Immunological pathways triggered by porphyromonas gingivalis and fusobacterium nucleatum: Therapeutic possibilities?

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Review Article

Immunological Pathways Triggered by *Porphyromonas gingivalis* and *Fusobacterium nucleatum*: Therapeutic Possibilities?

Kívia Queiroz de Andrade, Cássio Luiz Coutinho Almeida-da-Silva, and Robson Coutinho-Silva

1. Introduction

Oral bacteria in dental biofilms contribute to the initiation and progression of periodontal diseases (PD) via exacerbated host inflammatory responses to these bacteria [1, 2]. PD are chronic inflammatory diseases of the periodontium (supporting structures around the teeth: gingiva, periodontal ligaments, and alveolar bone [3]), including gingivitis and periodontitis. Gingivitis is the initial reversible inflammatory lesion in the soft tissues surrounding the teeth, and periodontitis results from a combination of factors that leads to periodontium destruction, often causing irreversible bone resorption and tooth loss [3]. It affects nearly half of the United States population [4], and severe periodontitis is the 6th most prevalent disease worldwide [5]. Periodontitis has a high impact on public health because of its long and expensive treatment. Furthermore, periodontitis is associated with several systemic diseases, including diabetes mellitus, cardiovascular diseases, and atherosclerosis, as we and others reviewed elsewhere [6, 7].

In healthy individuals, there is an established homeostasis between immunity and oral cavity microorganisms that do not cause disease [8]. Oral epithelial and immune cells contribute directly and indirectly to maintain this equilibrium [9]. The loss of homeostasis due to dental plaque formation along with genetic, hormonal, and host behavioral...
factors make the individual susceptible to PD. Furthermore, the absence or decrease of an effective innate immune response by some cells stimulated by *P. gingivalis* LPS can greatly increase the proliferation of various bacterial species with the formation of biofilms at the root of the tooth, leading to exacerbated inflammation in the tissues [10].

*P. gingivalis*, a nonmotile, non-spore-forming Gram-negative bacterium, has the ability to induce dysbiosis in the oral microbiota (an imbalance among microbial species) [3, 11, 12]. *F. nucleatum* is one of the most common species in the human gingival sulcus; its prevalence increases with the severity of PD and the progression of inflammation [13, 14]. *F. nucleatum* serves as a true bridge, connecting initial and later bacterial colonizers, thereby favoring the formation of dental plaques [15]. When *F. nucleatum* is not present, the number of late colonizers is significantly lower [15].

Conventional clinical treatment for periodontitis consists initially of mechanical bacterial removal (scaling and root planning), thereby reducing the contact of bacterial agents with inflammatory and noninflammatory cells in the oral cavity. However, this procedure may not be sufficient to generate clinical improvement. In this context, several signaling pathways are involved in the progression of PD; therefore, therapies that modulate these pathways may help prevent the development of PD and consequently avoid bone loss [16].

The exact molecular host response events against *P. gingivalis* and *F. nucleatum* are not fully understood; nevertheless, understanding of these mechanisms is essential for the identification of therapeutic targets aiming to prevent and treat periodontitis. In this context, there are some immunological pathways that have been demonstrated to be involved in the development of periodontitis and in infections with periodontopathogens. In this respect, we and others previously demonstrated the role of the NLRP3 inflammasome in the development of periodontitis [17, 18]. Furthermore, it is known that purinergic signaling via the P2X7 receptor is one of the important pathways for the activation of the NLRP3 inflammasome and control of intracellular infections, including *P. gingivalis* infections [19, 20]. The activation of this inflammasome leads to caspase-1 maturation, in turn leading to cleavage of the inactive form of interleukin- (IL-) 1β (pro-IL-1β) to its active form (IL-1β) [21]. Cytokine production is central to the host inflammatory response during PD and infection [20]. Other caspases involved in inflammation, although less studied and considered involved in the noncanonical inflammasome, are caspase-4, caspase-5, and caspase-11 that are important factors for counteracting Gram-negative bacterial pathogens via induction of cell death and IL-1 release [22, 23].

This review is aimed at illuminating advances in the study of mechanisms of innate and adaptive immune responses after *P. gingivalis* and *F. nucleatum* interactions with the host. We discuss the role of TLRs, the inflammasome, purinergic signaling, cytokines, and chemokines, as well as the innate and adaptive immune cells involved in host resistance to infections by these bacteria. Our review highlights the importance of understanding signaling pathways induced by *P. gingivalis* and *F. nucleatum* that could potentially serve as effective strategies for treating patients with PD.

2. *Porphyromonas gingivalis* and *Fusobacterium nucleatum*: Periodontopathogenic Pathogens

*P. gingivalis* is a well-adapted colonizing opportunistic pathogen with the ability to invade gingival epithelial cells [24], periodontal ligament fibroblasts [1], osteoblasts [25], and immune cells [26]. It requires anaerobic conditions for growth *in vitro*, as well as hemin and vitamin K in its nutrient medium. It appears as black-pigmented colonies in blood agar medium attributed to agglomeration of heme groups on its cell membranes [27, 28]. *P. gingivalis* obtains energy through the fermentation of amino acids, thereby allowing survival in periodontal pockets, where there are low sugar levels [11]. *P. gingivalis* is considered an “inflammo-philic” bacterium (from the Greek suffix -philic meaning “attracted to” or “loving”) [29]; it is thought that infections with this organism induce the production of proinflammatory cytokines that damage the host tissue, promoting bacterial survival [30, 31]. Therefore, the conditions of the inflamed tissue favors the nutritional needs of the dysbiotic community, caused by the release of products resulting from tissue destruction, including peptides and components containing heme groups [1, 19, 32]. *P. gingivalis* is normally found in 10%–25% of healthy subjects and 79%–90% of subjects with periodontitis [33, 34]. There is a positive correlation between the depth of the periodontal pocket and the presence of *P. gingivalis* [11].

*P. gingivalis* is considered the keystone pathogen of PD because of its ability to modify the normal oral microbiota composition to one with greater pathogenicity that intensively accelerates bone loss [3, 35]. This periodontopathogen is considered a master of immune system subversion that exploits several sabotage tactics allowing it to evade, weaken, or deceive the host’s immune system [36]. *P. gingivalis* possesses several virulence factors, including proteolytic enzymes (e.g., gingipains), capsule, lipopolysaccharide (LPS), fimbriae, nucleoside diphosphate kinase (NDK), ceramide, and outer membrane vesicles (OMVs) [37] (summarized in Table 1).

In a classic study, Socransky et al. analyzed over 13,000 periodontitis subgingival dental plaque samples and grouped the species into bacterial “complexes,” according to the relationship between the different species. The “red complex” is constituted by *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, presenting greater pathogenic potential, and being related to clinical measures of periodontal disease such as pocket depth and bleeding on probing. *F. nucleatum* is part of the “orange complex,” which is a core group that supports colonization of the “red complex” bacteria and is important for the progression of PD [38].

*F. nucleatum* is a human pathogen that is filamentous, Gram-negative, non-spore-forming, nonmotile, and anaerobic [13]. It is a heterogeneous species that belongs to the...
family *Fusobacteriaceae* and includes five proposed subspecies (ss): *ss animalis*, *ss fusiforme*, *ss nucleatum*, *ss polymorphum*, and *ss vincentii* [13, 39–41]. Despite the fact that *F. nucleatum* has been found in various tissues, the most common anatomical site in humans is the oral cavity [13]. As for *P. gingivalis*, this bacterium also presents virulence factors that make it a potential opportunistic pathogen in periodontal infections. *F. nucleatum* possesses several virulence factors, including adhesins (facilitating adhesion and invasion to various cell types, leading to colonization, dissemination, and triggering host immune responses) [13], endotoxins (e.g., LPS) [42, 43], and secretion of serine proteases (responsible for suppressing the nutritional needs of other oral microorganisms) (summarized in Table 2) [44].

There is evidence that synergistic and antagonistic interactions among various microorganisms influence the pathogenesis of PD [45, 46]. *P. gingivalis* suppresses apoptosis in gingival epithelial cells by activating the phosphatidylinositol-3-kinase (PI3K) signaling pathway, thereby favoring its own survival as well as intracellular survival of *F. nucleatum*, as observed in a coinfection subcutaneous chamber model (*F. nucleatum* and *P. gingivalis*) [29]. The interaction between these bacterial species may

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**Table 1: Virulence factors of *Porphyromonas gingivalis***

| Periodontopathogenic pathogen | Virulence factors | Function | Reference |
|-------------------------------|-------------------|----------|-----------|
| **Porphyromonas gingivalis** | Gingipains | (i) Activates host MMPs; degrades cell-cell components, complement system proteins, cytokines, immunoglobulins, integrins, and collagen; alters cell signal transduction and cellular function (ii) Cleaves T-cell receptors, including CD2, CD4, and CD8 and interferes with the cell-mediated immune response. Cleaves CD14, an endotoxin receptor, resulting in hyporesponsiveness to LPS (iii) Stimulates the expression of protease-activated receptors on neutrophils, gingival epithelial cells, gingival fibroblasts, and T cells, releasing proinflammatory cytokines, to increase vascular permeability and to cause bleeding at the periodontal site and influx of polymorphonuclear leukocytes and degrade fibrinogen | [11, 56–61] |
| | Capsule (CPS or K-antigen) | (i) Various serotypes of CPS differentially stimulate the release of chemokines by macrophages as well as cytokines by dendritic cells (ii) Encapsulated strains of *P. gingivalis* can also generate greater resistance to phagocytosis by polymorphonuclear leukocytes and differential capacity to adhere to the gingival epithelium (i) Favor adherence to saliva proteins, to the extracellular matrix, to eukaryotic cells, and to other bacteria, contributing to the biofilm generation | [62–64] |
| | Fimbriae | (i) Type I fimbriae act on the capacity for invasion and colonization. Type II fimbriae shows greater proinflammatory efficiency | [65–67] |
| | Lipopolysaccharide (LPS) | (i) Stimulates proinflammatory responses and bone resorption | [68, 69] |
| | Outer membrane vesicles (OMVs) | (i) The purified OMVs of *P. gingivalis* activates the production of proinflammatory cytokines, signaling of inflammasome, and pyroptosis in macrophages | [70] |
| | Nucleoside diphosphate kinase (NDK) | (i) Responsible for modulating purinergic signaling and inhibiting proapoptotic actions of primary oral epithelial cells | [71] |
| | Phosphoethanolamine dihydroceramide (PEDHC) and phosphoglycerol dihydroceramide (PGDHC) | (i) Promotes IL-1β-mediated release of prostaglandin E2 (PGE2) in primary cultures of gingival fibroblasts; induces apoptosis in chondrocytes and gingival fibroblasts; promotes osteoclastogenesis mediated by receptor activator of nuclear factor kappa-B ligand (RANKL) via interaction with Myh9 (nonmuscle myosin II-A) independently of Toll-like receptor 2/4 (TLR2/4) | [72] |
| | Serine phosphatase | (i) Involved in neutrophil subversion by causing dephosphorylation of the serine S536 of the p65 subunit of NF-κB and prevents translocation of NF-κB to the nucleus, consequently inhibiting IL-8 production | [73–75] |

Arg-X: arginine-specific; CPS: capsule; IgG: immunoglobulin G; LPS: lipopolysaccharide; Lys-X: lysine-specific; MMPs: metalloproteinases; Myh9: nonmuscle myosin II-A; NDK: nucleoside diphosphate kinase; NF-xB: nuclear factor kappa-light-chain-enhancer of activated B cells; OMVs: outer membrane vesicles; PAMPs: molecular pattern associated with the pathogen; PEDHC: phosphoethanolamine dihydroceramide; PGDHC: phosphoglycerol dihydroceramide; PGE2: prostaglandin E2; p65: also known as Rel A, nuclear factor NF-kappa-B p65 subunit; RANKL: receptor activator of nuclear factor kappa-B ligand; TLR2/4: Toll-like receptor 2/4.
Influence the mechanism of invasion and adhesion of these and other periodontal bacteria in human gingival epithelial cells [2]. *Fusobacterium nucleatum* was shown to be capable of increasing the invasion of *Porphyromonas gingivalis* in gingival epithelial cells [2, 47, 48]. In an animal model study, coinfection with *P. gingivalis* and *F. nucleatum* synergistically increased bone loss and exacerbated inflammatory responses when compared to that of monoinfection in rat periodontal tissues [49]. The consequences of these interactions may be caused not only by local inflammation but also by systemic manifestations.

