Update on the Adaptive Immune Responses of the Dental Pulp

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Abstract

Recent advances in immunology have disclosed the enormous complexity of the immune regulatory system. The dental pulp is equipped to mount adaptive immune responses to caries, which include at least antigen-presenting cells, lymphocytes, mast cells and their cytokines, and chemokines. The purpose of this review is to summarize our current understanding of the roles of these cellular and molecular components in the irreversibly inflamed pulp. The immunopathology of abscess formation and the mechanisms for painless pulpitis are also discussed. *(J Endod 2007;33:773–781)*

Key Words

Adaptive immunity, caries, chemokines, cytokines, dental pulp

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Adaptive immunity is antigen specific, and serves to enhance the protective mechanisms of nonspecific innate immunity. It is also known as *acquired immunity* to emphasize the fact that these potent protective responses are acquired or learned as a consequence of experience. Adaptive immunity includes antigen-specific lymphocytes (T and B cells) and their products, which include inflammatory chemokines, cytokines, and antibodies. Inflammatory chemokines direct the trafficking of immune cells and cytokines induced during T-cell activation to regulate immune and inflammatory responses. Many cytokines produced by innate immune cells are produced by activated T cells in adaptive immunity. A comparison of cytokines induced in the innate and adaptive immune responses is presented in Table 1. For example, interferon-gamma (IFN-γ), which augments phagocytosis, is secreted by natural killer (NK) cells in the innate immune response, and also by effector T cells in adaptive immunity. Transforming growth factor-beta (TGF-β), which attracts immune cells but inhibits T- and B-cell proliferation and macrophage functions, is produced by macrophages and odontoblasts in the innate response, and also by regulatory T cells (Treg) in the adaptive immune response.

Cytokines such as interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) are only induced by activated T cells. Antibodies produced by activated B cells are capable of neutralizing bacterial toxins or preventing bacterial adherence. Antibodies can also function as opsonins for phagocytosis of extracellular bacteria such as those found in caries. The end result of adaptive immunity is an exaggerated inflammatory response (immune inflammation) intended to eliminate the infection. However, if the source of infection (i.e., caries) is not eliminated, immune inflammation in pulpitis eventually leads to irreversible destruction of the pulp. The purposes of this review are to summarize recent developments in immunology pertaining to pulp biology and to critically examine our understanding of the mechanism of pulpitis. Because pain and inflammation are intimately related, the modulation of the pain experience is also discussed.

Chemokines in Irreversible Pulpitis

Inflammatory chemokines are produced by activated leukocytes and tissue cells during inflammation and they, together with the upregulation of adhesion molecules, determine the composition of inflammatory infiltrates. In the inflamed pulp, adhesion molecules such as platelet-endothelial cell adhesion molecule-1 (PECAM-1) and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells are upregulated to facilitate the extravasation of leukocytes (1). In deep caries samples, chemokine ligands such as monocyte chemotactic protein-1 (CCL2/MCP-1), macrophage inflammatory protein 3-alpha (CCL20/MIP-3α), and interleukin-8 (CXCL8/IL-8) are immunohistochemically localized mainly in macrophages (2, 3) (Fig. 1). CCL2/MCP-1, which is also produced by immature dendritic cells (DCs), endothelial cells, and lipoteichoic acid (LTA)-stimulated odontoblasts, recruits monocytes, immature DCs, memory T cells, and NK cells to amplify the inflammatory response (4). The secretion of CCL20/MIP-3α by macrophages in the inflamed pulp may account for the recruitment of CCR6+ memory T cells, particularly Th2 cells (5–7) and immature DCs (8) in irreversible pulpitis.

Interleukin-8 was induced in vitro when pulpal fibroblasts or human pulp stem cells were challenged by endodontic pathogens, substance P (SP), lipopolysaccharides (LPS), or tumor necrosis factor (TNF) (9–12). However, IL-8 in inflamed pulps is immunohistochemically localized in odontoblasts, lymphocytes, macrophages, and endothelial cells, but not in fibroblasts (2, 13). These findings suggest that IL-8 secreted

TABLE 1. Major cytokines in innate and adaptive immunity in the dental pulp

Cytokine	Cell source	Innate	Adaptive	Principal functions
TNF-α, IL-1	Mo/Mϕ	●	●	EC activation, inflammaion
IFN-γ	NK cells	●	●	Mϕ activation
	Th1, CD8		●	
IL-12	Mo/Mϕ	●	●	IFN-γ induction
	DCs	●	●	
IL-18	Mϕ	●	●	IFN-γ induction
TGF-β	Mo/Mϕ	●	●	Anti-inflammatory
	Odontoblasts	●	●	Chemotaxis
	Treg cells		●	
IL-10	Mo/Mϕ	●		Anti-inflammatory
	Th2		●	Mϕ deactivation
IL-6	Mo/Mϕ, EC, fibroblasts	●	●	Acute-phase protein synthesis in liver
	Activated T cells		●	B-cell proliferation
IL-2	Activated T cells		●	T, B, NK proliferation
IL-4, IL-13	Th2		●	Th2 differentiation, IgE
	Mast cells		●	Mast cell proliferation

DC, dendritic cell; EC, endothelial cell; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Mo, monocyte; Mϕ, macrophage; NK, natural killer; TGF, tumor growth factor; TNF, tumor necrosis factor; Th, T helper; Treg, T regulatory.

by fibroblasts may not be important in the initial stage of pulpitis. Interestingly, the abundant presence of IL-8 in the inflamed pulps did not effectively attract neutrophils (13). The authors suggested that the secretion of chemoattractants intravascularly by endothelial cells might inhibit their transmigration (14, 15).

