Inflammasomes and their regulation in periodontal disease

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INFLAMMASOMES AND THEIR REGULATION IN PERIODONTAL DISEASE: A REVIEW

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ABSTRACT

Interleukin-1β (IL-1β) which is secreted by host tissues leading to periodontal tissue inflammation is a major pro-inflammatory cytokine in the pathogenesis of periodontal disease. The conversion of pro-IL-1β into its biologically active form is controlled by multiprotein complexes named as inflammasomes which are key regulator of host defense mechanisms and inflammasome involved diseases, including the periodontal diseases. Inflammasomes are regulated by different proteins and processes, including pyrin domain (PYD)-only proteins (POPs), CARD-only proteins (COPs), tripartite motif family proteins (TRIMs), autophagy and interferons. A review of *in vitro*, *in vivo* and clinical data from these publications revealed that several inflammasomes including (NOD)-like receptor (NLR) pyrin domain containing 3 (NLRP3) and Absent in melanoma 2 (AIM2) have been found to be involved in periodontal disease pathogenesis. To the best of our knowledge, the current article provides the first review of the literature focusing on studies that evaluated both inflammasomes and their regulators in periodontal disease. An upregulation for inflammasomes and a downregulation of inflammasome regulator proteins including POPs, COPs and TRIMs have been reported in periodontal disease. Although Interferons (type I and II) and autophagy have been found to be involved in periodontal disease, their possible role in inflammasome activation has not evaluated yet. Modulating the excessive inflammatory response by the use of inflammasome regulators may have potential in the management of periodontal disease.

Key words: inflammasomes, periodontal disease, caspase activation and recruitment domains, pyrin domain, tripartite motif proteins, autophagy, interferons

1. INTRODUCTION

Periodontal disease is an inflammatory and infectious disease characterized by the destruction of the tooth's supporting tissues [1]. This tissue destruction is initiated by an excessive inflammatory host response to periodontal pathogens, such as *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*), which colonize the dental plaque [2]. The host response is characterized by the production of key proinflammatory cytokines, such as interleukin-1beta (IL-1β) [1], which is produced by various immune and tissue resident cells, including macrophages, oral fibroblasts, oral epithelial cells and osteoblasts. The release of IL-1β is a major step in the immune response due to its ability to induce the expression of a range of other inflammatory cytokines [1 2].

1.1 IL-1β and Periodontal Disease

During the pathogenesis of periodontal disease, IL-1β activates endothelial cells and enables the adhesion of eosinophils, thereby increasing the inflammatory response. IL-1β also regulates the destruction of the alveolar bone by promoting osteoclast formation and activity [3]. Clinical studies show that IL-1β levels are higher in the gingival crevicular fluid (GCF) of periodontitis patients compared with controls [4], and there is also a correlation between IL-1β gingival tissue levels and severity of periodontal disease [5]. The monitoring of IL1β levels in GCF has been proposed as a useful approach for evaluating the host response during disease initiation, progression, and for determining therapeutic outcomes [6].

Studies evaluating the effects of periodontal treatment, with or without antibiotics, on IL-1β levels in GCF have reported conflicting outcomes in patients. Some studies have reported a decrease in IL-1β levels after periodontal treatment for up to 6 months [7-10], while other studies found changes in IL-1β levels only at 3 months [11,12], and some studies found no change in IL-1β levels [6,13,14]. Thus, there is only some evidence suggesting that periodontal tissue breakdown

may be controlled by regulating IL-1β expression. In contrast, 1 month after non-surgical periodontal treatment, IL-1β levels increased in the GCF and mRNA expression was still present in the gingiva of periodontitis patients, although clinical improvements were noted [6]. Thus, inflammasomes, which regulate IL-1β production, appear important in periodontal disease pathogenesis.

1.2 Regulation of the Production of IL-1β by Inflammasomes in Periodontal Disease

IL-1β is initially produced as an inactive precursor, pro-IL-1β, following cellular stimulation with pathogen-associated molecular pattern molecules (PAMPs) and damage associated molecular pattern molecules (DAMPs). These molecules act through pattern recognition receptors (PRRs), which are located on cell membranes, and regulate gene expression pathways [15]. PRRs have several family members, including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [16].