In order to carry out studies with periodontopathogenic bacteria, several bacterial strains are used. The use of bacterial strains from a given type culture is more feasible due to easy access to commercially available sources, as opposed to the more difficult collection of bacteria from clinical subjects. It is important to appreciate that strains of the same bacterial species may have varying characteristics, and therefore, the results generated by one strain are not always transferable to another. It is advisable to use clinical microorganisms as well as those from collections (type cultures) to conduct these studies, in order to compare them with their standards. Notably, it was observed that there were no differences in LPS characteristics and cellular activation when using clinical samples from patients with periodontitis and ATCC strains [50].

*P. gingivalis* strains show genetic variations, and studies have associated these variations with virulence potential, in which certain strains present a greater virulence whereas other strains of the same species display more commensal behavior [51]. Examples of virulent strains of *P. gingivalis* are W83, W50, ATCC 49417, and A7A1, while 381, 33277, and 23A4 are characterized as less virulent strains [52–54]. Regarding *F. nucleatum*, it was shown that all strains significantly increased the phagocytic capacity of neutrophils as well as IL-8 and TNF-α production [55]. Interestingly, phagocytosis of *F. nucleatum ss polymorphum* was significantly greater than that of *F. nucleatum ss vincentii* and *ss nucleatum* [55]. These studies highlight the importance of choosing the correct strain to perform *in vitro* and *in vivo* studies that mimic the bacterial effects on human oral diseases.

### Table 2: Virulence factors of *Fusobacterium nucleatum*.

| Periodontopathogenic pathogen | Virulence factors | Function | Reference |
|------------------------------|-------------------|----------|-----------|
| *Fusobacterium nucleatum*    | Adhesins          | FadA is required for binding and invasion of host cells | [76–78] |
|                              | LPS               | Stimulates inflammation and bone resorption | [79, 80] |
|                              | Serine proteases  | Induces damage to host tissue and IgA degradation while favoring acquisition of nutrients | [44] |
|                              | Production of ammonium and butyrate | Butyrate and ammonium inhibits the proliferation of gingival fibroblasts | [81] |
|                              | Outer membrane proteins (Fap2 and RadD) | RadD and Fap2 function as adhesins, binding to a variety of Gram-positive species and *Porphyromonas gingivalis*, respectively. Both of them induces lymphocyte apoptosis | [82–84] |

FadA: *Fusobacterium* adhesin A; Fap2: fatty-acid-binding protein.

3. Immunological Mechanisms Triggered by *P. gingivalis* and *F. nucleatum*

Here, we reviewed pathways used by the host to control *P. gingivalis* and *F. nucleatum* infection as well as how these bacteria subvert these innate and adaptive immune responses.

3.1. Pattern Recognition Receptors (TLR2 and TLR4 pathways). Bacterial components induce innate immune responses through host recognition to pathogen-associated molecular patterns (PAMPs), which are evolutionarily conserved molecules shared by microorganisms, but which are absent in the host. These PAMPs alert the innate immune system to the presence of pathogens. In the same context, in a situation of tissue homeostasis alteration due to microbial invasion, necrosis, cell injury, or stress, the release of intracellular damage-associated molecular patterns (DAMPs) occurs. DAMPs are considered danger signals that alarm the innate immune system and are therefore alternatively called “alarmins” [85].

PAMPs and DAMPs are identified by a wide variety of pattern recognition receptors (PRRs), present in the plasma membrane, cytoplasm, or vesicles (such as endosomes) in inflammatory cells, as well as in resident cells. The recognition of PAMPs and DAMPs by the host results in the induction of signaling pathways such as activator protein 1 (AP-1) and factor nuclear kappa B (NF-κB), leading to the expression of proinflammatory cytokines [86]. The family of PRRs includes Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (retinoic acid-inducible gene-I-like receptors, or (RLRs)), and nucleotide oligomerization domain- (NOD-) like receptors (NLRs) [86].

TLRs are expressed in oral epithelial tissue, can be stimulated by commensal microorganisms, and also serve to protect the host against microbial infections [9]. The TLR signaling pathway involves the recruitment of the adapter protein containing the TIR (Toll-IL-1 receptor) domain and myeloid differentiation primary response 88 (MyD88) to the cytoplasmic region of TLR, with subsequent activation of NF-κB and induction of proinflammatory cytokines and host defense genes [87].
The interaction of LPS with TLRs is one of the mechanisms of manipulation of the host response used by *P. gingivalis* to facilitate its adaptation and survival [88]. LPS is composed of an antigen O of variable length, a polysaccharide core, and a lipid A moiety. Lipid A is the effector LPS portion that binds to TLR4/MD2/CD14 (TLR4 signaling requires additional costimulation by cluster of differentiation 14 (CD14) and myeloid differentiation protein 2 (MD-2)).

The molecular structure of LPS varies depending on the bacterial species [89]. The level of acylation of PAMPs allows the host to discriminate commensal from pathogenic bacteria [90]. *P. gingivalis* LPS differentiates from LPS of other bacterial species through modifications in the O-antigen structure [91–93], as well as modifications in the acylation patterns and in the receptor-activating capacities of the lipid A component [11]. Lipid A from *P. gingivalis* has a penta-acylated phosphorylated structure that activates TLR4 and a tetra-acylated monophosphorylated structure that antagonizes TLR4, thereby attenuating host immune responses. These differences of lipid A structures depend on the microenvironment and hemin concentrations [94]. At high hemin concentrations, conferring high degrees of inflammation, several tetra- and penta-acylated lipid A structures were observed in *P. gingivalis* as opposed to one major penta-acylated lipid A structure at low hemin levels [94] (see Figure 1). Another type of LPS was also identified in *P. gingivalis*, A-LPS, with an anionic polysaccharide linked to lipid A, responsible for serum resistance and cellular integrity; however, it was a weak inducer of cytokine release by human monocytes [91].

The literature remains conflicted regarding signaling of *P. gingivalis* LPS binding to TLR4 receptors; this conflict concerns the distinct portions of *P. gingivalis* lipid A that exhibit various receptor binding attributes [88].

*F. nucleatum* lipid A is a hexa-acylated fatty acid composed of tetradecanoate (C14) and hexadecanoate (C16) and is structurally similar to *Escherichia coli* lipid A [95]. This structural similarity may explain the fact that *F. nucleatum* has a strong activity via TLR4 [95] (see Figure 2). These structural differences in bacterial LPS composition may explain why *F. nucleatum* LPS stimulates IL-1β secretion more strongly than does *P. gingivalis* LPS [96].

The observations cited above explain why TLR2 predominates over TLR4 in terms of recognition by *P. gingivalis* [36]. Activation of TLR2 by *P. gingivalis* LPS induces two distinct signaling pathways, one of which leads to the synthesis of proinflammatory cytokines, and antimicrobial responses and represents the pathway of CXC chemokine receptor 4 (CXCR4) modulated by *P. gingivalis*. The other cascade involves the proadhesive capacity and pathway cross talk between TLR2 and the complement system [36]. It has been demonstrated in vivo and in vitro in neutrophils that *P. gingivalis* inhibits the TLR2/MyD88 signaling which is considered a host-protective pathway, thus avoiding the death of neutrophils infected with *P. gingivalis*. This inhibition occurs through ubiquitination and degradation of MyD88 via E3 ubiquitin ligase Smurf1 dependent of the
Crosstalk between TLR2 and the complement receptor C5aR (C5aR/TLR2). On the other hand, *P. gingivalis* activates the TLR2/Mal/PI3K signaling pathway that blocks phagocytosis and stimulates inflammation in neutrophils [29, 97]. Because of the effects of *P. gingivalis* gingipains during infection with this pathogen, there are high levels of C5a [97, 98] that could induce alteration of the PI3K pathway leading to inhibition of phagocytosis. The C5aR/TLR2 crosstalk generated by *P. gingivalis* inhibits the activation of RhoA (Ras homolog gene family, member A), cytoskeleton reorganization, and actin polymerization, all of which contribute to phagocytosis. All these effects result in a protective effect, not only for *P. gingivalis* but also for other bacteria of the oral cavity. These modulations by *P. gingivalis* contribute to the persistence of periodontal dysbiosis and the chronicity of inflammation in PD [29].

The crosstalk between the TLR2 and the CXCR4 in lipid rafts of macrophages is another form of subversion of the immune response by *P. gingivalis*, allowing their survival in vivo and in vitro. The fimbrae of *P. gingivalis* bind to CXCR4 and activate the cAMP-dependent protein kinase A pathway, inhibiting NF-κB and nitric oxide (NO) synthesis [67]. For this reason, fimbrae from *P. gingivalis* are considered molecules with antimicrobial properties [67].

*P. gingivalis* fimbrae induce the production of proinflammatory cytokines, including IL-6 and TNF-α, and they mediate the expression of adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1) [103]. The fimbrae exploit TLR2 signaling to interact with complement 3 (CR3), thereby allowing internalization of *P. gingivalis* fimbrae stimulated the expression of IL-8 in gingival epithelial cells via TLR2 [102].

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Both TLR2 and TLR4 are associated with bone loss in an animal model of periodontitis induced by *P. gingivalis* [106–108]. The phosphoglycerol dihydroceramide (PGDHC) from *P. gingivalis* also promotes receptor activator of nuclear factor kappa-B ligand- (RANKL-) mediated osteoclastogenesis, via interaction with Myh9 (nonmuscle myosin II-A) independently of TLR2/4 [72].

During *F. nucleatum* infection in macrophages, both TLR2 and TLR4 recognize this pathogen and are redundant...
in terms of the production of IL-6 and TNF-α [109]. In macrophages, MyD88 is required for cytokine secretion induced by *F. nucleatum* infection [109]. Furthermore, the same study showed that TLR2/TLR4 and MyD88 were required for the optimal activation of NF-κB and mitogen-activated protein kinases (MAPKs, including p38, extracellular signal-regulated kinase (ERK), and Jun N-terminal protein kinase (JNK)) in response to *F. nucleatum* [109].

Interestingly, using HEK293T cells, which lack endogenous TLRs, it was demonstrated that *F. nucleatum* invasion in the host cell was needed to induce IL-8 secretion, as was p38 MAPK signaling, but not NF-κB or NOD-1 [110]. On the other hand, TLR2-silenced Ca9-22 cells infected with *F. nucleatum* resulted in the absence of IL-6 and IL-8 responses, whereas induction of IL-1β remained, suggesting that this bacterium modulates the expression of these cytokines via TLR2 [90].

Antimicrobial peptides, including human beta-defensins (HBD), assist the barrier function of gingival tissue [111]. *F. nucleatum* increased the expression of the HBD-2 and HBD-3, and it is believed that this induction occurs via TLR2, considering that there was suppression of *F. nucleatum*-induced HBD when using TLR2 knockdown cells [112]. Furthermore, FomA, a porin protein from *F. nucleatum*, was shown to induce cytokine secretion through TLR2 signaling [112].

Therefore, it is important to understand the TLR2 and TLR4 downstream signaling pathways induced by oral bacteria in cells of the oral cavity and in animal models of PD, because they can potentially be targets of pharmacological treatments. Therapeutic drugs could be designed to target the inhibition of the signaling pathways used by *P. gingivalis* and *F. nucleatum* to survive, as suggested in Figure 3.

### 3.2. Inflammasomes
NLRs (nucleotide-binding oligomerization domain-like receptors) are part of the family of PRRs and can be a part of "inflammasomes" [86, 113, 114]. Inflammasomes are multiprotein complexes assembled in the host cell in response to infection and/or cellular stress that can ultimately lead to a type of cell death called "pyroptosis" and/or proinflammatory cytokine maturation and secretion [19, 115]. Canonical inflammasomes activate procaspase-1 into the mature form caspase-1 while noncanonical inflammasomes involve caspase-11 (in mice) or caspase-4/5 (in humans) [21]. Canonical inflammasomes can be activated by various ligands, and they are named according to the receptor involved in the stress recognition: NLPR3, NLRP1, NLRC4, and AIM2. By contrast, noncanonical inflammasomes are only activated by cytosolic LPS [21]. In the case of the NLRP3 and AIM2 inflammasomes, a PYD-CARD adaptor protein ASC (apoptosis-associated protein with caspase recruitment domain) is required for the assembly and stabilization of these inflammasomes, as reviewed elsewhere [21, 115].