A limited number of studies have examined the chemokine induction of leukocytes by oral bacteria. IL-8 and CCL2/MCP-1 were secreted by peripheral blood mononuclear cells (PBMCs) upon stimulation with *Streptococcus mutans* (16). Neutrophils stimulated with *Porphyromonas endodontalis* yielded high titers of CCL3/MIP-1α and CCL4/MIP-1β (17). Both CCL3/MIP-1α and CCL4/MIP-β attract monocytes, macrophages, immature DCs, and T helper-1 (Th1) cells, but their presence in irreversible pulpitis tissues has not been examined. Human Th1 cells selectively express CXCR3 and CCR5. Their chemokine ligands (CXCL9, CXCL10, CXCL11, CCL3, CCL4, CCL5, and CCL8) are produced by neutrophils, activated macrophages, and immature DCs (18). Interestingly, CXCL10/IP-10 (IFN-γ-inducing protein) secretion by LTA-stimulated odontoblasts was recently reported (19). Th2 cells expressing CC chemokine receptors (CCR3, CCR4, and CCR8) respond to CCL20/MIP-3α and CCL21 chemokines (6, 7). CD8+ effector T cells express CCR2 as well as similar receptors (CCR5 and CXCR3) to those of Th1 cells (20, 21). Antigen-specific immunoglobulin (IgG)-secreting B cells also express CXCR3, and are targeted to inflamed nonmucosal tissues (22). However, the specificity of B-cell chemokine receptors and the inflammatory chemokines for T cells in reversible as well as irreversible pulpitis tissues has not been determined. A comprehensive study of the chemokine profile in pulps beneath shallow and deep caries is needed for a better understanding of their role in pulpitis.

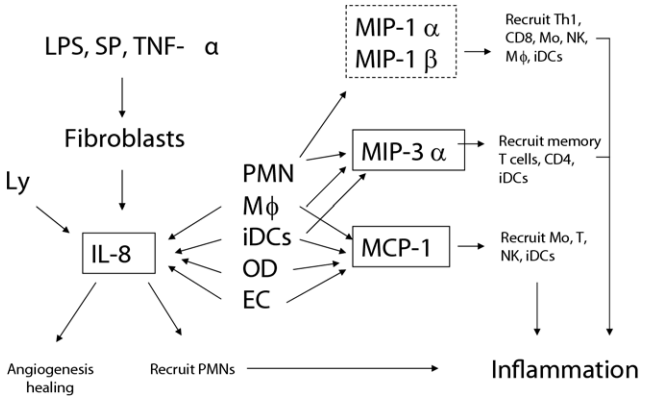

Figure 1. Functions and the cellular origin of chemokines in irreversible pulpitis. Chemokines such as IL-8, MIP-3α, and MCP-1 have been reported in the endodontic literature (solid-line text boxes). MIP-1α and MIP-1β are implicated but not yet identified in the inflamed pulp (dashed-line text box). Abbreviations: EC, endothelial cell; iDC, immature dendritic cell; Ly, lymphocyte; Mo, monocyte; Mϕ, macrophage; OD, odontoblast; PMN, neutrophil.

Antigen-Presenting Cells in Adaptive Immunity

The response of antigen-specific T cells to protein antigens requires the participation of antigen-presenting cells (APCs), which capture, process, and present the antigens to T cells. APCs can be further classified into "professional" APCs (e.g., bone marrow–derived DCs), which possess the unique ability to activate and induce clonal expansion of naive and memory T cells, and "nonprofessional" APCs, which include any major histocompatibility complex (MHC)-II bearing cells (e.g., B cells, monocytes, macrophages, and endothelial cells) capable of interacting locally with memory T cells (23, 24). DCs are migratory and are more effective in antigen presentation than other APCs. Macrophages have a profound capacity for endocytosis, but the low level of MHC-II expression on activated macrophages in part explains why macrophages are generally less efficient at antigen presentation and T-cell stimulation than either B cells or DCs (25). Antigen-specific B cells bind soluble protein antigens on their membrane Ig molecules and internalize, process, and present antigens to CD4+ T cells during humoral immune responses (26). The numbers of pulpal DCs and macrophages increase greatly in early pulpitis samples (27, 28). The majority of T cells in inflamed pulps are CD45RO+ memory cells (27, 29). It is likely, therefore, that both DCs and macrophages participate in antigen presentation in early pulpitis. The number of B cells increases significantly only in irreversible pulpitis samples when antigen from caries bacteria binding to specific Ig on B cells can then be presented to T cells.

