meryon SD, Brook AM. Penetration of dentine by three oral bacteria in vitro and their associated cytotoxicity. Int Endod J 1990;23:196–202.
141. Meryon SD, Jakeman KJ, Browne RM. Investigation of the cytotoxicity of bacterial species implicated in pulpal inflammation. Int Endod J 1986;19:11–20.
142. Sedgley CM, Molander A, Flannagan SE, et al. Virulence, phenotype and genotype characteristics of endodontic Enterococcus spp. Oral Microbiol Immunol 2005;20:10–9.
143. Kayaoglu G, Orstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Crit Rev Oral Biol Med 2004;15:308–20.
144. Baumgartner C. Pulpal infections including caries. In: Hargreaves KM, Goodis HE, eds. Seltzer and Bender's dental pulp. Chicago: Quintessence Publishing; 2002:298–9.
145. Murray PE, Smith AJ, Windsor LJ, Mjor IA. Remaining dentine thickness and human pulp responses. Int Endod J 2003;36:33–43.
146. Stanley HR. Design for a human pulp study. II. Oral Surg Oral Med Oral Pathol 1968;25:756–64.
147. Stanley HR. Design for a human pulp study. I. Oral Surg Oral Med Oral Pathol 1968;25:633–47.
148. Pissiotis E, Spangberg L. Dentin as inhibitor of bacterial toxicity on pulpal cells in vitro. J Endod 1992;18:166–71.
149. Pissiotis E, Spangberg LS. Dentin permeability to bacterial proteins in vitro. J Endod 1994;20:118–22.
150. Damen JJ, de Soet JJ, ten Cate JM. Adsorption of [3H]-lipoteichoic acid to hydroxyapatite and its effect on crystal growth. Arch Oral Biol 1994;39:753–7.
151. Reeves R, Stanley HR. The relationship of bacterial penetration and pulpal pathosis in carious teeth. Oral Surg Oral Med Oral Pathol 1966;22:59–65.
152. 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;40:609–14.
153. McLachlan JL, Sloan AJ, Smith AJ, Landini G, Cooper PR. S100 and cytokine expression in caries. Infect Immun 2004;72:4102–8.
154. Socransky SS, Gibbons RJ, Dale AC, Bortnick L, Rosenthal E, Macdonald JB. The microbiota of the gingival crevice area of man. I. Total microscopic and viable counts and counts of specific organisms. Arch Oral Biol 1963;8:275–80.
155. Welin J, Wilkins JC, Beighton D, et al. Effect of acid shock on protein expression by biofilm cells of Streptococcus mutans. FEMS Microbiol Lett 2003;227:287–93.
156. Svensater G, Welin J, Wilkins JC, Beighton D, Hamilton IR. Protein expression by planktonic and biofilm cells of Streptococcus mutans. FEMS Microbiol Lett 2001;205:139–46.
157. Hahn CL, Overton B. The effects of immunoglobulins on the convective permeability of human dentine in vitro. Arch Oral Biol 1997;42:835–43.