NLRP3 is currently the best characterized inflammasome and is associated with various chronic inflammatory diseases, including type II diabetes, obesity, and intestinal diseases...
It is well accepted that two signals are needed to activate the NLRP3 inflammasome: (1) the recognition of a PAMP via PRRs such as TLRs induces NF-κB activation and subsequent transcription of genes encoding NLRP3 and inactive forms of the proinflammatory cytokines, including pro-IL-1β and pro-IL-18 [21, 115]; and (2) crystals/particles (such as uric acid and silica), β-amyloid, bacteria, viruses, fungi, protozoa, pore-forming toxins, and DAMPs such as adenosine triphosphate (ATP) [23, 115, 117] are recognized by the host cell and activate the inflammasome [115]. Activation of the NLRP3 inflammasome results in the activation of caspase-1 that is responsible for cleaving pro-IL-1β and pro-IL-18 to their biologically active forms, IL-1β and IL-18 and/or for inducing pyroptosis (to be discussed later in this review [115]). Recent studies showed that activated caspase-1 cleaves gasdermin D, inducing pore formation in the plasma membrane and leading to IL-1β/IL-18 release and/or pyroptosis [118, 119].

Studies have examined inflammasome components and their byproducts during periodontitis and infection of cells with periodontopathogens. Human monocytic cells (Mono-Mac-6 cells) infected with *P. gingivalis* showed increased levels of NLRP3 and IL-1β/IL-18 but decreased levels of ASC [120]. In THP-1 cells, *P. gingivalis* activated the NLRP3 inflammasome through the TLR2 and TLR4 pathways [121]. This bacterium may downregulate ASC as a mechanism of survival, because ASC is involved in cell death and consequent clearance of intracellular bacteria [122].

We and others demonstrated that *P. gingivalis* infection induced intracellular pro-IL-1β production but not IL-1β secretion in human gingival epithelial cells [123] and murine macrophages [20, 96, 124]. In fact, in human gingival epithelial cells and murine macrophages, we and others showed that purinergic P2X7 receptor activation by extracellular ATP is necessary for IL-1β release after *P. gingivalis* infection [20, 96, 123, 124]. Furthermore, we found that the intracellular processing of pro-IL-1β was NLRP3-dependent in murine macrophages [124]. Alternatively, some studies have shown that caspase-8 can also be involved in NLRP3 inflammasome activation and cleaved pro-IL-1β in response to TLR4 activation [125, 126].

Furthermore, we showed *P. gingivalis* infection in vivo induced IL-1β production in order to restrain bacterial infection in a manner that was dependent on caspase-1/11, P2X7 receptor, and autocrine IL-1 receptor signaling [20]. Interestingly, it was shown that *P. gingivalis* mediated inflammasome repression when macrophages were infected with *P. gingivalis* and *F. nucleatum* through a mechanism involving reduced endocytosis [96]. These studies highlight the importance of studying infection models in order to understand the pathogenesis and immune responses during periodontitis.

In fact, the inflammasome is very important in the pathophysiology of periodontitis. It is already known that the NLRP3 inflammasome [18] and secretion of IL-1β [68] are crucial for the development of PD, because in the absence of NLRP3 or IL-1β, there is no periodontitis induced by *P. gingivalis* in mouse models. IL-1β also plays a prominent role in promoting tissue pathology and inflammatory responses in periodontal lesions and stimulating the loss of connective tissue and bone [68]. Furthermore, various studies demonstrated increases in the expression of NLRP3, AIM2, IL-1β, and IL-18, but not ASC or NLRP2, in gingival tissue from periodontitis patients when compared to healthy individuals [120, 121, 127].

Unlike *P. gingivalis* infections, in murine macrophages and gingival epithelial cells, *F. nucleatum* infections activate the NLRP3 inflammasome leading to pyroptosis and IL-1β/IL-18 secretion even in the absence of extracellular ATP, suggesting that *F. nucleatum* provides both PAMPS and a danger signal [96, 128]. In gingival epithelial cells, *F. nucleatum* leads to NF-κB activation, culminating in enhanced expression of the proinflammatory cytokine IL-8 [129] and IL-1β secretion [128]. In these cells, *F. nucleatum* infection was sufficient to induce caspase-1 activation in a NLRP3-dependent manner and the secretion of the danger signals ASC and high-mobility group box 1 protein (HMGB1) [128]. Interestingly, we showed that NLRX1 has a dual effect on *F. nucleatum*-infected gingival epithelial cells by upregulating NLRP3-dependent caspase-1 activation but downregulating NF-κB activation and IL-8 production [129]. These results show that inflammasome activation after *F. nucleatum* infection is a robust but complex regulated process. In oral infection in mice, we showed that *F. nucleatum* induced the expression and secretion of several proinflammatory cytokines, including the inflammasome-dependent IL-1β [130]. In agreement with *in vitro* and mouse model studies, the expression of the components of the NLRP3 inflammasome was also shown to be increased in periapical lesions in human subjects with periapical periodontitis [131]. Despite the fact that *F. nucleatum* infection in vivo was evaluated in some studies, the effects of this bacterial infection *in vivo* with respect to inflammasome activation and modulation still needs to be investigated, because it may be an important therapeutic target.

Regarding inflammasomes other than the NLRP3, it was demonstrated that NLRP6 was more highly expressed in gingival tissues of patients with periodontitis than in healthy controls [132]. The same study showed that pyroptosis of gingival fibroblasts induced by *P. gingivalis* infection was dependent on the NLRP6 inflammasome and caspase-1 activation. Moreover, *P. gingivalis*-infected gingival fibroblasts showed increased levels of IL-1β and IL-18 secretion in an NLRP6 and caspase-1-dependent manner [132]. Furthermore, the expression of the AIM2 inflammasome (that recognizes double-stranded DNA) was also more highly expressed in the gingival tissue of periodontitis patients than in controls, as well as in human macrophages infected with *P. gingivalis* [121]. In human macrophages, *P. gingivalis* induced pyroptosis and IL-1β secretion in a caspase-1/AIM2 inflammasome-dependent manner [121]. In agreement with these studies, the AIM2 inflammasome was also found in high levels in periapical lesions and was primarily distributed in inflammatory cells [131]. These data suggest that the AIM2 and NLRP6 inflammasomes are involved in the development of PD, highlighting the need to study these immunological pathways during these diseases and periodontopathogen infections, in order to
develop therapeutic targets. Together, these data using cell lines, mouse models, and clinical samples demonstrate that inflammasomes play important roles in the pathophysiology of periodontitis as well as during control of *P. gingivalis* and *F. nucleatum* infections. This supports the concept that inflammasome components and/or its byproducts could be potential therapeutic targets for modulating the development of periodontitis and/or controlling infection by periodontopathogens.

3.3. Purinergic Signaling. ATP acts as a signaling molecule in various physiological processes, including synaptic transmission, bone formation and resorption, blood pressure regulation, and inflammation [133–135]. Extracellular nucleotides bind to purinergic receptors [136], and these receptors and their subtypes are found in virtually all cell types in mammals [137]. As mentioned above, extracellular ATP is considered a DAMP that can be released from stressed/damaged, infected, or dying cells via various proposed mechanisms, including pannexin-1 hemichannels, connexin hemichannels, or even through the purinergic P2X7 receptors, with consequent autocrine and paracrine cell signaling [138–142].

Purinergic receptors are classified as P1 and P2 receptors [136]. P1 receptors are G-protein-coupled metabotropic receptors that exclusively recognize extracellular adenosine [143, 144]. Their role during periodontopathogen infections will be discussed later in this review. There are two subfamilies of purinergic P2 receptors: P2X receptors that are ionic channels activated by ATP and P2Y receptors that are G-protein-coupled receptors activated by ATP, ADP, UTP, UDP, and UDP-glucose [143, 145]. Currently, seven P2X protein-coupled receptors activated by ATP, ADP, UTP, and P2Y receptors that are G-proteins of purinergic P2 receptors: P2X receptors that are ionic channels activated by ATP and P2Y receptors that exclusively recognize extracellular adenosine [136]. P1 receptors are G-protein-coupled metabotropic receptors [136], and these receptors and their subtypes are found in virtually all cell types in mammals [137]. As mentioned above, extracellular ATP is considered a DAMP that can be released from stressed/damaged, infected, or dying cells via various proposed mechanisms, including pannexin-1 hemichannels, connexin hemichannels, or even through the purinergic P2X7 receptors, with consequent autocrine and paracrine cell signaling [138–142].

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We showed that *P. gingivalis* infection induces higher expression of the P2X7 receptor in infected murine macrophages [124] and in the maxilla of orally infected mice [17]. Corroborating our data in mice, we also demonstrated that P2X7 receptor expression levels were lower in patients after periodontal treatment than prior to treatment [17], suggesting that the P2X7 receptor may play a role during PD and infection. Interestingly, P2X7 receptor distribution in macrophages may be modulated by *P. gingivalis* fimbriae, because we showed that receptor expression had a distinct pattern of focus formation in the absence of *P. gingivalis* fimbriae [124]. These data suggest that *P. gingivalis* infection induces P2X7 receptor expression and that *P. gingivalis* expresses virulence factors that allow this pathogen to modulate receptor distribution in the host cell.

In a model of *P. gingivalis* infection *in vivo*, we showed that the P2X7 receptor was required for IL-1β production, leukocyte recruitment to the site of infection, and bacterial clearance [20]. Interestingly, the P2X5 receptor was shown to be required for efficient production of IL-1β and osteoclast maturation *in vitro*, and that P2X5 receptor deficiency, but not P2X7 receptor deficiency, led to decreased bone loss in an animal model of PD [149]. These data suggest that the P2X7 receptor might be dispensable for the development of periodontitis but it is required for the induction of immune responses and microbial clearance during periodontogenic bacterial infection in murine models. Together, these results suggest that the P2X5 and P2X7 receptors may be novel therapeutic targets in this oral disease.

The ability of *P. gingivalis* to adapt to the gingival epithelium has not yet been fully understood. Among several virulence factors harbored by *P. gingivalis*, it seems that this pathogen evolved to protect itself against extracellular ATP through its nucleotide-diphosphate-kinase enzyme (NDK). NDK is an important virulence factor of *P. gingivalis* that has been shown to cleave extracellular ATP molecules [150]. After *P. gingivalis* infection in gingival epithelial cells, release of ATP occurs and the P2X7/pannexin 1 receptor is activated by autocrine action of ATP; the NDK from *P. gingivalis* accumulates in the cytoplasm [151]. NDK from *P. gingivalis* is carried along myosin-9 filaments and actin filaments to the host cell periphery. Upon translocation to the extracellular environment through the formation of the P2X7/pannexin 1 channel, NDK hydrolyzes ATP, thereby reducing the activation signal of the P2X7 receptor and its downstream signaling events [151]. During infection in gingival epithelial cells, NDK from *P. gingivalis* inhibited ATP-induced reactive oxygen species (ROS) generation, thereby contributing to bacterial persistence [152]. NDK also decreased ATP-induced IL-1β release [153] and inhibited ATP-induced host cell death after infection with *P. gingivalis* [150]. Inhibition of these pathways by *P. gingivalis*, by means of its NDK, contributes to intracellular bacterial survival and persistence [151].

*P. gingivalis* also modulates the transcription of forkhead box protein 1 (FOXO-1) genes, leading to the synthesis of antioxidant enzymes such as superoxide dismutase and catalase, restoring the redox balance and preventing long-term oxidative damage. With the inhibition of the toxic response, *P. gingivalis* can survive, replicate, and translocate through adjacent cells, notwithstanding its ability to adapt to the oxidative stress environment [152, 154]. Intracellular *P. gingivalis* inhibited NADPH oxidase (NOX) 2-ROS, followed by suppression of hypochlorous acid production in gingival epithelial cells; these bacteria were present in ER-rich/LC3-positive autophagic vacuoles, considered a new mechanism of bacterial survival [24].