Macrophages in Pulpitis

In inflammation, macrophages have three major functions: antigen presentation, phagocytosis, and immunomodulation through the production of various cytokines and growth factors. Macrophages are activated and deactivated during the inflammatory process (30). CD4+ Th1 and CD8+ T cells can both activate macrophages by IFN-γ and CD40L-CD40 interactions. Activated macrophages produce TNF-α, IL-1, IL-12, IL-10, chemokines, and short-lived lipid mediators such as platelet activating factor (PAF), prostaglandins, and leukotrienes to or-

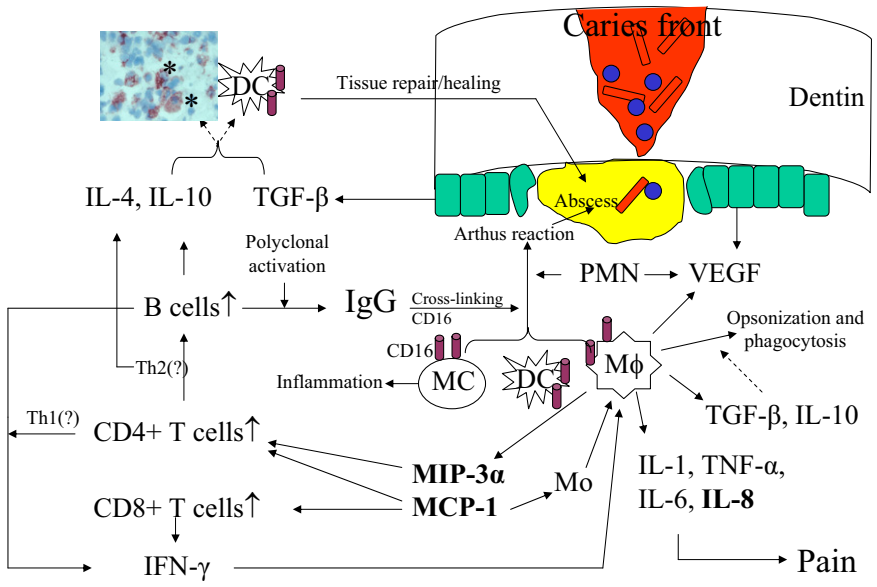

Figure 2. Chemokines, immune cells, and mast cells in the pathogenesis of a local abscess beneath deep caries. Negative feedback of TGF-β and IL-10 is depicted with a dashed line. Chemokines secreted by macrophages are indicated in bold. The presence of Th1 and Th2 cells in the inflamed pulp has not been determined and is indicated with a question mark (?). Photo inset: Macrophages (*asterisks*) in a frozen section from one irreversible pulpitis sample. Positive cells were stained with mouse anti-human macrophage antibody (DakoCytomation, Carpinteria, CA), followed by an indirect immunoperoxidase staining procedure (not previously published). Abbreviations: DC, dendritic cell; MC, mast cell; Mo, monocyte; Mϕ, macrophage; PMN, neutrophil.

chestrate a local inflammation. Izumi et al. (28) observed a higher number of macrophages than DCs in all stages of caries invasion. In irreversible pulpitis, significantly increased titers of TNF-α and IL-1 were reported (31, 32), and IL-1 was mainly localized in macrophages and pulp connective tissue stroma (31). IL-10 and TGF-β deactivate macrophages, which results in matrix deposition and tissue remodeling. IL-4 or IL-13 alternatively activates macrophages to promote a type-2 response with humoral immunity and tissue repair (8, 33). In chronic inflammation, such as tuberculoid leprosy, continuous extracellular release of antimicrobial molecules such as oxygen radicals, nitric oxide, and proteases by frustrated macrophages causes tissue damage (34). Blocked by the dentin, frustrated macrophages in the dental pulp are not able to eliminate the bacteria in deep caries, and their products contribute to pulpal tissue destruction. The roles of macrophages in irreversible pulpitis are summarized in Fig. 2.

Dendritic Cells in Pulpitis

The number of pulpal DCs (HLA-DR+, FXIIIa+) increases with the advance of caries (27, 28). However, the maturity of these pulpal DCs in the steady state or in pulpitis has not been examined. Mature DCs express HLA class II, CD83, and DC-lysosome-associated membrane glycoprotein (DC-LAMP) as well as costimulatory molecules such as CD86 and CD40 (35, 36). Trace levels of mature DC markers (CD83, DC-LAMP) have been detected in the odontoblastic layer and pulp core, respectively (37, 38), but their biologic significance is not understood. After capturing antigens, immature DCs undergo maturation and migrate to lymph nodes to present antigens to T cells (39). A recent study confirmed that a subgroup of mouse pulpal DCs with enhanced CD86 expression migrated to regional lymph nodes in response to injury (40).

