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Review Article

Molecular pathogenesis of bacteria-induced periodontitis

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Abstract: Periodontitis, one of the most common inflammatory diseases, is induced by bacteria such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola. Of these, P. gingivalis is a keystone pathogen of periodontitis and has a characteristic ability to invade various host cells, including nonphagocytic epithelial cells and fibroblasts. This invasion facilitates the adaptation and survival of P. gingivalis in the gingival environment. The pathogenesis of periodontitis arises essentially from a complex interplay between bacterial and host factors, leading to a range of presentations that depend on the balance of this interaction. However, the exact roles or the pathogenesis of specific microorganisms are not currently understood in terms of periodontitis. Therefore, the aim of this review was to summarize the findings from current literature regarding the role of microorganisms like P. gingivalis in periodontitis. Our hope is that this will direct future research toward a better understanding of the molecular pathogenesis of periodontitis and to further define the concepts of endodontic treatment.

Keywords: Periodontitis, pathogenesis, porphyromonas gingivalis

Introduction

Periodontitis is one of the most common inflammatory diseases induced by bacteria such as *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola* [1, 2]. It manifests as a breakdown of tooth-supporting tissues with ulceration of the periodontal pockets that potentially allow bacteria to gain access to the blood stream [3-7]. Periapical lesions are classified histologically as acute apical periodontitis, chronic apical periodontitis, and periapical cysts [8]. Apical periodontitis is characterized by inflammation and destruction of periodontal tissues caused by the etiological agents of periodontitis [9-11] and results from a complex interplay of microbial factors and host defense in response to microbial invasion [12]. Persisting microorganisms located in the periapical tissues continually induce periapical inflammation [13, 14]. Infection of the root canal system has been established as the primary cause of apical periodontitis [15-18].

There are no completely effective therapeutic or preventive methods currently known, despite many studies on periodontitis. Therefore, the aim of this review is to summarize findings in the current literature regarding the role of microorganisms in apical periodontitis and to direct future research toward the establishment of a better understanding of the molecular pathogenesis of periodontitis, further defining the concepts of endodontic treatment [12].

Microbiologic role of periodontitis

A review of molecular studies revealed a range of species such as *Actinomyces, Propionibacterium, Peptostreptococcus, Eubacterium, Fusobacterium, Prevotella, Campylobacter, Treponema*, and *Porphyromonas (including P. endodontalis)* [19]. This review provides a general overview of the molecular pathogenesis of bacteria-induced periodontitis, especially that of *P. gingivalis*, the most important pathogen of periodontitis.

The oral cavity houses an estimated 10^{10} bacteria, comprising more than 500 species [20, 21]. However, the diversity of endodontic microbiota and lack of understanding of specific bacterial species leads to uncertainties regarding the exact pathogenic roles of these microbial spe-