P1 receptors recognize extracellular adenosine; they are subdivided into A1, A2a, A2b, and A3, exhibiting varying degrees of sensitivity to adenosine [155]. During gingival epithelial cell infection with *P. gingivalis*, treatment with the specific A2a receptor agonist (CGS-21680) led to bacterial proliferation and increased cAMP levels. However, when a broad-spectrum adenosine agonist (NECA) was used, minimal effects on intracellular *P. gingivalis* levels were observed [156]. These data suggest that adenosine signaling may attenuate inflammatory processes associated with bacterial
infection [156], a mechanism of bacterial survival in the buccal mucosa. These results demonstrate the need for further studies aimed at the participation of P1 and P2 receptors in the context of infection by periodontopathogenic bacteria, as well as PD. Furthermore, even though there are no studies of purinergic signaling and *F. nucleatum* to date, the results from our group and others on *P. gingivalis* infection suggest that *F. nucleatum* infection can also be modulated by purinergic signaling infection. In this context, future studies are needed regarding *F. nucleatum* infection and purinergic signaling. Figure 4 suggests therapeutic targets in the pathways of the activation of purinergic P2X7 receptor and inflammasome by the bacterium *F. nucleatum* or *P. gingivalis* and possible therapeutic targets.

3.4. Regulation of Caspases Involved in Apoptosis and Pyroptosis by *P. gingivalis* and *F. nucleatum*. Caspases are endoproteases that cleave peptide bonds in a cysteine-dependent and aspartate-directed manner [157]. Caspases can mediate substrate activation and inactivation, and they may also generate active signaling molecules that participate in immune responses, including cell death and inflammation [157]. To date, 11 caspases have been found in humans (caspase-1 to caspase-10 and caspase-14), whereas 10 have been found in mice (caspase-1, 2, 3, 6, 7, 8, 9, 11, 12, and 14) [157]. Caspases are categorized according to their roles in physiology: apoptosis (caspase-3, 6, 7, 8, and 9 in mammals) and inflammation/pyroptosis (caspase-1, 4, and 5 in humans and caspase-1 and 11 in mice).

Caspase-11 has a protective effect during infections in which the bacterium invades the cytosol [158]. This caspase directly binds the hexa-acylated lipid portion of LPS from Gram-negative bacteria, the same component structure that activates TLR4, through its CARD domain [22]. However, species with four acyl groups, although capable of binding, have not been shown to activate caspase-11 [159]. There are some pathogenic bacteria that alter the acylation status of its lipid A, thereby avoiding recognition by cells of the immune system, minimizing the inflammatory process [22]. In this context, it is important to carry out studies that evaluate the possibility of caspase-11 controlling the intracellular proliferation of *P. gingivalis* and *F. nucleatum*.

Apoptosis is a programmed cell death that involves the controlled dismantling of intracellular components while avoiding inflammation and damage to surrounding cells [157]. By contrast, pyroptosis is a nonapoptotic type of cell death that involves plasma membrane rupture and release of proinflammatory intracellular contents [160]. For this reason, pyroptosis usually occurs via noncanonical inflammasome activation by Gram-negative bacteria and is mediated by inflammatory caspase-1 and caspase-4/5 in humans, or by caspase-1 and caspase-11 in mice [118, 119]. Pyroptosis is characterized by the formation of membrane pores, cell edema, and osmotic lysis and release of the cytosolic contents into the extracellular medium [22]. This pathway contributes to intracellular bacterial clearance and destroys any niche formed by intracellular bacterial replication because it causes intracellular bacterial exposure to the extracellular compartment, making bacteria more susceptible to antibodies and attacks by phagocytes such as neutrophils [159]. In this sense, it is advantageous to prevent or delay host cell death in order

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**Figure 4:** Activation of purinergic P2X7 receptor and inflammasome by the bacterium *F. nucleatum* or *P. gingivalis* and possible therapeutic targets.
to preserve their intracellular environment, thereby favoring microbial persistence [19].

Induction or inhibition of apoptosis by bacteria varies according to cell type, bacterial species and strains, duration of infection, and presence of bacterial components (e.g., LPS, proteinases). Cell death by *P. gingivalis* was observed in B cells and human gingival fibroblasts, and inhibition of apoptosis provoked by this bacterium was demonstrated in human monocytes, macrophages, neutrophils, and primary gingival epithelial cells [161–168]. One of the pathways in which *P. gingivalis* prevents the death of gingival epithelial cells either by necrosis or by apoptosis is through the activation of the JAK1/Akt transducer and transcriptional activator 3 (STAT3) pathway, causing upregulation of miR-203 that leads to inhibition of the suppressor of cytokine signaling 3 (SOCS3) negative regulator and subsequent suppression of apoptosis [168–170]. Furthermore, at the mitochondrial membrane, proapoptotic Bad is inhibited and the ratio of Bcl2: Bax increases, decreasing cytochrome c levels and caspase-3/9 activation, thereby reducing the effect of apoptosis [171].

During infection in murine macrophages, we and others showed that *P. gingivalis* itself did not induce the expression of the activated form of caspase-1, pyroptosis [70], and IL-1β [17, 20, 96, 124], requiring a second signal for the activation of these molecules [(17, 20, 96, 124)]. However, OMVs isolated from *P. gingivalis* induced the activation of caspase-1 (including pyroptosis) and IL-1β in murine and human macrophages [70], suggesting that live *P. gingivalis* negatively modulates inflammasome and caspase-1 activation in favor of its own survival. By contrast, studies using PMA-primed THP-1 cells demonstrated that *P. gingivalis* infection per se induced the activation of the NLRP3 and AIM2 inflammasomes, promoting caspase-1 [121] and caspase-4 activation, leading to pyroptosis in a MOI-dependent manner [172]. Interestingly, high levels of *P. gingivalis* infection did not induce caspase-1 activation or cell death due to a mechanism believed to involve gingipains [70, 173]. Therefore, *P. gingivalis* can modulate activation of caspases involved in apoptosis and pyroptosis depending on the cell line and model of infection. These data agree with those of a study showing enhanced levels of NLRP3 and pyroptosis along with the active forms of caspase-1 and IL-1β in the gingival stroma of periodontitis specimens compared to those of healthy samples [174].

*In vitro* studies showed that while *P. gingivalis* can persist intracellularly in macrophages for up to 63 h [175], this bacterium was able to survive intracellularly for up to 8 days in gingival epithelial cells [73]. Indeed, *P. gingivalis* possesses several virulence factors that inhibit host cell death induced by various proapoptotic agents [168, 169]. *P. gingivalis* triggered rapid and reversible surface phosphatidylycerine exposure (an apoptosis marker) through a mechanism requiring caspase activation [168]. This opportunistic pathogen can manipulate the host machinery to facilitate its long-term survival by inhibiting the intrinsic apoptotic pathway (cytochrome c release and caspase-3/9 activation) [168, 171]. In fact, it was demonstrated that *P. gingivalis* inhibited chemically induced apoptosis in primary cultures of gingival epithelial cells by blocking the activation of the effector caspase-3 via manipulation of the JAK/STAT pathway that controls intrinsic mitochondrial cell death pathways [169].

Even though *P. gingivalis* does not induce pyroptosis in murine macrophages, *F. nucleatum* infection induces caspase-1 and pyroptosis that is inhibited if *P. gingivalis* is added to the culture [96]. Together, these studies show that *P. gingivalis* is well adapted to survive in various cell types by avoiding the induction of host cell death. These data suggest the need for studies to verify how these bacteria modulate caspases in favor of their own survival, to develop therapeutic targets, and to generate effective treatments for PD.

### 3.5. Adaptive Immunity.

The adaptive immune system acts in the context of the chronicity of PD, reinforcing the protection of the host through cellular and noncellular mechanisms [176]. During the development of periodontitis, various T helper subsets predominate in several stages [177]. B cells were observed to predominate in the progression of periodontal lesions, together with Th2 cell profiles [178, 179]. B cells and their antibodies act by preventing bacterial adhesion, by inactivating bacterial toxins, and by acting as opsonins for neutrophil phagocytosis [180].

CD4⁺ T cells are involved directly and indirectly in osteoclastic reabsorption during PD. Various subsets of CD4⁺ T cells promote or suppress immune responses of the host during the progression of periodontitis [181, 182]. Baker et al. demonstrated that animals without MHC-II-restricted CD4⁺ T cells but not MHC-I-restricted CD8⁺ T cells were resistant to oral alveolar bone loss induced by *P. gingivalis* infection, suggesting that CD4⁺ T cells contribute to bone demineralization [183]. Throughout the development of periodontitis, Th1 and Th2 responses characterize disease progression [184], acting as essential immunoregulators and mediators of the initial lesion. Th17 cells may directly or indirectly exacerbate inflammation by modulating Th1 cells or by increasing the synthesis of inflammatory molecules from gingival fibroblasts [185]. By contrast, Treg cells act on the balance of periodontal lesions and become a therapeutic target, allowing the modulation of host immune response, thereby attenuating the tissue damage associated with periodontitis [186]. *P. gingivalis* stimulates increased production of the proinflammatory cytokine IL-17 produced by Th17 cells [187] that act by stimulating the production of inflammatory molecules such as cytokines, chemokines, and other effector compounds, inducing RANKL by osteoblasts, thereby influencing reabsorption of bone [188].

*P. gingivalis* and *F. nucleatum* also induce and evade various adaptive immune responses generated in the infected host. *P. gingivalis* was shown to suppress IFN-γ-stimulated release of CXCL9, CXCL10, and CXCL11 from epithelial cells. The inhibition of chemokine expression occurred at the level of gene transcription and was associated with downregulation of interferon regulatory factor 1 (IRF1) and decreased STAT1 expression [189]. In accordance with this idea, *P. gingivalis*-stimulated antigen-presenting cells enhanced Th17 but not Th1 polarization because the bacteria favored the generation of Th17-related cytokines such
as IL-1β, IL-6, and IL-23, but not the Th1-related IL-12 [190]. Furthermore, when mice were subcutaneously vaccinated with formalin-killed \( P. \) gingivalis and then were orally challenged with \( P. \) gingivalis, the vaccination protected the mice from alveolar bone resorption and inflammation. This was due to the downregulation of Th17 immune responses and upregulation of Treg, IL-10, and TGF-\( \beta \) production [182]. Regarding the production of the T cell growth factor IL-2, \( P. \) gingivalis targets its expression at the protein level by inhibiting AP-1 and NF-\( \kappa \)B activity, impeding the ability of T cells to sustain stable IL-2 accumulation by means of the bacterial gingipain Rgp [191]. Interestingly, \( P. \) gingivalis gingipain Kgp was also demonstrated to hydrolyze IgG1 and IgG3 heavy chains in vitro [192]. Moreover, cleavage of IgG1 was identified in gingival crevicular fluid from patients with aggressive periodontitis and chronic periodontitis, while no cleavage was detected in healthy controls [193]. In a study involving individuals with untreated and successfully treated chronic periodontitis, both individuals expressed sIgA, IgA, IgG1, and IgG4 against \( F. \) nucleatum at the same levels but untreated individuals presented sIgA and Th1- (IFN-\( \gamma \)- and IgG1-) dominant immune responses [194].

Further studies are needed, primarily in vivo, to evaluate the role of \( P. \) gingivalis and \( F. \) nucleatum in the profiles of immunoinflammatory cells, correlating them with the chronicity of PD such that effective treatments may be developed to prevent the exacerbation of the tissue injury and bone loss. Figure 5 demonstrates the results discussed in this review.