Both pathogens and host tissue-derived factors promote DC maturation. We recently demonstrated that oral streptococci could rapidly transform monocytes into mature DCs in vitro (41). Host-derived factors such as inflammatory mediators, cytokines (e.g., IL-1 and TNF-α) and vasoactive intestinal polypeptide (VIP) in pulpitis can promote DC maturation (42, 43). Neuropeptides such as calcitonin gene-related peptide (CGRP) and VIP can inhibit the migration of mature DCs to regional lymph nodes (44). Furthermore, mature DCs are found in chronically inflamed skin and periodontal tissues (7, 45, 46). Our preliminary study indicated the presence of CD83+ mature DCs in inflamed pulps (unpublished data).

The significance of local antigen presentation by mature DCs in peripheral tissues is not clear, but mature DCs may play a role in continuously recruiting effector leukocytes to infection sites (7). It is reasonable that these monocyte-derived DCs may join tissue-resident pulpal DCs in antigen uptake and either migrate to regional lymph nodes or mature locally. Mature DCs that fail to migrate to lymph nodes could produce an array of chemokines to attract the appropriate effector lymphocytes (7) and present antigens locally to memory T cells in inflamed pulps (27–29, 47, 48). A model of monocyte and DC trafficking in pulpitis is proposed in Fig. 3.

Recent studies demonstrate that immature DCs in peripheral tissues in the steady state can capture tissue antigens from apoptotic cells and become migratory semimature DCs (tolerogenic DCs) that induce Treg to promote tolerance in draining lymph nodes (49–51). Immunosuppressive mediators such as TGF-β, IL-10, and vascular endothelial cell growth factor (VEGF) can alter DC maturation to restore them to their immature phenotype to participate in tissue healing (52–56). The expression of these mediators is elevated in irreversible pulpitis tissues (57–59). It is speculated that tolerogenic DCs can be induced in pulpitis to promote healing. Functional studies of the heterogenic DC population are necessary to elucidate its roles in pulpitis. Better understanding of the intricate immune responses in the inflamed pulp may result in more efficacious and predictable vital pulp therapy.

T Cells and Their Cytokines in Adaptive Immunity

After T cells are activated by antigens presented on APCs and/or cytokines, they secrete cytokines and differentiate into various effector cells: CD4+ T helper cells, cytotoxic CD8+ T cells, Treg cells, or memory cells (Fig. 4). The nature of the adaptive response is deter-

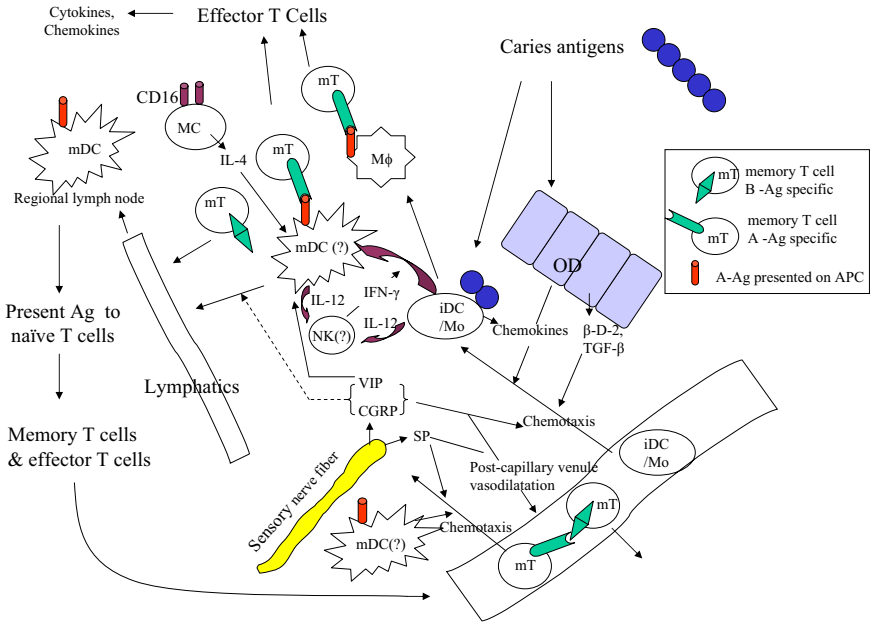

Figure 3. Cellular and molecular mechanisms in T-cell and dendritic cell/monocyte trafficking. Caries antigens can directly stimulate odontoblasts and resident immature DCs to secrete chemotactic agents to attract immature DCs or monocytes. An A-antigen processed by DCs or macrophages can be presented to memory T cells with the specific A-antigen receptors and T cells become effector cells. T cells with the B-antigen receptor may return through lymphatic vessels to the circulation. Factors that might contribute to local maturation of DCs are illustrated. The presence of mDC and NK cells has not been confirmed and is indicated with a question mark (?). Maturing DCs may also migrate through lymphatics to present antigens to T cells at local lymph nodes. Inhibition of maturing DC migration by VIP and CGRP is depicted with a dashed line. Leukocyte chemotaxis is promoted by neuropeptides (such as VIP and CGRP), TGF-β and β-D-2 from odontoblasts, and chemokines from mature DCs as well as odontoblasts. Abbreviations: CGRP, calcitonin gene-related peptide; iDC, immature DC; MC, mast cell; mDC, mature DC; Mo, monocyte; Mϕ, macrophage; mT, memory T cell; NK, natural killer; OD, odontoblast; VIP, vasoactive intestinal polypeptide.

