Overlapping Protective and Destructive Regulatory Pathways in Apical Periodontitis

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Abstract

Introduction: Protective and destructive immunoreactions take place simultaneously in apical periodontitis. However, the same reactions defending the periapical area from infection-derived damage may also result in host tissue injury. **Methods:** The inflammatory reaction of the periapical tissues is self-limited. Regeneration of the injured tooth-supporting structures may follow elimination of the causative microbial irritation. **Results:** Recent experimental and clinical observations have identified important interplay between positive and negative regulatory pathways. A network of stimulatory and inhibitory feedback loops may influence the intensity of the defense and inflammatory responses and the balance between bone resorption and regeneration, resulting in lesion expansion or healing of apical periodontitis. **Conclusions:** We critically discuss research data on regulatory mechanisms that control the activity of host effector cells and signaling molecules during interactions with pathogenic microbes. *(J Endod 2014;40:155–163)*

Key Words

Apical periodontitis, bone resorption, cytokines, immunity, inflammation, stem cells

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Apical periodontitis, an inflammatory response within the periapical tissues, most frequently results from polymicrobial infection of pulpal origin. The disease may take an acute or chronic course. Chronic apical periodontitis may present as dental granuloma or periradicular cyst (1). Based on clinical and experimental reports from the 80s and 90s, we have proposed that protective and destructive immunoreactions take place simultaneously in chronic periapical lesions (2). However, "destructive" and "protective" terms need to be carefully defined because the same reactions responsible for host defense by the elimination of invading microorganisms may also result in tissue injury. Moreover, tissue injury can be prevented by the inhibition of antimicrobial immunoinflammatory reactions, albeit at the price of overwhelming infection (3, 4). Here, we review the results of recent animal and human studies and laboratory experiments that contribute to a better understanding of the periapical inflammatory processes. Furthermore, we analyze regulatory mechanisms that control the activity of effector cells and molecules. These mechanisms can influence the fine-tuned balance between protection from apical infection, lesion progression, and repair. Table 1 summarizes the major subclasses of immune cells playing an important role in apical periodontitis, their major actions, and examples of major released mediators.

Regulation of the Initial Inflammatory Cell Assembly

Proliferative activity is restricted to epithelial cells (ECs) in periapical lesions (5, 6). A robust influx of leukocytes is initiated by the interaction between microorganisms of the infected root canal and the periodontal ligament (PDL) (7–10). PDL cells constitutively express low levels of adhesion molecules and chemokines. They provide a weak stimulus for recruiting and activating leukocytes, thereby maintaining the homeostasis of healthy periapical tissues (11–14). Pathogenic bacteria and bacterial molecules released from the infected root canal stimulate PDL cells to up-regulate a broad set of inflammatory mediators. These include chemoattractant molecules, which stimulate the influx of inflammatory cells to the lesion site (7, 9, 15, 16). Human PDL fibroblasts (PDLFs) express toll-like receptor (TLR)-related molecules (ie, TLR2, TLR4, MD-2, and MyD88) and CD14. Using the CD14/TLR pathway, PDLFs challenged with lipopolysaccharide (LPS) and *Staphylococcus epidermidis* peptidoglycan initiate an interleukin (IL)-8/CXCL8 response, the key signal for polymorphonuclear (PMN) leukocyte chemoattraction (17) (Fig. 1). As shown in cultured cells, TLR receptors may stimulate inflammation independently of the CD14 molecule. The synthetic peptidoglycan analog muramyl dipeptide induces a positive regulatory loop via nucleotide-binding oligomerization domain-like receptors in apical periodontitis (17–19). *Porphyromonas gingivalis* and LPS extracted from *P. gingivalis* and from Escherichia *coli* increase gene expression of both pro- and anti-inflammatory cytokines in human PDLF cell cultures. The former ones are represented by tumor necrosis factor alpha (TNF-α), IL-6, monocyte chemoattractant protein (MCP)-1/CCL2, the chemoattractant "regulated upon activation, normal T-cell expressed and secreted" (RANTES)/CCL5, and stromal-derived factor 1 and the latter ones by transforming growth factor-beta (TGF-β). The pattern and intensity of cytokine gene expression by PDLFs shows marked variation both between different individuals and depending on the source of the stimulatory agent (20–22). In lesion-derived human PDLF extracts, 54% of the genes investigated with an inflammatory gene array were significantly up-regulated compared with healthy PDLF extracts (23).