### 4. Challenges and Perspectives

The role of \( P. \) gingivalis and \( F. \) nucleatum in the pathogenesis of PDs is well-documented in the literature; nevertheless, the exact molecular mechanisms induced by these bacteria are not yet fully understood. Studies on TLR2/4 activation by \( P. \) gingivalis LPS remain to be clarified, because of controversies in the literature. Additional studies in human cells are necessary because the inflammatory potential is higher in these cells than in animal cells. Further studies are also needed to investigate how \( F. \) nucleatum manipulates downstream TLR2/TLR4 pathways in order to survive and replicate intracellularly. It is known that inflammasomes are involved in the pathogenesis of periodontitis; however, it remains necessary to determine which inflammasomes, in addition to NLRP3, actually contribute to the pathogenesis of PD induced by \( P. \) gingivalis and \( F. \) nucleatum. Even though we know that P2X7 receptor is involved in the immune responses against \( P. \) gingivalis, the role of the purinergic signaling in the context of \( F. \) nucleatum infection remains unknown. Further research needs to address the role of caspases, especially caspase-11, in the context of inflammation and cell death in in vivo and in vitro models of PD. More studies that clarify the differences in the immune response of various cells of the oral cavity involved in infections by \( P. \) gingivalis and \( F. \) nucleatum are also needed, considering time of infection, MOI, and types of strains to be representative in PD in humans. Because models of coinfections and cocultures are more representative of human periodontitis, it is important that future
studies investigate models of in vivo coinfection with *P. gingivalis* and *F. nucleatum* in order to better understand the host immune response. Therefore, with more solid literature on these signaling pathways and immune responses during infection with these bacteria, effective treatments for PD may emerge.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| AP-1 | Activator protein 1 |
| ATP | Adenosine triphosphate |
| ASC | Apoptosis-associated protein with caspase recruitment domain |
| CPS | Capsule |
| CLRs | C-type lectin receptors |
| CARD | Caspase recruitment domain |
| CD14 | Cluster of differentiation 14 |
| CR3 | Complement 3 |
| CXCL | Chemokine (C-X-C motif) |
| CXC4 | C-X-C chemokine receptor type 4 |
| DAMPs | Patterns associated with damage |
| ERK | Extracellular signal-regulated kinase |
| FaDA | Fusobacterium adhesin A |
| Fap2 | Fatty-acid-binding protein 2 |
| FomA | Porin protein |
| FOXO-1 | Forkhead box protein O1 |
| GCF | Gingival crevicular fluid |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| HBD | Beta-defensins |
| HMGB1 | High-mobility group box 1 protein |
| ICAM-1 | Intercellular adhesion molecule 1 |
| IgG | Immunoglobulin G |
| IL | Interleukin |
| IRF1 | Interferon regulatory factor 1 |
| JNK | Jun N-terminal protein kinase |
| LPS | Lipopolysaccharide |
| LRRs | Leucine-rich repeats |
| MAPKs | Mitogen-activated protein kinases |
| MD-2 | Myeloid differentiation protein 2 |
| MMPs | Matrix metalloproteinases |
| MOI | Multiplicity of infection |
| MyD88 | Myeloid differentiation primary response 88 |
| Myh9 | Nonmuscle myosin II-A |
| NDK | Nucleoside diphosphate kinase |
| NF-kB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| NLRs | Nucleotide-binding oligomerization domain-like receptors |
| NOD | Nucleotide oligomerization domain |
| NLRBs | NOD-like receptors or baculovirus inhibitor (BtR) repeats |
| NO | Nitric oxide |
| OS | Oxidative stress |
| OMVs | Outer membrane vesicles |
| PAMPs | Molecular pattern associated with the pathogen |
| PBMCs | Peripheral blood mononuclear cells |
| PGDHC | Phosphoglycerol dihydroceramide |
| PEDHC | Phosphoethanolamine dihydroceramide |
| PRRs | Pattern recognition receptors |
| PYD | Pyridine domain |
| PGE2 | Prostaglandin E2 |
| PI3K | Phosphatidylinositol-3-kinase |
| PD | Periodontal disease |
| P2X | The ATP-gated P2X receptor cation channel family |
| P2Y | Family of purinergic G protein-coupled receptors |
| p65 | Also known as Rel A, nuclear factor NF-kappa-B p65 subunit |
| Rabs | Ras superfamily of monomeric G protein |
| RANKL | Receptor activator of nuclear factor kappa-B ligand |
| Rel A | v-rel reticuloendotheliosis viral oncogene homolog A |
| RLRs | RIG-I-like receptors (retinoic acid-inducible gene-I-like receptors) |
| RhoA | Ras homolog gene family, member A |
| ROS | Reactive oxygen species |
| STAT3 | Signal transducer and activator of transcription 3 |
| SOCS3 | Suppressor of cytokine signaling 3 |
| TRAF6 | Tumor necrosis factor receptor-associated factor 6 |
| TRIF | TIR-domain-containing adapter-inducing interferon-β |
| TRAM | TRIF-related adaptor molecule |
| TIR | Toll/interleukin-1 receptor |
| TLRs | Toll-like receptors |
| TNF-α | Tumor necrosis factor-α |

**Disclosure**

The opinions, findings, and conclusions herein are those of the author and not necessarily represent those of The Regents of the University of California or any of its programs.

**Conflicts of Interest**

The authors declare no conflict of interest.

**Authors’ Contributions**

Kívia Queiroz de Andrade conceived the project, reviewed the literature, wrote the manuscript, and prepared the figures. Cássio Luiz Coutinho Almeida da Silva reviewed the literature and critically reviewed and wrote the manuscript. Robson Coutinho-Silva critically reviewed the paper and supervised the project.

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References

[1] J. Liu, X. Tang, C. Li et al., “Porphyromonas gingivalis promotes the cell cycle and inflammatory cytokine production in periodontal ligament fibroblasts,” Archives of Oral Biology, vol. 60, no. 8, pp. 1153–1161, 2015.

[2] Y. Li, H. Guo, X. Wang, Y. Lu, C. Yang, and F. Yang, “Coinfection with Fusobacterium nucleatum can enhance the attachment and invasion of Porphyromonas gingivalis or Aggregatibacter actinomycetemcomitans to human gingival epithelial cells,” Archives of Oral Biology, vol. 60, no. 9, pp. 1387–1393, 2015.

[3] G. Hajishengallis, R. P. Darveau, and M. A. Curtis, “The keystone-pathogen hypothesis,” Nature Reviews Microbiology, vol. 10, no. 10, pp. 717–725, 2012.

[4] P. I. Eke, B. A. Dye, L. Wei, G. O. Thornton-Evans, and R. J. Genco, “Prevalence of periodontitis in adults in the United States: 2009 and 2010,” Journal of Dental Research, vol. 91, no. 10, pp. 914–920, 2012.

[5] M. S. Tonetti, S. Jepsen, L. Jin, and J. Otomo-Corgel, “Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: a call for global action,” Journal of Clinical Periodontology, vol. 44, no. 5, pp. 456–462, 2017.

[6] R. Nakao, H. Hasegawa, B. Dongying, M. Ohnishi, and H. Senpuku, “Assessment of outer membrane vesicles of periodontopathic bacterium Porphyromonas gingivalis as possible mucosal immunogen,” Vaccine, vol. 34, no. 38, pp. 4626–4634, 2016.

[7] F. Q. Bui, C. L. C. Almeida-da-Silva, B. Huynh et al., “Association between periodontal pathogens and systemic disease,” Journal of Biomedical Science, vol. 42, no. 1, pp. 27–35, 2019.

[8] W. G. Wade, “The oral microbiome in health and disease,” Pharmacological Research, vol. 69, no. 1, pp. 137–143, 2013.

[9] A. E. Delitto, F. Rocha, A. M. Decker, B. Amador, H. L. Sorsenon, and S. M. Wallet, “MyD88-mediated innate sensing by oral epithelial cells controls periodontal inflammation,” Archives of Oral Biology, vol. 87, pp. 125–130, 2018.

[10] B. W. Bainbridge and R. P. Darveau, “Porphyromonas gingivalis lipopolysaccharide: an unusual pattern recognition receptor ligand for the innate host defense system,” Acta Odontologica Scandinavica, vol. 59, no. 3, pp. 131–138, 2001.

[11] N. Bostanci and G. N. Belibasakis, “Porphyromonas gingivalis: an invasive and evasive opportunistic oral pathogen,” FEMS Microbiology Letters, vol. 333, no. 1, pp. 1–9, 2012.

[12] A. Gmiterek, A. Klopot, H. Wojtowicz, S. C. Trindade, M. Olczak, and T. Olczak, “Immune response of macrophages induced by Porphyromonas gingivalis requires HmuY protein,” Immunobiology, vol. 221, no. 12, pp. 1382–1394, 2016.

[13] Y. W. Han, “Fusobacterium nucleatum: a commensal-turned pathogen,” Current Opinion in Microbiology, vol. 23, pp. 141–147, 2015.

[14] S. E. Whitmore and R. J. Lamont, “Oral bacteria and cancer,” PLOS Pathogens, vol. 10, no. 3, article e1003933, 2014.

[15] Q. Ding and K. S. Tan, “The danger signal extracellular ATP is an inducer of Fusobacterium nucleatum biofilm dispersal,” Frontiers in Cellular and Infection Microbiology, vol. 6, p. 155, 2016.

[16] B. A. Di, I. Gigante, S. Colucci, and M. Grano, “Periodontal disease: linking the primary inflammation to bone loss,” Clinical and Developmental Immunology, vol. 2013, Article ID 503754, 2013.

[17] E. S. Ramos-Junior, A. C. Morandini, C. L. C. Almeida-da-Silva et al., “A dual role for P2X7 receptor during Porphyromonas gingivalis infection,” Journal of Dental Research, vol. 94, no. 9, pp. 1233–1242, 2015.

[18] Y. Yamaguchi, T. Kurita-Ochiai, R. Kobayashi, T. Suzuki, and T. Ando, “Regulation of the NLRP3 inflammasome in Porphyromonas gingivalis-accelerated periodontal disease,” Inflammation Research, vol. 66, no. 1, pp. 59–65, 2017.

[19] C. L. C. Almeida-da-Silva, A. C. Morandini, H. Ulrich, D. M. Ojcius, and R. Coutinho-Silva, “Purinergic signaling during Porphyromonas gingivalis infection,” Journal of Biomedical Science, vol. 39, no. 4, pp. 251–260, 2016.

[20] C. L. C. Almeida-da-Silva, E. S. Ramos-Junior, A. C. Morandini et al., “P2X7 receptor-mediated leukocyte recruitment and Porphyromonas gingivalis clearance requires II–1β production and autocrine II–1 receptor activation,” Immunology, vol. 224, no. 1, pp. 50–59, 2019.

[21] M. Lamkanfi and V. M. Dixit, “Mechanisms and functions of inflammasomes,” Cell, vol. 157, no. 5, pp. 1013–1022, 2014.

[22] A. J. Russo, B. Behl, I. Banerjee, and V. A. K. Rathinam, “Emerging insights into noncanonical inflammasome recognition of microbes,” Journal of Molecular Biology, vol. 430, no. 2, pp. 207–216, 2018.

[23] Y. S. Yi, “Caspase-11 non-canonical inflammasome: a critical sensor of intracellular lipopolysaccharide in macrophage-mediated inflammatory responses,” Immunology, vol. 152, no. 2, pp. 207–217, 2017.

[24] K. Lee, J. A. S. Roberts, C. H. Choi, K. R. Atanasova, and Ö. Yilmaz, “Porphyromonas gingivalis traffics into endoplasmic reticulum-rich-antiphagosomes for successful survival in human gingival epithelial cells,” Virulence, vol. 9, no. 1, pp. 845–859, 2018.

[25] Y.-K. J. Zhang, R. L. Yeager, Y. Tanaka, and C. D. Klaassen, “Enhanced expression of Nrf2 in mice attenuates the fatty liver produced by a methionine- and choline-deficient diet,” Toxicology and Applied Pharmacology, vol. 245, no. 3, pp. 326–334, 2010.

[26] H. Guyodo, V. Meuric, L. Pottier et al., “Colocalization of Porphyromonas gingivalis with CD4+ T cells in periodontal disease,” FEMS Immunology & Medical Microbiology, vol. 64, no. 2, pp. 175–183, 2012.

[27] X. Liu, A. Sroka, J. Potempa, and C. A. Genco, “Coordinate expression of the Porphyromonas gingivalis lysine-specific gingipain proteinase, Kgp, arginine-specific gingipain proteinase, RgpA, and the heme/hemoglobin receptor, HmuR,” Biological Chemistry, vol. 385, no. 11, pp. 1049–1057, 2004.

[28] J. W. Smalley et al., “The HbA2 haemagglutinin domain of the lysine-specific gingipain (Kgp) of Porphyromonas gingivalis promotes micro-oxo bishaem formation from monomeric iron(III) protoporphyrin IX,” Microbiology, vol. 152, no. 6, pp. 1839–1845, 2006.

[29] T. Maekawa, J. L. Krauss, T. Abe et al., “Porphyromonas gingivalis manipulates complement and TLR signaling to
uncouple bacterial clearance from inflammation and promote dysbiosis,” *Cell Host & Microbe*, vol. 15, no. 6, pp. 768–778, 2014.

[30] E. Gemmell, R. I. Marshall, and G. J. Seymour, “Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease,” *Periodontology 2000*, vol. 14, no. 1, pp. 112–143, 1997.

[31] G. P. Garlet, “Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints,” *Journal of Dental Research*, vol. 89, no. 12, pp. 1349–1363, 2010.

[32] X. Ke, L. Lei, H. Li, H. Li, and F. Yan, “Manipulation of necrotosis by *Porphyromonas gingivalis* in periodontitis development,” *Molecular Immunology*, vol. 77, pp. 8–13, 2016.