mined by the preceding innate immune reaction. Immature DCs are able to polarize T helper cells into functional subsets (according to their cytokine pattern) of Th1, Th2, or inducible Treg cells, depending on the dose, affinity, and nature of the antigens and the type and concentration of cytokines in the tissue microenvironment (7, 60). For instance, an increased dose of peptide antigen elicits a shift from a Th1 to a Th2 response (61). *S. mutans* associated with the initial caries lesions is a Th1 inducer, whereas *Pseudoramibacter alactolyticus*, usually isolated from deep caries, is a Th2 inducer (62). Th1 cells secrete IFN-γ, and Th2 cells secrete IL-4 and IL-10 (63). It is generally accepted that Th1 and Th2 cytokines are mutually inhibitory at the T-cell level (64, 65). The pathology of most immune-mediated diseases can be explained by cross regulation between Th1 and Th2 cytokines. For example, the lepromatous form of leprosy, found in patients with impaired cell-mediated immunity, exhibits primarily a Th2 cytokine profile in skin lesions, with numerous mycobacterial organisms present. Conversely, the tuberculoid form of leprosy, found in patients having strong cell-mediated immunity, is characterized by a type of Th1 cell response, with few organisms present.

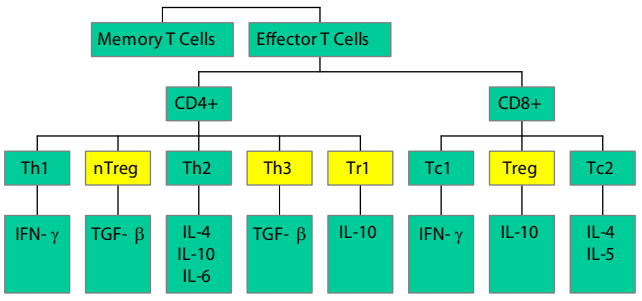

Figure 4. Classification of activated T cells according to their phenotypes, cytokine profile, and functions. Subtypes of regulatory T cells are depicted in yellow.

The concept of cross regulation between Th1 and Th2 cytokines fails to adequately explain certain preclinical outcomes (66, 67). For instance, the alleviation of an allergic (Th2) response was not achieved by a shift to a Th1 response in a murine model (68). Recent studies identified Treg as an important suppressor T-cell subset in both Th1 and Th2 responses that prevents infection-induced pathosis and prolongs pathogen persistence (69, 70). A shortage of Treg may result in an excessive Th1 or Th2 response with associated immunopathology. Excess Treg may suppress protective cell-mediated or humoral immunity (71, 72). The immunosuppressive function of Treg is mediated either through direct cell contact or through cytokines, and these cells are different from the originally defined suppressor T cells (73, 74). The best-studied Treg subset expressing CD4+, CD25+, and transcription factor FoxP3+ is a naturally occurring T-cell subset (nTreg) that exerts its suppressive function mainly via cell-cell contact. These cells are considered a component of the innate immune response, and play a key role in the maintenance of self-tolerance. Inducible regulatory T cells, including Treg type 1 (Tr1) and T helper type 3 (Th3) subsets, exhibit cytokine-mediated suppression with IL-10 and TGF-β (70). The role of Treg in the pathogenesis of pulpitis is yet to be determined.

The terms *type-1* and *type-2 cytokines* are used in this review in preference to *Th1* and *Th2 cytokines* because inflammatory cytokines in cell-mediated and humoral immunity are also produced by non-CD4+ Th cells, such as Treg, CD8, or NK cells. Type-1 cytokines, including IFN-γ, IL-2, IL-12, and TNF-α, orchestrate strong cellular immune responses and inhibit synthesis of type-2 cytokines. Type-2 cytokines include IL-10 and IL-4, which suppress macrophage activation and stimulate B cells to proliferate and to differentiate into plasma cells, respectively, an indication of homeostasis or chronicity of the

disease state (75, 76). Both the concentration of type-1 (TNF-α and IFN-γ) and type-2 (IL-10, IL-4) cytokine proteins and their mRNA levels are elevated in inflamed pulps (32, 58, 77). The titer of IL-2 was reported to be increased in pulpitis by one study (77), but this finding was not supported by two other studies (31, 78). The discrepancy could result from the different microbial flora used or by LTA interference (79).