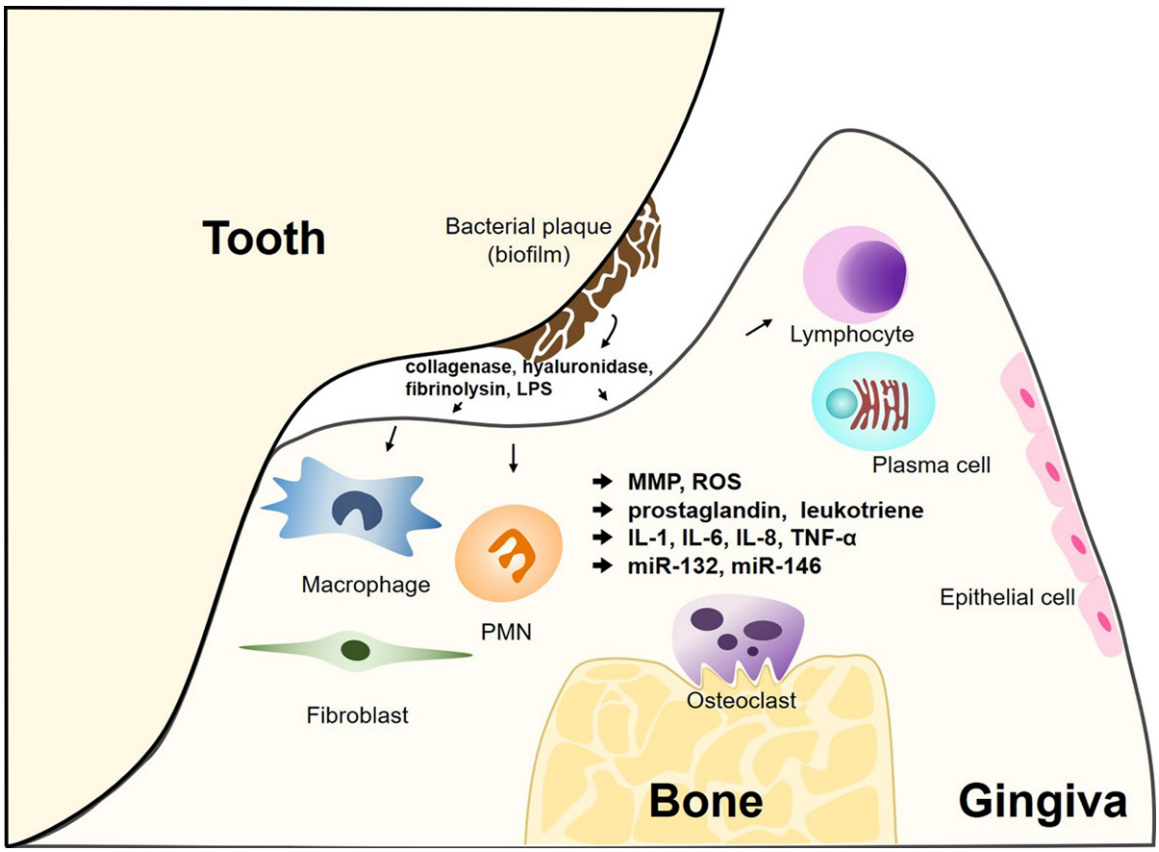

Figure 1. Main cellular and molecular mechanisms involved in the pathogenesis of bacteria-associated periodontitis. LPS, Lipopolysaccharide; MMP, matrix metalloproteinase; ROS, reactive oxygen species; miR, microRNA; PMN, polymorphonuclear leukocyte.

cies [12]. A high prevalence of enterococci and streptococci has been found across studies [12], with *Enterococcus faecalis* reported as the most prevalent species by one molecular study [22]. Other prevalent species include *Lactobacillus spp., Actinomyces spp., Peptostreptococcus spp., Pseudoramibacter alactolyticus, Propionibacterium propionicum, Filifactor alocis, Porphyromonas gingivalis, and Candida albicans* [20, 23, 24].

The pathogenicity of microbial species is influenced by several factors including bacterial interactions, host evasion, release of lipopolysaccharide (LPS), and production of proteolytic enzymes [11, 25]. The main ecological factors affecting survival of bacterial species in the endodontic environment are availability of nutrients, the oxygen level, and pH [21, 25, 26]. Advancement of the infection into deeper parts of the root canal system and the consequent decrease in oxygen [26] and fermentable carbohydrates leads to the flourishing of anaerobic bacteria, which survive on proteins and glycopeptides from the breakdown of pulpal tissues and serum proteins [20, 21]. The breakdown of serum proteins leads to a rise in the pH of the environment [23], thereby favoring proliferation of late-stage bacteria. Production of enzymes like collagenase, hyaluronidase, and fibrinolysins (**Figure 1**) such as those from the *Porphyromonas* and *Prevotella* species [10, 11], aid breakdown of host tissues and the spread of the microorganisms.

Most microorganisms in the root canal system congregate in biofilm forms [27]. A biofilm is a sessile multi-cellular microbial community characterized by cells that are firmly attached to a surface [28]. Microorganisms in the endodontic flora show an enhancement in overall pathogenicity when they occur in biofilm communities rather than as individual bacterial cells [21, 29]. Thus, previous work has shown a more severe inflammatory response when a mixture of species was introduced into the root

canal system [10, 17]. High concentrations of bacterial virulence factors at the biofilm-tissue interface can downregulate host defense barriers, while lower bacterial concentrations deeper in periodontal tissues can have a stimulatory effect on inflammatory responses [30]. Several advantages are afforded to bacteria living in a biofilm configuration [27, 28, 31] such as the creation of niches for survival of different species, enhanced metabolic efficiency, protection from host defense and anti-microbial agents, and retention of water for hydration of the microbial cells.