[33] C. O. Igboin, A. L. Griffen, and E. J. Leys, “*Porphyromonas gingivalis* strain diversity,” *Journal of Clinical Microbiology*, vol. 47, no. 10, pp. 3073–3081, 2009.

[34] H. W. Yang, Y. F. Huang, and M. Y. Chou, “Occurrence of *Porphyromonas gingivalis* and Tannerella forsythensis in periodontally diseased and healthy subjects,” *Journal of Periodontology*, vol. 75, no. 8, pp. 1077–1083, 2004.

[35] K. Honda, “*Porphyromonas gingivalis* sinks teeth into the oral microbiota and periodontal disease,” *Cell Host Microbe*, vol. 10, no. 5, pp. 423–425, 2011.

[36] J. L. Krauss, J. Potempa, J. D. Lambris, and G. Hajishengallis, “Complementary Tolls in the periodontium: how periodontal bacteria modify complement and Toll-like receptor responses to prevail in the host,” *Periodontology*, vol. 52, no. 1, pp. 141–162, 2010.

[37] G. Hajishengallis, “Immune evasion strategies of *Porphyromonas gingivalis*,” *Journal of Oral Biosciences*, vol. 53, no. 3, pp. 233–240, 2011.

[38] S. S. Socransky, A. D. Haffajee, M. A. Cugini, C. Smith, and R. L. Kent, “Microbial complexes in subgingival plaque,” *Journal of Clinical Periodontology*, vol. 25, no. 2, pp. 134–144, 1998.

[39] S. E. Gharbia, H. N. Shah, P. A. Lawson, and M. Haapasalo, “Distribution and frequency of Fusobacterium nucleatum subspecies in the human oral cavity,” *Oral Microbiology and Immunology*, vol. 5, no. 6, pp. 324–327, 1990.

[40] S. E. Gharbia and H. N. Shah, “Fusobacterium nucleatum subsp. fusiforme subsp. nov. and Fusobacterium nucleatum subsp. animalis subsp. nov. as additional subspecies within Fusobacterium nucleatum,” *International Journal of Systematic Bacteriology*, vol. 42, no. 2, pp. 296–298, 1992.

[41] S. Nie, B. Tian, X. Wang et al., “Fusobacterium nucleatum subspecies identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry,” *Journal of Clinical Microbiology*, vol. 53, no. 4, pp. 1399–1402, 2015.

[42] S. Hase, T. Hofstad, and E. T. Rietschel, “Chemical structure of the lipid A component of lipopolysaccharides from Fusobacterium nucleatum,” *Journal of Bacteriology*, vol. 129, no. 1, pp. 9–14, 1977.

[43] T. Hofstad, N. Skaug, and T. Bjornland, “O-Antigenic cross-reactivity in Fusobacterium nucleatum,” *Acta Pathologica Microbiologica Scandinavica Section B Microbiology*, vol. 87B, no. 1-6, pp. 371–374, 1979.

[44] G. Bachrach, G. Rosen, M. Bellalou, R. Naor, and M. N. Sela, “Identification of a Fusobacterium nucleatum 65 kDa serine protease,” *Oral Microbiology and Immunology*, vol. 19, no. 3, pp. 155–159, 2004.

[45] F. Feuille, J. L. Ebersole, L. Kesavalu, M. J. Stepfen, and S. C. Holt, “Mixed infection with *Porphyromonas gingivalis* and Fusobacterium nucleatum in a murine lesion model: potential synergistic effects on virulence,” *Infection and Immunity*, vol. 64, no. 6, pp. 2094–2100, 1996.

[46] L. Kesavalu, S. Sathishkumar, V. Bakhtavatchalu et al., “Rat model of polymicrobial infection, immunity, and alveolar bone resorption in periodontal disease,” *Infection and Immunity*, vol. 75, no. 4, pp. 1704–1712, 2007.

[47] A. Saito, S. Inagaki, R. Kimizuka et al., “Fusobacterium nucleatum enhances invasion of human gingival epithelial and aortic endothelial cells by *Porphyromonas gingivalis*,” FEMS *Immunology and Medical Microbiology*, vol. 54, no. 3, pp. 349–355, 2008.

[48] A. Saito, E. Kokubu, S. Inagaki et al., “*Porphyromonas gingivalis* entry into gingival epithelial cells modulated by Fusobacterium nucleatum is dependent on lipid rafts,” *Microbial Pathogenesis*, vol. 53, no. 5–6, pp. 234–242, 2012.

[49] D. Polak, A. Wilensky, L. Shapira et al., “Mouse model of experimental periodontitis induced by *Porphyromonas gingivalis*/Fusobacterium nucleatum infection: bone loss and host response,” *Journal of Clinical Periodontology*, vol. 36, no. 5, pp. 406–410, 2009.

[50] F. C. Martinho, F. R. M. Leite, L. M. M. Nóbrega et al., “Comparison of Fusobacterium nucleatum and *Porphyromonas gingivalis* lipopolysaccharides clinically isolated from root canal infection in the induction of pro-inflammatory cytokines secretion,” *Brazilian Dental Journal*, vol. 27, no. 2, pp. 202–207, 2016.

[51] G. D. Tribble, G. J. Lamont, A. Profulskse-Fox, and R. J. Lamont, “Conjugal transfer of chromosomal DNA contributes to genetic variation in the oral pathogen *Porphyromonas gingivalis*,” *Journal of Bacteriology*, vol. 189, no. 17, pp. 6382–6388, 2007.

[52] D. Grenier and D. Mayrand, “Selected characteristics of pathogenic and nonpathogenic strains of Bacteroides gingivalis,” *Journal of Clinical Microbiology*, vol. 25, no. 4, pp. 738–740, 1987.

[53] M. L. Laine and A. J. Winkelhoff, “Virulence of six capsular serotypes of *Porphyromonas gingivalis* in a mouse model,” *Oral Microbiology and Immunology*, vol. 13, no. 5, pp. 322–325, 1998.

[54] M. E. Neiders, P. B. Chen, H. Suido et al., “Heterogeneity of virulence among strains of Bacteroides gingivalis,” *Journal of Periodontal Research*, vol. 24, no. 3, pp. 192–198, 1989.

[55] S. Kurgan, D. Kansal, D. Nguyen et al., “Strain-specific impact of Fusobacterium nucleatum on neutrophil function,” *Journal of Periodontology*, vol. 88, no. 4, pp. 380–389, 2017.

[56] M. A. Curtis, J. Aduse-Opoku, and M. Rangarajan, “Cysteine proteases of *Porphyromonas gingivalis,*” *Critical Reviews in Oral Biology & Medicine*, vol. 12, no. 3, pp. 192–216, 2001.

[57] Y. Guo, K. A. Nguyen, and J. Potempa, “Dicthotomy of gingipains action as virulence factors: from cleaving substrates with the precision of a surgeon’s knife to a meat chopper-like brutal degradation of proteins,” *Periodontology 2000*, vol. 54, no. 1, pp. 15–44, 2010.

[58] A. Baba, N. Abe, T. Kadowaki et al., “Arg-gingipain is responsible for the degradation of cell adhesion molecules of human gingival fibroblasts and their death induced by *Porphyromonas gingivalis*,” *Biological Chemistry*, vol. 382, no. 5, pp. 817–824, 2001.
Mediators of Inflammation

[59] N. M. O’Brien-Simpson, R. A. Paolini, B. Hoffmann, N. Slakeski, S. G. Dashper, and E. C. Reynolds, “Role of RgpA, RgpB, and Kgp proteinases in virulence of Porphyromonas gingivalis W50 in a murine lesion model,” Infection and Immunity, vol. 69, no. 12, pp. 7527–7534, 2001.

[60] T. Imamura, “The role of gingipains in the pathogenesis of periodontal disease,” Journal of Periodontology, vol. 74, no. 1, pp. 111–118, 2003.

[61] S. Sugawara, E. Nemoto, H. Tada, K. Miyake, T. Imamura, and H. Takada, “Proteolysis of human monocyte CD14 by cysteine proteinases (gingipains) from Porphyromonas gingivalis leading to lipopolysaccharide hyposensitiveness,” Journal of Immunology, vol. 165, no. 1, pp. 411–418, 2000.

[62] G. Sundqvist, M. T. Baer, and F. C. Gibson, “Phagocytosis and virulence of different strains of Porphyromonas gingivalis,” Scandinavian Journal of Dental Research, vol. 99, no. 2, pp. 117–129, 1991.

[63] R. Vernal, R. Leon, A. Silva, A. J. van Winkelhoff, J. A. Garcia-Sanz, and M. Sanz, “Differential cytokine expression by human dendritic cells in response to different Porphyromonas gingivalis capsular serotypes,” Journal of Clinical Periodontology, vol. 36, no. 10, pp. 823–829, 2009.

[64] G. d’Empaire, P. M. Bartold, N. J. Gully, P. S. Zilm, and A. H. Rogers, “The serine phosphatase SerB of Porphyromonas gingivalis suppresses IL-8 production by dephosphorylation of NF-κB,” PLoS Pathogens, vol. 9, no. 4, article e1003326, 2013.

[65] Y. W. Han, W. Shi, G. T.-J. Huang et al., “Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells,” Infection and Immunity, vol. 68, no. 6, pp. 3140–3146, 2000.

[66] K. Sven and N. Skaug, “Bone resorption stimulated by lipopolysaccharides from Bacteroides, Fusobacterium and Veillonella, and by the lipid A and the polysaccharide part of Fusobacterium lipopolysaccharide,” Scandinavian Journal of Dental Research, vol. 88, no. 6, pp. 535–542, 1980.

[67] G. Hajishengallis, M. Wang, S. Liang, M. Triantafillou, and K. Triantafillou, “Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function,” Proceedings of the National Academy of Sciences U S A, vol. 105, no. 36, pp. 13532–13537, 2008.

[68] C. Y. Chiang, G. Kyritsis, D. T. Graves, and S. Amar, “Interleukin-1 and tumor necrosis factor activities partially account for calvarial bone resorption induced by local injection of lipopolysaccharide,” Infection and Immunity, vol. 67, no. 8, pp. 4231–4236, 1999.

[69] E. Nishida, Y. Haru, T. Kaneko, Y. Ikeda, T. Ukai, and I. Kato, “Bone resorption and local interleukin-1alpha and interleukin-1beta synthesis induced by Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis lipopolysaccharide,” Journal of Periodontal Research, vol. 36, no. 1, pp. 1–8, 2001.

[70] A. J. Fleetwood, M. K. S. Lee, W. Singleton et al., “Metabolic remodeling, inflammasome activation, and pyroptosis in macrophages stimulated by Porphyromonas gingivalis and its outer membrane vesicles,” Frontiers in Cellular and Infection Microbiology, vol. 7, p. 351, 2017.

[71] K. R. Atanasova and O. Yilmaz, “Prelude to oral microbes and chronic diseases: past, present and future,” Microbes and Infection, vol. 17, no. 7, pp. 473–483, 2015.

[72] H. Kanazaki, A. Movila, R. Kayal et al., “Phosphoglycerol dihydroceramide, a distinctive ceramide produced by Porphyromonas gingivalis, promotes RANKL-induced osteoclas-
[85] D. Tang, R. Kang, C. B. Coyne, H. J. Zeh, and M. T. Lotze, “PAMPs and DAMPs: signal 0s that spur autophagy and immunity,” *Immunological Reviews*, vol. 249, no. 1, pp. 158–175, 2012.

[86] N. Said-Sadier and D. M. Ojcius, “Alarmins, inflammasomes and immunity,” *Journal of Biomedical Science*, vol. 35, no. 6, pp. 437–449, 2012.

[87] A. Bahrami, N. Parsamanesh, S. L. Atkin, M. Banach, and A. Sahebkar, “Effect of statins on toll-like receptors: a new insight to pleiotropic effects,” *Pharmacological Research*, vol. 135, pp. 230–238, 2018.

[88] L. A. Belfield, J. H. Bennett, W. Abate, and S. K. Jackson, “Exposure to *Porphyromonas gingivalis* LPS during macrophage polarisation leads to diminished inflammatory cyto- kine production,” *Archives of Oral Biology*, vol. 81, pp. 41–47, 2017.

[89] P. M. Chilton, C. A. Embry, and T. C. Mitchell, “Effects of differences in lipid A structure on TLR4 pro-inflammatory signaling and inflammasome activation,” *Frontiers in Immunology*, vol. 3, p. 154, 2012.