Like CD4+ T cells, cytotoxic CD8+ effector T cells can be classified into T cytotoxic-1 (Tc1), Tc2, and CD8+ Treg subsets according to their cytokine pattern (80). Tc1 cells secrete IFN-γ but not IL-4 or IL-5, whereas Tc2 cells secrete IL-4 and IL-5, but not IFN-γ (81). CD8 Treg cells secrete mainly IL-10 to suppress immune responses (69). Both Tc1 and Tc2 cells induce strong inflammatory reactions, and Tc2 cells are more potent in enhancing B-cell proliferation but less cytotoxic than Tc1 cells (81). The distribution of Tc1, Tc2, and Treg cells in inflamed dental pulps and their antigenic specificity in reversible pulpitis samples are not known. Few of these (but a significantly higher number of CD8+ T cells than CD4+ T cells) are present in reversible pulpitis samples (82, 83). This preferential attraction of CD8+ T cells could be caused by the nature of the antigens, chemokines, or cytokines. We demonstrated that *S. mutans* preferentially activates CD8+ over CD4+ T cells in vitro (58). A recent study demonstrated that CXCL11 (a CXCR3 ligand) preferentially attracts CD8+ T cells (84). CXCL11 is secreted by many cell types, including monocytes and fibroblasts, on stimulation with IFN-γ and IL-1 (85). The significantly higher expression of IFN-γ mRNA compared to IL-4 or IL-10 in reversible pulpitis tissue supports the notion that the induction of CXCL11 in early pulpitis may contribute to the accumulation of CD8+ T cells (58). Although the cytotoxic nature of CD8+ T cells in the inflamed dental pulp remains undetermined, lack of adequate CD4+ T cells in early pulpitis might render CD8+ T cells less cytolytic or noncytolytic (86).

The significantly higher numbers of B cells and plasma cells found in irreversible pulpitis samples together with an increased CD4/CD8 ratio (82, 83) support a type-2 immune response beneath deep caries. Approximately 60% of deep caries pulp samples contain IL-10 mRNA, and its prevalence is significantly higher in these samples than in pulps collected from shallow caries (58). IL-10, mainly secreted by activated lymphocytes and macrophages, is perhaps the most antiinflammatory cytokine known (87). IL-10 effectively inhibits IFN-γ secretion, phagocytosis, and maturation of immature DCs, and promotes Tr1 induction (88). In symptomatic pulps, elevated levels of the mRNA of IL-18 (a type-1 cytokine) and IL-6, which is considered a type-2 cytokine by some researchers, were also detected (89). Although Th cell subsets (Th1 and Th2) in inflamed pulps have not been identified, there appears to be a shift from the cellular immunity associated with shallow caries to a mixed cellular/humoral immunity in irreversibly inflamed pulps beneath deep caries. Several host and bacterial factors can contribute to the shift of the cellular population. An increased dose of peptide antigen elicits a shift from a Th1 toward a Th2 response (61). Bacterial antigens such as *P. alactolyticus* found in deep caries polarize peripheral blood leukocytes toward a type-2 cytokine profile, and other deep-caries associated bacteria such as *Lactobacillus casei*, *Actinomyces viscosus,* and *Prevotella intermedia* elicit a mixed or a weak type-2 profile (62). Furthermore, activated Th1 cells naturally switch to secrete type-2 cytokines after multiple divisions to allow homeostasis to be reestablished (90).

Messenger RNA expression of anti-inflammatory cytokines such as IL-4 and IL-10 is elevated in the later stage of pulpitis (58). In addition, IL-1ra antagonizes the proinflammatory effects of IL-1α and IL-β and is expressed by pulpal endothelial cells and leukocytes in inflamed pulps (91). TGF-β, another family of host-derived anti-inflammatory factors, also counteracts the effects of proinflammatory cytokines by inhibiting lymphocyte proliferation and macrophage functions. TGF-β1, secreted by antigen-activated T cells, Treg, LPS-activated monocytes, and odontoblasts (59), causes synthesis of extracellular matrix proteins such as collagens, matrix metalloproteinases, and integrins. Activated macrophages remove dead tissue and secrete growth factors to stimulate fibroblast proliferation and revascularization. These actions may promote tissue repair after local immune and inflammatory reactions have been controlled, but their anti-inflammatory efforts in irreversible pulpitis may be too late to be beneficial. Although dental pulps can recover from localized abscesses in animals (92, 93), it is not clear whether a similar healing capacity also exists in humans.