Porphyromonas gingivalis is a gram-negative, anaerobic, and rod-shaped microbe with a variety of virulence factors including LPS, capsular polysaccharide, fimbriae, and gingipains [32]. *P. gingivalis* can disengage bacterial clearance mechanisms by promoting cross-talk between toll-like receptor (TLR)-2 and C5a receptors (C5aR) in neutrophils [33]. The pathogenicity of *P. gingivalis* has been widely studied, including its ability to colonize surfaces of oral tissues, interact with other oral bacteria, induce a destructive immune response, and invade host cells [34]. Fimbriae were found to increase the tissue invasiveness and pro-inflammatory ability of *P. gingivalis* [30]. Cell invasion by *P. gingivalis* occurs in oral epithelial cells, gingival fibroblasts, aortic and heart endothelial cells, and vascular smooth muscle cells [34-38]. *P. gingivalis* invasion is believed to protect the bacteria against environmental challenges including innate immune surveillance systems and antibiotic treatment [39]. This likely plays a pivotal role in chronic bacterial infection [40]. Previous work has shown that binding of *P. gingivalis* to red blood cells (RBCs) restricts phagocytosis of the bacterium by monocytes and neutrophils. This has led to the hypothesis that RBCs may also affect *P. gingivalis*-stimulated release of pro-inflammatory cytokines and production of intracellular reactive oxygen species (ROS) by neutrophils [41].

P. gingivalis is regarded as a significant contributor to the pathogenesis of periodontitis and certain other systemic diseases, including atherosclerosis. *P. gingivalis* can translocate from periodontal pockets into the circulation, where it adheres to RBCs. This may protect the bacterium, which is more prevalent in patients with rheumatoid arthritis (RA) than in those without [42]. Several studies have clearly demonstrated an epidemiologic association between periodontitis and RA [43]. In addition, RA improves after periodontal treatment, as does periodontitis after treatment of RA [44, 45]. Furthermore, DNA from a variety of oral bacteria, including *P. gingivalis*, has been detected in synovial fluid of patients with active RA [46]. Some evidence supports the invasion of cardiovascular cells and tissues by *P. gingivalis*, causing inflammation [47]. *P. gingivalis* may be an important pathogen in Alzheimer's disease (AD) by contributing to brain inflammation [48]. *P. gingivalis* was also demonstrated in the livers of patients with non-alcoholic steatohepatitis (NASH). Dental infection with *P. gingivalis* is also thought to promote progression of NASH [30]. *P. gingivalis* infection also shows a close association with squamous cell carcinoma [49]. Therefore, additional studies are required to verify the pathogenic relationship between periodontitis and other systemic inflammatory diseases.

Related cells and chemical mediators

Acute apical periodontitis is characterized by the presence, mainly, of neutrophils and macrophages [10, 11, 50]. Prolonged irritation favors the predominance of macrophages, lymphocytes, and plasma cells as well as production of the connective tissue and epithelial cells (**Figure 1**) [10, 11], characteristic of chronic apical periodontitis. Neutrophil-mediated inflammation and tissue injury manifests in the periodontium as degradation of collagen by matrix metalloproteinases of neutrophil origin, resulting in an abundance of collagen peptides [3, 51, 52]. Neutrophils help eliminate bacterial cells and products via the release of oxygen free radicals [11, 53] which damage both bacterial and host tissues [10].

Dendritic cells (DCs) are important sentinels of the immune system that sample the microenvironment for potential microbial antigens and initiate an immune response when necessary. They colocalize with *P. gingivalis* proteins in the oral mucosa of patients with periodontitis [54]. These DCs could be involved in the dissemination of bacteria to distal sites such as atherosclerotic plaques or the synovium, where many researchers have found DNA from *P. gingivalis* when studying patients with RA [46, 55, 56].