[90] A. Peyret-Lacombe, G. Brunel, M. Watts, M. Banach, and A. Sahebkar, “Effect of statins on toll-like receptors: a new insight to pleiotropic effects,” *Pharmacological Research*, vol. 135, pp. 230–238, 2018.

[91] N. A. Paramonov, D. Bailey, M. Rangarajan et al., “Structural analysis of the polysaccharide from the lipopolysaccharide of *Porphyromonas gingivalis* strain W50,” *European Journal of Biochemistry*, vol. 268, no. 17, pp. 4698–4707, 2001.

[92] A. Peyret-Lacombe, G. Brunel, M. Watts, M. Charveron, and H. Duplan, “TLR2 sensing of F. nucleatum and S. sanguinis distinctly triggered gingival innate response,” *Cytokine*, vol. 46, no. 2, pp. 201–210, 2009.

[93] N. Paramonov, D. Bailey, M. Rangarajan et al., “Structural analysis of the core region of O-lipopolysaccharide of *Porphyromonas gingivalis* from mutants defective in O-antigen ligase and O-antigen polymerase,” *Journal of Bacteriology*, vol. 191, no. 16, pp. 5272–5282, 2009.

[94] M. N. Al-Qutub, P. H. Braham, L. M. Karimi-Naser, X. Liu, C. A. Genco, and R. P. Darveau, “Hemin-dependent modulation of the lipid A structure of *Porphyromonas gingivalis* lipopolysaccharide,” *Infection and Immunity*, vol. 74, no. 8, pp. 4474–4485, 2006.

[95] Y. Asai, Y. Makimura, A. Kawabata, and T. Ogawa, “Soluble CD14 discriminates slight structural differences between lipid A that lead to distinct host cell activation,” *Journal of Immunology*, vol. 179, no. 11, pp. 7674–7683, 2007.

[96] D. J. Taxman, K. V. Swanson, P. M. Broglie et al., “*Porphyromonas gingivalis* mediates inflammasome repression in polymicrobial cultures through a novel mechanism involving reduced endocytosis,” *Journal of Biological Chemistry*, vol. 287, no. 39, pp. 32791–32799, 2012.

[97] S. Liang, J. L. Krauss, H. Domon et al., “The C5a receptor impairs IL-12-dependent clearance of *Porphyromonas gingivalis* and is required for induction of periodontal bone loss,” *The Journal of Immunology*, vol. 186, no. 2, pp. 869–877, 2011.

[98] M. Wang, J. L. Krauss, H. Domon et al., “Microbial hijacking of complement-toll-like receptor crosstalk,” *Science Signaling*, vol. 3, no. 109, pp. ra11–ra11, 2010.

[99] I. J. Savitri, K. Ouahara, T. Fujita et al., “Irsogladine maleate inhibits *Porphyromonas gingivalis*-mediated expression of toll-like receptor 2 and interleukin-8 in human gingival epithelial cells,” *Journal of Periodontal Research*, vol. 50, no. 4, pp. 486–493, 2015.

[100] O. Andrukhov, S. Ertlschweiger, A. Moritz, H. P. Bantleon, and X. Rausch-Fan, “Different effects of P. gingivalis LPS and E. coli LPS on the expression of interleukin-6 in human gingival fibroblasts,” *Acta Odontologica Scandinavica*, vol. 72, no. 5, pp. 337–345, 2014.

[101] P. L. Wang, Y. Azuma, M. Shinohara, and K. Ohura, “Toll-like receptor 4-mediated signal pathway induced by *Porphyromonas gingivalis* lipopolysaccharide in human gingival fibroblasts,” *Biochemical and Biophysical Research Communications*, vol. 273, no. 3, pp. 1161–1167, 2000.

[102] Y. Asai, Y. Ohyama, K. Gen, and T. Ogawa, “Bacterial fimbriae and their peptides activate human gingival epithelial cells through Toll-like receptor 2,” *Infection and Immunity*, vol. 69, no. 12, pp. 7387–7395, 2001.

[103] G. Hajishengallis, M. Wang, and S. Liang, “Induction of distinct TLR2-mediated proinflammatory and proadhesive signaling pathways in response to *Porphyromonas gingivalis* fimbriae,” *Journal of Immunology*, vol. 182, no. 11, pp. 6690–6696, 2009.

[104] G. Hajishengallis, M. Wang, E. Harokopakis, M. Triantafilou, and K. Triantafilou, “*Porphyromonas gingivalis* fimbriae proactively modulate beta2 integrin adhesive activity and promote binding to and internalization by macrophages,” *Infection and Immunity*, vol. 74, no. 10, pp. 5658–5666, 2006.

[105] E. Harokopakis, M. H. Albzreh, M. H. Martin, and G. Hajishengallis, “TLR2 transmodulates monocyte adhesion and transmigration via Rac1- and PI3K-mediated inside-out signaling in response to *Porphyromonas gingivalis* fimbriae,” *Journal of Immunology*, vol. 176, no. 12, pp. 7645–7656, 2006.

[106] E. Burns, G. Bachrach, L. Shapiro, and G. Nussbaum, “Cutting edge: TLR2 is required for the innate response to *Porphyromonas gingivalis*: activation leads to bacterial persistence and TLR2 deficiency attenuates induced alveolar bone resorption,” *Journal of Immunology*, vol. 177, no. 12, pp. 8296–8300, 2006.

[107] J. Lin, L. Bi, X. Yu et al., “*Porphyromonas gingivalis* exacerbates ligation-induced, RANKL-dependent alveolar bone resorption via differential regulation of Toll-like receptor 2 (TLR2) and TLR4,” *Infection and Immunity*, vol. 82, no. 10, pp. 4127–4134, 2014.

[108] G. Papadopoulos, E. O. Weinberg, P. Massari et al., “Macrophage-specific TLR2 signaling mediates pathogen-induced TNF-dependent inflammatory oral bone loss,” *The Journal of Immunology*, vol. 190, no. 3, pp. 1148–1157, 2013.

[109] S. R. Park, D. J. Kim, S. H. Han et al., “Diverse Toll-like receptor mediates cytokine production by Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans in macrophages,” *Infection and Immunity*, vol. 82, no. 5, pp. 1914–1920, 2014.

[110] S. Y. Quah, G. Bergenholtz, and K. S. Tan, “Fusobacterium nucleatum induces cytokine production through Toll-like receptor-independent mechanism,” *International Endodontic Journal*, vol. 47, no. 6, pp. 550–559, 2014.

[111] S. Ji, J. E. Shin, Y. S. Kim, J. E. Oh, B. M. Min, and Y. Choi, “Toll-like receptor 2 and NALP2 mediate induction of human beta-defensins by fusobacterium nucleatum in...
gingival epithelial cells,” *Infection and Immunity*, vol. 77, no. 3, pp. 1044–1052, 2009.

[112] D. N. Toussi, X. Liu, and P. Massari, “The FomA porin from *Fusobacterium nucleatum* is a Toll-like receptor 2 agonist with immunoadjuvant activity,” *Clinical and Vaccine Immunology*, vol. 19, no. 7, pp. 1093–1101, 2012.

[113] M. Bawa and V. A. Saraswat, “Gut-liver axis: role of inflammasomes,” *Journal of Clinical and Experimental Hepatology*, vol. 3, no. 2, pp. 141–149, 2013.

[114] E. A. Miao, J. V. Rajan, and A. Aderem, “Caspase-1-induced pyroptotic cell death,” *Immunological Reviews*, vol. 243, no. 1, pp. 206–214, 2011.

[115] P. Broz and V. M. Dixit, “Inflammasomes: mechanism of assembly, regulation and signalling,” *Nature Reviews Immunology*, vol. 16, no. 7, pp. 407–420, 2016.

[116] M. A. Pettengill, A. Abdul-Sater, R. Coutinho-Silva, and D. M. Ojcius, “Danger signals, inflammasomes, and the intricate intracellular lives of clamydiae,” *Journal of Biomedical Science*, vol. 39, no. 5, pp. 306–315, 2016.

[117] G. Lordén, I. Sanjuán-García, N. de Pablo et al., “Lipin-2 regulates NLRP3 inflammasome by affecting P2X7 receptor activation,” *The Journal of Experimental Medicine*, vol. 214, no. 2, pp. 511–528, 2017.

[118] J. Shi, Y. Zhao, K. Wang et al., “Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death,” *Nature*, vol. 526, no. 7575, pp. 660–665, 2015.

[119] N. Kayagaki, I. Sanjuán-García, N. de Pablo et al., “Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling,” *Nature*, vol. 526, no. 7575, pp. 666–671, 2015.

[120] N. Bostanci, G. Emingil, B. Saygan et al., “Expression and regulation of the NALP3 inflammasome complex in periodontal diseases,” *Clinical and Experimental Immunology*, vol. 157, no. 3, pp. 415–422, 2009.

[121] E. Park, H. S. Na, Y. R. Song, S. Y. Shin, Y. M. Kim, and J. Chung, “Activation of NLRP3 and AIM2 inflammasomes by *Porphyromonas gingivalis* infection,” *Infection and Immunity*, vol. 82, no. 1, pp. 112–123, 2014.

[122] T. Ohitsu, H. Ryu, Y. A. Mimamishma et al., “ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway,” *Nature Cell Biology*, vol. 6, no. 2, pp. 121–128, 2004.

[123] O. Yilmaz, A. A. Sater, L. Yao, T. Koutouzis, M. Pettengill, and D. M. Ojcius, “ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by *Porphyromonas gingivalis*,” *Cellular Microbiology*, vol. 12, no. 2, pp. 188–198, 2010.

[124] A. C. Morandini, E. S. Ramos-Junior, J. Potempa et al., “*Porphyromonas gingivalis* fimbiae dampen P2X7-dependent interleukin-1β secretion,” *Journal of Innate Immunity*, vol. 6, no. 6, pp. 831–845, 2014.

[125] C. L. Case, L. J. Kohler, J. B. Lima et al., “Caspase-11 stimulates rapid flagellin-independent pyroptosis in response to *Legionella pneumophila*,” *Proceedings of the National Academy of Sciences USA*, vol. 110, no. 5, pp. 1851–1856, 2013.

[126] C. N. Casson, A. M. Copenhagen, E. E. Zwack et al., “Caspase-11 activation in response to bacterial secretion systems that access the host cytosol,” *PLOS Pathogens*, vol. 9, no. 6, article e1003400, 2013.

[127] F. Xue, R. Shu, and Y. Xie, “The expression of NLRP3, NLRP1 and AIM2 in the gingival tissue of periodontitis patients: RT-PCR study and immunohistochemistry,” *Archives of Oral Biology*, vol. 60, no. 6, pp. 948–958, 2015.

[128] F. Q. Bui, L. Johnson, J. Roberts et al., “*Fusobacterium nucleatum* infection of gingival epithelial cells leads to NLRP3 inflammasome-dependent secretion of IL-1β and the danger signals ASC and HMGB1,” *Cellular Microbiology*, vol. 18, no. 7, pp. 970–981, 2016.

[129] S. C. Hung, P. R. Huang, C. L. C. Almeida-da-Silva, K. R. Atanasova, O. Yilmaz, and D. M. Ojcius, “NLRX1 modulates differentially NLRP3 inflammasome activation and NF-kB signaling during *Fusobacterium nucleatum* infection,” *Microbes and Infection*, vol. 20, no. 9–10, pp. 615–625, 2018.

[130] L. Johnson, C. L. C. Almeida-da-Silva, C. M. Takiya et al., “Oral infection of mice with *Fusobacterium nucleatum* results in macrophage recruitment to the dental pulp and bone resorption,” *Journal of Biomedical Science*, vol. 41, no. 3, pp. 184–193, 2018.

[131] S. Ran, B. Liu, S. Gu, Z. Sun, and J. Liang, “Analysis of the expression of NLRP3 and AIM2 in periapical lesions with apical periodontitis and microbial analysis outside the apical segment of teeth,” *Archives of Oral Biology*, vol. 78, pp. 39–47, 2017.

[132] W. Liu, J. Liu, W. Wang, Y. Wang, and X. Ouyang, “NLRP6 induces pyroptosis by activation of caspase-1 in gingival fibroblasts,” *Journal of Dental Research*, vol. 97, no. 12, pp. 1391–1398, 2018.

[133] J. K. Crane, T. M. Nacher, S. S. Choudhari, and E. M. Giroux, “Two pathways for ATP release from host cells in enteropathogenic Escherichia coli infection,” *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 289, no. 3, pp. G407–G417, 2005.