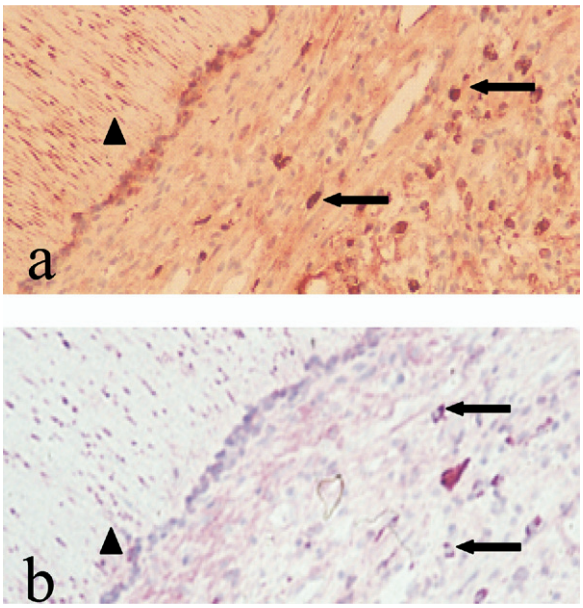

Figure 5. IgG1 is the predominant subtype in inflamed pulps. Adjacent paraffin sections from an irreversible pulpitis sample were stained with mouse anti-human IgG1 (*A*) and IgG2 (*B*) antibodies respectively (not previously published). The IgG-containing cells (*arrow*) are located in the tissue, and IgG1 and IgG2 molecules are localized in the dentinal tubules (*arrowhead*).

B Cells and Antibodies

A few B cells appear in early pulpitis, but their number increases significantly along with CD4+ T cells when the caries approaches the pulp. In addition to their main function of producing antibodies, B cells can also function as APCs, modulate DC functions, and produce cytokines such as IL-10, IL-4, and IFN-γ in response to pathogens (94, 95). Speer et al. (96) reported that inflamed pulps contained higher levels of IgG and IgA than noninflamed pulps, using pooled tissues. Because the serum levels of immunoglobulins of subjects vary with sex, age, and race (97–99), the lack of adjustment of serum levels in their study makes the interpretation of their results difficult.

An immunohistochemical study of inflamed pulps revealed IgG to be the predominant class, followed by IgA and IgE (100). Our preliminary study suggests that IgG1, rather than IgG2, may be the dominant subclass among immunoglobulin-bearing cells (Fig. 5). The determination of IgG subclasses may help to identify the predominant caries antigens. For example, protein antigens elicit an IgG1 response with the help of CD4+ T cells, whereas carbohydrate antigens such as polysaccharide and LPS mainly elicit an IgG2 response, which may occur without the help of T cells (101). The latter are known as thymus-independent or T-independent antigens, which generally elicit antibodies of low affinity, mainly IgM and some IgG subtypes. Most T-independent anti-

gens are polyvalent, with many repeated antigenic epitopes such as nucleic acids and bacterial cell wall components (e.g., LPS, LTA). These components in deep carious lesions may induce IgM and IgG2 expression in irreversible pulpitis tissues.

The specificity of antibodies in pulpits samples was examined using supernatant fluids from tissue explants (102, 103). Levels of antibodies reacting with 2 strains (*S. mutans* and *L. casei*) out of a total of 16 isolates implicated in caries and endodontic infection were significantly elevated over those in normal pulps. When reacted with eight clinical caries isolates, levels of antibodies to *S. mutans* and *Lactobacillus acidophilus* were elevated but failed to reach statistical significance (103). It appears that antibody titers against *S. mutans* and lactobacilli may be elevated in irreversible pulpitis. Because of the diversity of the caries flora and the small quantities of antibodies found in dental pulps, molecular approaches are required to better correlate the caries flora with their corresponding antibodies in pulpal tissue. On the other hand, B cells can also be activated polyclonally by mitogens such as pokeweed. In fact, most oral bacteria themselves or their cellular sonicates are polyclonal B-cell activators that require CD4+ helper T cells and need to be processed by APCs (104, 105). As a group, Gram-negative oral bacteria are more potent polyclonal B-cell activators than are Gram-positive bacteria (106–108). Because the number of Gram-negative bacteria increases in certain deep carious lesions, it is reasonable to assume that the antibodies secreted by B cells and plasma cells beneath deep caries are either antigen specific or polyclonally activated.

It is not known whether antibodies secreted by B cells and plasma cells beneath deep caries are protective. If antibodies are effective opsonins in promoting bacterial clearance by macrophages, the disease will not progress. If they are not protective, the lesion will persist, and a continuous B-cell activation would promote large amounts of IL-1 secretion and hence tissue destruction (109–111) (Fig. 2). Even if they are protective, it may be too late to reverse the damage in the case of irreversible pulpitis.

Mast Cells

Normal human pulps contain few if any mast cells, whereas these cells are abundant in inflamed pulps (112–114). Because the titer of IgE in the inflamed pulp is low (100), classic Type I hypersensitivity mediated by IgE and mast cells is unlikely to be important in the pathogenesis of pulpitis. However, mast cells contain proinflammatory cytokines (TNF-α, IL-1) and release potent immunoregulatory mediators (115). Release of IL-4 from activated mast cells would promote DC maturation and a transition from acute to chronic inflammation. SP induces the release of various autacoids, such as histamine and 5-hydroxytryptamine released from mast cells (116, 117), which are able to sensitize or activate afferent neurons and produce vasodilatation and protein exudation in the postacute phase of neurogenic inflammation. Recent studies have demonstrated the important role of mast cells in eliciting an Arthus reaction (next section). It is reasoned that mast cells and the mediators they produce are important in abscess formation during a later stage of pulpitis (Fig. 2).