Osteoclasts are also important since bony destruction is a characteristic of apical peri-

odontitis. The presence of inflammatory upregulators triggers the osteoblasts in periapical tissues to stimulate the proliferation of osteoclasts and upregulate their activity to resorb surrounding bone [10, 53]. Cellular injury causes the metabolism of arachidonic acid found on the host cell membranes into compounds such as prostaglandins and leukotrienes (**Figure 1**) [10, 11, 53]. Prostaglandins upregulate the activity of osteoclasts to resorb bone, while leukotrienes enhance the chemotaxis of neutrophils and their adhesion to the endothelium. Specific antibodies act by binding with antigens to form antigen-antibody complexes which then act as attractants for other immune cells [10, 11].

Differential regulation of inflammatory cytokines in response to infection is important for the recruitment of immune cells in periodontitis and is required for orchestration or activation of innate and adaptive immune responses [57]. Pro-inflammatory cytokines involved in periodontitis include interleukin (IL)-1, IL-6, and IL-8 as well as tumor necrosis factor (TNF)-α (**Figure 1**) [10, 11, 50, 58]. Macrophages destroy microorganisms via phagocytosis [11] and are also responsible for the release of mediators such as IL-1, interferons, TNF-α, and other growth factors [10, 11, 53]. T-lymphocytes are capable of producing a variety of cytokines that reduce expression of pro-inflammatory cytokines and induce connective tissue growth factors [12]. Neutrophils also secrete cytokines such as TNF-α, IL-1β, and IL-6 when stimulated with LPS from *P. gingivalis* [59].

P. gingivalis expresses various virulence factors including LPS, fimbriae, lipoproteins, and gingipains which modulate the expression of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α in human and murine monocytes and macrophages [60]. Fimbriae are potent stimulators of inflammation [61] and *P. gingivalis* fimbriae has significantly induced TNF-α production via TLR-2 in macrophages [62, 63]. Previous studies have reported that subgingival biofilms downregulate nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) and IL-1β expression in human gingival fibroblasts. LPS from *P. gingivalis* is directly toxic to host tissues and signals endothelial cells to express adhesion molecules and to activate macrophages to produce TNF-α and interleukins [10, 11, 41, 53, 64, 65]. Other researchers have reported that neutrophils readily release pro-inflammatory cytokines and chemokines and produce intracellular ROS upon stimulation with *P. gingivalis*.

Among the various cytokines, TNF-α affects many functions ranging from inflammation to tumor promotion and anti-microbial immunity [66]. Expression of TNF-α was higher in periodontitis patients than in healthy controls and periodontal bacteria stimulated expression of TNF-α [67]. In the periodontium, increased levels of IL-6 and TNF-α due to *P. gingivalis* infection may upregulate osteoclastic activity via the RANK/RANKL/OPG pathways [67]. These cytokines may also spill over from the periodontium into the circulation [68] to promote synthesis of acute-phase proteins such as C-reactive protein, serum amyloid P protein, and pentraxins [41]. TNF-α has the additional ability to cause direct cytotoxic damage to both bacterial and host cells.

IL-8 acts as a chemotactic agent that encourages the influx of neutrophils in acute stages of apical periodontitis [10, 53, 69]. Similar to TNF-α, IL-1β is a pro-inflammatory cytokine critical to the inflammatory response against pathogens with well-known involvement in periodontal disease [57]. Previous work has shown that levels of IL-1β and inflammasome components are increased in patients with periodontitis and that *P. gingivalis* induces the release of IL-1β via activation of the TLR-2/4-NLRP3/absent in Melanoma 2 (AIM2) inflammasome-caspase-1 pathway. In THP-1 (leukemic monocytic) cells, *P. gingivalis* induced both IL-1β secretion and inflammatory cell death through activation of caspase-1 [70].