[134] M. F. Jarvis and B. S. Khakh, “ATP-gated P2X cation-channels,” *Neuropharmacology*, vol. 56, no. 1, pp. 208–215, 2009.

[135] M. T. Young, “P2X receptors: dawn of the post-structure era,” *Trends in Biochemical Sciences*, vol. 35, no. 2, pp. 83–90, 2010.

[136] G. Burnsstock, “Purine and pyrimidine receptors,” *Cellular and Molecular Life Sciences*, vol. 64, no. 12, pp. 1471–1483, 2007.

[137] G. Burnsstock and G. E. Knight, “Cellular distribution and functions of P2 receptor subtypes in different systems,” *International Review of Cytology*, vol. 240, pp. 31–304, 2004.

[138] W. G. Junger, “Immune cell regulation by autocrine purinergic signalling,” *Nature Reviews Immunology*, vol. 11, no. 3, pp. 201–212, 2011.

[139] R. Coutinho-Silva and D. M. Ojcius, “Role of extracellular nucleotides in the immune response against intracellular bacteria and protozoan parasites,” *Microbes and Infection*, vol. 14, no. 14, pp. 1271–1277, 2012.

[140] M. Idzko, D. Ferrari, and H. K. Eltzschig, “Nucleotide signalling during inflammation,” *Nature*, vol. 509, no. 7500, pp. 310–317, 2014.

[141] P. Pellegrati, S. Falzoni, P. Pinton, R. Rizzato, and F. Di Virgilio, “A novel recombinant plasma membrane-targeted luciferase reveals a new pathway for ATP secretion,” *Molecular Biology of the Cell*, vol. 16, no. 8, pp. 3659–3665, 2005.

[142] L. E. B. Savio, P. de Andrade Mello, C. G. da Silva, and R. Coutinho-Silva, “The P2X7 receptor in inflammatory diseases: angel or demon?,” *Frontiers in Pharmacology*, vol. 9, p. 52, 2018.

[143] M. P. Abbracchio, G. Burnsstock, A. Verkhratsky, and H. Zimmermann, “Purinergic signalling in the nervous system.”
system: an overview,” Trends in Neurosciences, vol. 32, no. 1, pp. 19–29, 2009.

[144] B. B. Fredholm, A. P. IJzerman, K. A. Jacobson, K. N. Klotz, and J. Linden, “International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors,” Pharmacological Reviews, vol. 53, no. 4, pp. 527–552, 2001.

[145] M. P. Abbracchio, G. Burnstock, J. M. Boeymans et al., “International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy,” Pharmacological Reviews, vol. 58, no. 3, pp. 281–341, 2006.

[146] R. Coutinho-Silva, G. Correa, A. A. Sater, and D. M. Ojcius, “The P2X(7) receptor and intracellular pathogens: a continuing struggle,” Purinergic Signalling, vol. 5, no. 2, pp. 197–204, 2009.

[147] R. Coutinho-Silva, C. M. da Cruz, P. M. Persechini, and D. M. Ojcius, “The role of P2 receptors in controlling infections by intracellular pathogens,” Purinergic Signalling, vol. 3, no. 1-2, pp. 83–90, 2007.

[148] A. C. Morandini, L. E. Savio, and R. Coutinho-Silva, “The role of P2X7 receptor in infectious inflammatory diseases and the influence of ectonucleotidases,” Biomedical Journal, vol. 37, no. 4, pp. 169–177, 2014.

[149] H. Kim, T. Kajikawa, M. C. Walsh et al., “The purinergic receptor P2X5 contributes to bone loss in experimental periodontitis,” BMB Reports, vol. 51, no. 9, pp. 468–473, 2018.

[150] O. Yilmaz, L. Yao, K. Maeda et al., “ATP scavenging by the intracellular pathogen Porphyromonas gingivalis inhibits P2X7-mediated host-cell apoptosis,” Cellular Microbiology, vol. 10, no. 4, pp. 863–875, 2008.

[151] K. Atanasova, J. Lee, J. Roberts, K. Lee, D. M. Ojcius, and O. Yilmaz, “Nucleoside-diphosphate-kinase of P. gingivais is secreted from epithelial cells in the absence of a leader sequence through a pannexin-1 interactome,” Scientific Reports, vol. 6, no. 1, article 37643, 2016.

[152] C. H. Choi, R. Spooner, J. DeGuzman, T. Koutouzis, D. M. Ojcius, and O. Yilmaz, “Porphyromonas gingivalis-nucleoside-diphosphate-kinase inhibits ATP-induced reactive-oxygen-species via P2X7 receptor/NAPDH-oxidase signaling and contributes to persistence,” Cellular Microbiology, vol. 15, no. 6, pp. 961–976, 2013.

[153] L. Johnson, K. R. Atanasova, P. Q. Bui et al., “Porphyromonas gingivalis attenuates ATP-mediated inflammasome activation and HMGB1 release through expression of a nucleoside-diphosphate kinase,” Microbes and Infection, vol. 17, no. 5, pp. 369–377, 2015.

[154] Q. Wang, M. Sztkowska, A. Ojo, D. A. Scott, H. Wang, and R. J. Lamont, “FOXO responses to Porphyromonas gingivalis in epithelial cells,” Cellular Microbiology, vol. 17, no. 11, pp. 1605–1617, 2015.

[155] K. A. Jacobson and Z. G. Gao, “Adenosine receptors as therapeutic targets,” Nature Reviews Drug Discovery, vol. 5, no. 3, pp. 247–264, 2006.

[156] R. Spooner, J. DeGuzman, K. L. Lee, and O. Yilmaz, “Danger signal adenosine via adenosine 2a receptor stimulates growth of Porphyromonas gingivalis in primary gingival epithelial cells,” Molecular Oral Microbiology, vol. 29, no. 2, pp. 67–78, 2014.

[157] D. R. McIlwain, T. Berger, and T. W. Mak, “Caspase functions in cell death and disease,” Cold Spring Harbor Perspectives in Biology, vol. 7, no. 4, p. 7, 2015.

[158] Y. Aachoui, I. A. Leaf, J. A. Hagar et al., “Caspase-11 protects against bacteria that escape the vacuole,” Science, vol. 339, no. 6122, pp. 975–978, 2013.

[159] J. A. Roberts and O. Yilmaz, “Dangerous liaisons: caspase-11 and reactive oxygen species cross-talk in pathogen elimination,” International Journal of Molecular Sciences, vol. 16, no. 10, pp. 23337–23354, 2015.

[160] A. de Gassart and F. Martinon, “Pyroptosis: caspase-11 unlocks the gates of death,” Immunity, vol. 43, no. 5, pp. 835–837, 2015.

[161] Z. Chen, C. A. Casiano, and H. M. Fletcher, “Protease-active extracellular protein preparations from Porphyromonas gingivalis W83 induce N-cadherin proteolysis, loss of cell adhesion, and apoptosis in human epithelial cells,” Journal of Periodontology, vol. 72, no. 5, pp. 641–650, 2001.

[162] D. R. Geatch, J. I. Harris, P. A. Heasman, and J. J. Taylor, “In vitro studies of lymphocyte apoptosis induced by the periodontal pathogen Porphyromonas gingivalis,” Journal of Periodontal Research, vol. 34, no. 2, pp. 70–78, 1999.

[163] E. Gemmell, S. Prajaneh, D. A. Grieco, J. J. Taylor, and G. J. Seymour, “Apoptosis in Porphyromonas gingivalis-specific T-cell lines,” Oral Microbiology and Immunology, vol. 14, no. 6, pp. 331–338, 1999.

[164] M. Hiroi, T. Shimojima, M. Kashimata et al., “Inhibition by Porphyromonas gingivalis LPS of apoptosis induction in human peripheral blood polymorphonuclear leukocytes,” Anticancer Research, vol. 18, no. 5A, pp. 3475–3479, 1998.

[165] S. F. Nakhjiri, Y. Park, O. Yilmaz et al., “Inhibition of epithelial cell apoptosis by Porphyromonas gingivalis,” FEMS Microbiology Letters, vol. 200, no. 2, pp. 145–149, 2001.

[166] K. Ozaki and S. Hanazawa, “Porphyromonas gingivalis fimбриae inhibit caspase-3-mediated apoptosis of monocyte THP-1 cells under growth factor deprivation via extracellular signal-regulated kinase-dependent expression of p21 Cip/WAF1,” Infection and Immunity, vol. 69, no. 8, pp. 4944–4950, 2001.

[167] P. M. Preshaw, R. E. Schifferle, and J. D. Walters, “Porphyromonas gingivalis lipopolysaccharide delays human polymorphonuclear leukocyte apoptosis in vitro,” Journal of Periodontal Research, vol. 34, no. 4, pp. 197–202, 1999.

[168] O. Yilmaz, T. Jungas, P. Verbeke, and D. M. Ojcius, “Activation of the phosphatidylinositol 3-kinase/Akt pathway contributes to survival of primary epithelial cells infected with the periodontal pathogen Porphyromonas gingivalis,” Infection and Immunity, vol. 72, no. 7, pp. 3743–3751, 2004.

[169] S. Yao, J. Park, Y. Hasegawa et al., “Intrinsic apoptotic pathways of gingival epithelial cells modulated by Porphyromonas gingivalis,” Cellular Microbiology, vol. 9, no. 8, pp. 1997–2007, 2007.

[170] C. E. Moffatt and R. J. Lamont, “Porphyromonas gingivalis induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells,” Infection and Immunity, vol. 79, no. 7, pp. 2632–2637, 2011.

[171] L. Yao, C. Jermanus, B. Barbetta et al., “Porphyromonas gingivalis infection sequesters pro-apoptotic Bad through Akt in primary gingival epithelial cells,” Molecular Oral Microbiology, vol. 25, no. 2, pp. 89–101, 2010.

[172] H. K. Jun, Y. J. Jung, and B. K. Choi, “Treponema denticola, Porphyromonas gingivalis, and Tannerella forsythia induce cell death and release of endogenous danger signals,” Archives of Oral Biology, vol. 73, pp. 72–78, 2017.
Y. Jin, H. K. Jun, and B. K. Choi, “Contradictory roles of Porphyromonas gingivalis gingipains in caspase-1 activation,” Cellular Microbiology, vol. 17, no. 9, pp. 1304–1319, 2015.

R. Cheng, W. Liu, R. Zhang, Y. Feng, N. A. Bhowmick, and T. Hu, “Porphyromonas gingivalis-derived lipopolysaccharide combines hypoxia to induce caspase-1 activation in periodontitis,” Frontiers in Cellular and Infection Microbiology, vol. 7, p. 474, 2017.

M. Wang, M. A. Shakhateeh, D. James et al., “Fimbrial proteins of Porphyromonas gingivalis mediate in vivo virulence and exploit TLR2 and complement receptor 3 to persist in macrophages,” The Journal of Immunology, vol. 179, no. 4, pp. 2349–2358, 2007.

A. Cekici, A. Kantarci, H. Hasturk, and T. E. Van Dyke, “Inflammatory and immune pathways in the pathogenesis of periodontal disease,” Periodontology 2000, vol. 64, no. 1, pp. 57–80, 2014.

T. Yucel-Lindberg and T. Bage, “Inflammatory mediators in the pathogenesis of periodontitis,” Expert Reviews in Molecular Medicine, vol. 15, p. e7, 2013.

T. Okui, Y. Aoki-Nonaka, T. Nakajima, and K. Yamazaki, “The role of distinct T cell subsets in periodontitis—studies from humans and rodent models,” Current Oral Health Reports, vol. 1, no. 2, pp. 114–123, 2014.

T. Morinuschi, D. E. Lopatin, N. Van Poperin, and Y. Ueda, “The potential role of interleukin-17 in the immunopathology of periodontal disease,” Journal of Clinical Periodontology, vol. 32, no. 4, pp. 369–374, 2005.

G. P. Garlet, C. R. Cardoso, F. S. Mariano et al., “Regulatory T cells attenuate experimental periodontitis progression in mice,” Journal of Clinical Periodontology, vol. 37, no. 7, pp. 591–600, 2010.

T. Oda, H. Yoshie, and K. Yamazaki, “Porphyromonas gingivalis antigen preferentially stimulates T cells to express IL-17 but not receptor activator of NF-kappaB ligand in vitro,” Oral Microbiology and Immunology, vol. 18, no. 1, pp. 30–36, 2003.

S. L. Gaffen, “Biolo...