Immunopathology in Experimental Pulpitis

Previous studies on the pathogenesis of pulpitis by caries bacteria were often conducted in an acute inflammatory environment (deep cavity preparation or mechanical pulp exposure) with neutrophil accumulation shortly after the procedure (118–120). These results supported the toxicity of bacteria and/or their by-products. However, caries elicits long-term chronic inflammation in vivo, and obvious neutrophil accumulation usually does not occur until the pulp is nearly exposed by the caries.

An acute localized abscess is induced immunologically when bovine serum albumin (BSA) or ovalbumin (OVA) is applied to freshly cut dentinal cavity preparations ($\leq$1.2 mm remaining dentin thickness) in teeth of BSA- or OVA-immunized animals, respectively (121, 122). In contrast, no tissue destruction is induced when OVA is applied to BSA-immunized animals. An Arthus reaction was thought to be responsible for neutrophil accumulation and abscess formation, but decomplementation did not seem to influence the severity of tissue destruction in later studies (118, 123). The authors interpreted these findings as indicating that the bacterial products themselves were chemotactic for neutrophils. Levin et al. (37) further proposed that bacterial products stimulated the adjacent odontoblasts to secrete IL-8, which causes the accumulation of neutrophils. A reduction of the C3 level in inflamed pulps was interpreted as indicating its consumption during inflammation (96). Sylvestre and Ravetch (124) showed that the presence of the intact complement cascade is neither necessary nor sufficient to trigger or propagate the Arthus reaction. Instead, IgG immune complex cross-linking of FcγR, particularly FcγRIII (CD16), expressed on mast cells, monocytes, macrophages, DCs, and neutrophils, is required for the Arthus reaction (125–127). Therefore, IgG immune complex cross-linking CD16 could contribute to neutrophil accumulation and thus abscess formation beneath deep caries (Fig. 2).

Painless Pulpitis

Traditionally, pulpal pain has been associated with pulpal inflammation. Most inflammatory mediators activate or sensitize peripheral neurons. The hyperalgesia and allodynia caused by inflammation of the pulp or periapical tissues is responsible for many clinical symptoms, such as throbbing pain, spontaneous pain, and percussion sensitivity (128). However, many studies have failed to correlate pain symptoms with the histopathologic appearance of the pulp (129–131). Some painless pulpitis teeth have chronically inflamed pulps with acute inflammation histologically, whereas other teeth with severe spontaneous pain contain chronically inflamed pulps without evidence of acute inflammation (131). Evidently, pulpal inflammation is not the only factor that causes pulpal pain. It is believed that host-derived and/or bacteria-derived local factors modulate pain intensity (132, 133), which may explain the discrepancy between clinical symptoms and the histopathologic status of the pulp. These local factors include bacterial metabolites in carious lesions (134); host-derived modifiers, such as endogenous opioids (132); and the adrenergic sympathetic and nitric oxide systems (134). Endogenous opioids, produced by lymphocytes (135), and enkephalins, induced by bradykinin in the inflamed pulp (136, 137), suppress pain sensation.

There are several ways that sympathetic nerves can modify pain sensation by their effects on vasculature and neuropeptides (133). It is well accepted that an increase in pulpal blood flow results in increased pulpal tissue pressure and thus pulpal pain in a low-compliance environment (138). Vasoconstriction and reduced pulpal blood flow depend on activation of sympathetic nerve fibers and the release of endothelin from endothelial cells (139) and somatostatin from sensory nerves (140). Adrenergic sympathetic fibers innervate arterioles to precapillary sphincters and regulate the release of sensory neuropeptides (133). Stress-induced sympathetic vasoconstriction may decrease pulpal pain intensity, which may explain why some patients find that their pulpal pain disappears on the way to the dentist.

Future Directions

There are many gaps in our current understanding of caries antigens and pulpal immune responses. The antigenicity of T and B cells or possible polyclonal activation in inflamed pulps warrant more study.

The quantitative analyses of NK cells, mature and maturing DCs, and inflammatory cytokine and chemokine profiles in relation to microbes may elucidate the immunopathologic mechanism of pulpitis. The knowledge gained in these areas might then be applied to an immunology-based vital pulp therapy in the future. Experimental studies have succeeded in blocking antidromic induced pulp vasodilatation using systemic SP- or CGRP-receptor antagonists (133, 141). It is conceivable that a topical receptor antagonist could be used therapeutically in the management of inflamed hyperemic pulp. The understanding of the biochemical and molecular networks and pathways involved in early reversible and later irreversible pulpitis may then be applied clinically to keep the dental pulp vital and healthy.

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