Herpesviruses including Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6, but not human herpesvirus 8, contribute to the etiopathogenesis of human apical

TABLE 1. Major Subclasses of Immune Cells and Their Major Actions

Subclasses of immune cells	Major actions	Major released mediators
Antigen presenting cells (APC)	Uptake and processing of antigens and displaying them to T cells	Depending on the subtype of APC (see below)
B cells/lymphocytes	Formation of antigen-specific antibodies; performing as APCs	Fully activated B cells (plasma B cells/ plasma cells) secrete antibodies
DCs	Function as professional APCs, innate recognition of microbes by TLR, regulate other immune cells including T and B cells	mDCs produce TNF-α and cytokines of the IL-1 and IL-12 families; pDCs produce type I interferons
LCs	mDCs of the mucosa and skin	Similar to other mDCs
Macrophages (together with monocytes, macrophages are called MNPs)	Develop from monocytes; function as professional phagocytes, and APCs; release toxic compounds that destroy microbes and innocent bystander host cells; regulate immune cells	Cytokines: IFN-β, IFN-γ, IL-1α, IL-1β, IL-6, IL-10, IL-15, IL-18, migration inhibitory factor, TGF-β TNF-α; chemokines: CCL-2CCL-3,CCL-4, CCL-5, CCL-22, IL-8, macrophage inflammatory proteins; reactive oxygen and nitrogen intermediates; eicosanoids; proteases
Mast cells	Anaphylactic type of allergic reactions; nonspecific antimicrobial defense; wound healing; immunoregulation	Histamine, serotonine; eicosanoids; cytokines: IL-1β, IL-3, IL-4, IL-5, IL-13, GM-CSF, SCF; chemokines: CCL-2, CCL-5, eotaxins
MOs	Phagocytosis, antigen presentation, give rise to mDCs and macrophages	Similar to mDCs and macrophages
NK cells	Cytotoxic lymphocytes of innate immunity, contribute to self- tolerance and immune memory	Cytotoxins: perforin, cytotoxins: perforin, chemokines CCL3, CCL4, and CCL5; cytokines IFN-γ, GM-CSF, and TNF-α
NKT cells	CD1d-restricted T cells; recognize lipids and glycolipids; rapid release of soluble regulatory mediators	Cytotoxins as NK cells; IL-2, IL-4, IL-13, IL-17, IL-21, IFN-γ, GM-CSF, TNF-α
PMNs	Of the 3 types of PMNs (basophilic, eosinophilic, and neutrophilic), neutrophils play a major role in apical periodontitis: provide first line of defense against pathogens by phagocytosis; attract and stimulate further PMNs, MOs, and macrophages; destroy periapical tissue components	Reactive oxygen and nitrogen intermediates; proteins with antimicrobial activities: acid hidrolases, defensins, lactoferrin, lysozyme; proteases destroying microbes and host tissues; eicosanoids; cytokines and chemokines: IL-1β, TNF-α, IL-8, and CXCL-10
T cells/lymphocytes	Participate in cell-mediated immune reactions as effectors and regulatory cells	Depending on the subtype of T cells
Cytotoxic T cells (CD8-positive T cells)	MHC class I–restricted cells, destruction of infected cells	Cytotoxins: NK cells; cytokines: IFN-γ, IL-2, GM-CSF; TNF-α; chemokines: CCL-3, CCL-4, CCL-5
Memory T cells (CD45RO-positive cells)	Rapid expansion to effector T cells upon antigen re-exposure	IFN-γ, IL-4, IL-17
Helper T cells (Th) (CD4-positive T cells)	MHC class I–restricted cells;promote maturation of B cells, activation of T cells and macrophages; differentiate into several subtypes, which produce stimulatory and inhibitory cytokines	Activated Th cells: IL-2; subset of activated Th cells: IL-17; Th1 cytokines: IFN-γ, TGF-β; Th2 cytokines: IL-4, IL-5, IL-6, IL-10, IL-13; Th17 cytokines: IL-1, IL-6, IL-17, TNF-α
Regulatory T cells (Treg) ($CD4^+/CD25^{hi}$/ $Foxp3^+$ cells)	Suppress activation of the immune system by secreting regulatory cytokines and by cell-to-cell contacts	TGF-β and IL-10

APCs, antigen-presenting cells; DCs, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-β, interferon beta; IFN-γ, interferon gamma; IL, interleukin; LCs, Langehans cells; MDCs, myeloid dendritic cells; MNPs, mononuclear phagocytes; MOs, monocytes; NK, natural killer; NKT, natural killer T; pDCs, plasmacytoid dendritic cells; PMN, polymorphonuclear leukocyte; TLR, Toll-like receptor; TNF-α, tumor necrosis factor alpha.

periodontitis by stimulating an increased production of early inflammatory cytokines and chemokines (24–27). Synergistic interactions between herpesviruses and endodontopathogenic bacteria may exacerbate the severity of the inflammatory response within the periradicular area (28–30). An aggressive type of pathologic picture and a symptomatic manifestation often characterize herpesvirus-productive periapical infection.

In vivo and *in vitro* studies showed that the production of chemoattractant molecules can be induced by irritation from dental materials, implants, and orthodontic tooth movement (31–33). Dentin proteins can augment the migration of neutrophil leukocytes by the induction of KC/CXCL1 and MIP-2/CXCL2 in mice (34). Sensory neuropeptide substance P, which is released during painful stimuli, has been shown to induce the expression of MIP-3α/CCL20 in immortalized PDLF cells. Concomitantly, substance SP stimulated the expression of the stress enzyme heme oxygenase 1 as a down-regulatory signal (35).

The first wave of chemokines released from pulpal and PDL cells reach the blood vessels, increasing their permeability and selectively attracting white blood cells to the site of inflammation (8, 16, 36). Chemokines bind to the molecules of the extracellular matrix within