TLRs are of crucial importance in the detection of pathogens, as they recognize distinct microbial pathogen-associated molecular patterns. This allows them to participate in the first line of defense against invading pathogens, thereby playing a significant role in inflammation and immune cell regulation [30]. Crosstalk between C5aR-and TLR-2 also inhibits phagocytosis of *P. gingivalis* and bystander bacteria and it stimulates a robust inflammatory response [33]. More scientific research is expected in the future on the pathogenesis of related cytokines and TLRs for periodontitis.

microRNA in periodontitis

microRNAs (miRNAs) are small single-stranded noncoding RNAs, usually 18-25 nt in length, that post-transcriptionally regulate gene expression by binding to the 3' untranslated region (UTR) of target mRNA to promote mRNA degradation and translational repression [71]. More than 1,000 human miRNAs have been identified in mammals, to date [72], and they are associated with diverse biological processes including cell differentiation/proliferation, metabolism, tumorigenesis, and immunity [73-75]. Several lines of evidence suggest that miRNAs play a critical role in a number of chronic inflammatory diseases [76, 77]. In the innate immune response, specific miRNAs can be up- or down-regulated by inflammatory stimuli. miRNAs with significant linkage to inflammatory responses were miR-9, miR-21, miR-132, miR-146, and miR-155 [78, 79].

Several miRNAs related to periodontal inflammation have been reported. For example, Lee et al. [80] reported differential miRNA expression in healthy and periodontitis tissues. miR-132 and miR-146 are inflammatory miRNAs related to periodontal bacterial infection (**Figure 1**). miR-146 expression increased in periodontal-disease gingival tissue [80, 81], THP-1 cells/THP-1-derived macrophages [82], and human gingival fibroblasts [83].

At present, the involvement of gingipain and fimbriae in miR-132 expression has not been investigated. Nevertheless, gingipain and fimbriae could possibly contribute to production of TNF-α via miR-132 induction. Chung's study demonstrated that live *P. gingivalis* induced miR-132 and its regulation was dependent on TLRs [57]. TLRs recognize pathogens and activate signaling pathways, including immune and inflammatory responses, to regulate cytokines, chemokines, and adhesion molecules and eliminate pathogens. Several reports have revealed that TLR signaling modulates various miRNAs [84, 85]. However, only a few studies have investigated TLR-mediated miR-146 expression in periodontal disease [83, 86]. Inhibition of TLR signaling or NF-κB abolished *P. gingivalis*-induced miR-132 expression [57], suggesting that miR-132 may be directly regulated by the TLR-2/4-NF-κB signaling pathway. However, further studies are needed to establish the exact mechanism.

Nuclear factor of activated T cells 5 (NFAT5) is a transcription factor that functions in the immune response by regulating TNF-α expression through direct binding to the TNF-α promoter and enhancement of TNF-α transcription [57, 87]. Previous work investigated the ability of *P. gingivalis* to affect the expression of inflammatory miRNAs involved in networks that control innate immunity and extend beyond TLR regulation in THP-1 derived macrophages. This work confirmed that *P. gingivalis* induced miR-132 via TLR-2/4 and NF-κB signaling and inhibition of miR-132 expression strongly suppressed the production of TNF-α. The expression of two inflammatory miRNAs, miR-132 and miR-146, and the role of miR-132 in live *P. gingivalis*-challenged THP-1-derived macrophages were also examined. *P. gingivalis* induced miR-132 expression via TLR-2/4 and NF-κB signaling and the enhancement of miR-132 expression was closely associated with increased production of TNF-α [57]. More studies are needed in the future to determine the pathogenic role of miRNAs in periodontitis.

Inflammasome and periodontitis

The inflammasome, introduced by Tschopp et al. [88], is a relatively new concept in innate immunity. Many of the interactions occurring between inflammasomes and the innate immune system are still unknown. Nevertheless, current evidence indicates that the inflammasome and its constituents are likely to have crucial functions in the initiation of periodontal disease and in several of the chronic systemic diseases associated with periodontitis [30]. Inflammasomes, which are multiprotein complexes localized within the cytoplasm of the cell, are engaged in the maturation of pro-inflammatory cytokines such as IL-1β and IL-18 [89]. After infection or cellular stress, inflammasomes are assembled, activated, and deployed in host defense, indicating an involvement in the pathophysiology of diseases [90]. Inflammasome activation is important to host defense but excessive inflammasome activation can be detrimental to health.

Periodontal bacteria has been postulated to suppress inflammasome activation as a mechanism to explain their low immune-stimulatory activity [91]. This specific inhibition appears to affect IL-1β and IL-18 processing and cell death in the macrophages of both humans and mice.

P. gingivalis has several ways of suppressing innate immunity and inflammasome activity. *P. gingivalis* infection also reduces endocytosis by preferentially suppressing endocytic pathways toward inflammasome activation [30]. Much higher levels of inflammasome components were found in gingival tissues from patients with chronic periodontitis than from healthy controls [70]. A reasonable assumption is, therefore, that the inflammasome is an operational part of innate immunity against periodontitis. Efforts should now be focused on determining how inflammasomes might affect the ecology of the dental plaque microbiota. This will require more study on the role of inflammasomes in periodontitis.

Conclusion

Invasion of pathogenic bacteria into host cells is an important feature of opportunistic infections, as bacteria are able to establish pathogenic reservoirs and evade host defense mechanisms [92, 93]. *P. gingivalis*, a non-motile organism, utilizes invasion as a major strategy to break through the oral epithelial barrier and spread within periodontal tissues. The invasion process of *P. gingivalis* begins with an interaction between the bacteria and oral epithelial cells, followed by cytoskeleton-associated internalization [39]. There are no effective inhibitory agents that directly target *P. gingivalis* invasion yet reported. Disinfection of the residing endodontic microflora is important but prevention of ingress of bacteria by closely adhering to asepsis is equally vital, as is placement of well-adapted temporary or permanent restorations [14, 20]. Despite the relatively high predictability of endodontic treatment outcomes, complete bacterial eradication remains impossible and not all treatments can resolve apical periodontitis.

For these reasons, the search for ideal materials, instruments, and techniques should continue for further enhancement of the success of endodontic treatment. Many experimental trials are now aimed at the eradication of bacteria responsible for periodontitis, using natural products such as bee venom [94] as well as antibiotics.

Use of chemical agents as an adjunct to mechanical instrumentation has the additional aim of achieving further disinfection without compromising the integrity of the tooth [20, 95]. Sodium hypochlorite (NaOCl), which is widely used as an effective anti-microbial agent, is also able to dissolve organic substances [95]. Ethylene diamine tetra-acetic acid (EDTA), commonly used in conjunction with NaOCl, assists in the removal of the smear layer on dentine and increases penetration of anti-microbial agents into dentinal tubules. Medications such as calcium hydroxide and chlorhexidine also enhance disinfection and prevent regrowth of the endodontic microflora [96, 97]. In fact, use of calcium hydroxide for at least 1 week has been reported to render more than 90% of the canals bacteria-free [96].

Various experimental methods involving molecular techniques are presently being tested for the treatment of periodontitis. For example, a recent evaluation of two small molecules, Alop1 and dynasore [34], revealed potent inhibitory activities of both molecules against invasion of *P. gingivalis* and its outer membrane vesicles (OMVs). Differential microtubule rearrangements were induced in oral epithelial cells by Alop1 and dynasore, which could precede microtubule-dependent internalization and intracellular trafficking of *P. gingivalis* [34]. Duncan et al. also suggested that *P. gingivalis* invasion may depend on a clathrin-mediated endocytosis based on the observed interaction between a binding domain of gingipains and the clathrin of epithelial cells [98]. Clathrin-mediated endocytosis was postulated as being responsible for the invasion of oral epithelial cells by *P. gingivalis* and its OMVs. Unlike the case for dynasore, the biological mechanism of Alop1 is not well understood, despite previous reports of its various biological activities [99-101].

In summary, the aim of this review was to summarize findings from current literature regarding the etiologic role of microorganisms in periodontitis. Our hope is that amalgamation of this knowledge will help to direct future research toward obtaining a better understanding of the etiology and pathogenesis of periodontitis, further consolidating the concepts of endodontic treatment.

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Disclosure of conflict of interest

None.

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