The inflammasome is a central signalling regulator of the innate immune system that rapidly recognizes and triggers the body's response to infections and foreign substances that are potentially harmful to the host [17]. The inflammasome is a multiprotein complex that consists of a PRR, an adaptor protein or ASC [apoptosis-related speck like protein containing a CARD (caspase activation and recruitment domain)] and an active form of caspase-1 [18]. Its mechanism of action is mediated by the PRR connecting to the ASC via oligomerization with its ligand, and then ASC converts pro-caspase-1 to its active form of caspase-1 with its adaptor CARD. The active caspase-1 subsequently cleaves pro-IL-1β, pro-IL-18 and pro-IL-33 into their biologically active forms [19].

Several different types of inflammasomes have been identified, including Nod-like receptor pyrin domain-containing protein 1 (NLRP1), NLRP2, NLRP3, NLRP6, NLRP12,

nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) containing a caspase activating and recruitment domain (CARD) 4 (NLRC4), Ice protease-activating factor (IPAF), NLRC5, PYHINS, AIM2 (Absent in melanoma 2), and Gamma-interferon-inducible protein (Ifi-16). [20]. Of the NLRPs, the first discovered was the **NLRP1 inflammasome,** which is activated primarily by the lethal toxin from *Bacillus anthracis*. It differs from the other NLRPs in that it contains both CARD and PYD domains. NLRP1 mediates intracellular signalling processes that include caspase-1 activation [21]. The role of the NLRP1 inflammasome has been evaluated in periodontal disease [22-25] and similar expression levels have been reported in chronic periodontitis (CP), aggressive periodontitis (AgP) and periodontally healthy controls [26]. Yilmaz et al [22] reported that infection with *Porphyromonas gingivalis (Pg)* of human gingival epithelial cells did not significantly activate the NLRP1 inflammasome and Bostanci et al. [23] also reported no differences in NLRP1 activation in gingival fibroblasts infected with either subgingival or supragingival biofilms. Similarly Belibasakis et al.[24] found that NLRP1 was also not effected by *Aggregatibacter actinomycetemcomitans* (*Aa)* in human mononuclear leukocyte cells. Huck et al. [25] reported no differences in NLRP1 inflammasome activity in human umbilical vein endothelial cells exposed to *Pg* or *Pg* lipopolysaccharide (LPS). Notably Guo et al. [27] reported a downregulation of NLRP1 in human gingival epithelium infected with *Pg*.

The **NLRP2 inflammasome** inhibits NF-κB activation [28] and is suggested to be a target to inhibit inflammation induced by the central nervous system [29]. The gingival levels of the NLRP2 inflammasome in patients with CP, AgP and gingivitis (G) were reportedly significantly higher compared with healthy controls [30]. *Pg* challenge induced a downregulation in the NLRP2 inflammasome in a human monocytic cell line. However, no effect was reported for NLRP2 in human oral epithelial cells infected with *Pg* [27]. In addition, no NLRP2 expression was reported in human gingival fibroblasts infected with subgingival or supragingival biofilms

[23]. Lastly, *Aa* infection did not effect NLRP2 inflammasome activity in human mononuclear leukocytes [24].

The **NLRP3 inflammasome** can be activated by a variety of pathogenic microbial components, such as LPS, peptidoglycans [31], as well as range of bacteria and viruses, including *Staphylococcus aureus*, *Klebsiella pneumonia*, influenza A virus [32] and *Pg* [33]. In addition molecules of endogenous and environmental origins that are released during tissue injury, such as extracellular ATP, hyaluronan, amyloid-β fibrils and uric acid crystals [34] have also been reported to activate inflammasome NLR3 . Three different mechanisms have been proposed in NLRP3 activation, including potassium efflux, the generation of mitochondrial-derived ROS, and phagolysosomal destabilization after the digestion of large particulate agonists, such as monosodium urate [35].

The role of NLRP3 in periodontal disease has been extensively reported within the literature. NLRP3 expression has been detected at higher levels in the gingiva of patients with CP, [36,37] AgP [38] and G compared with periodontally healthy participants [30]. Relatively high levels for NLRP3 were also detected in the saliva of CP and AgP groups compared with those in the periodontally healthy group [39]. *Pg* reportedly upregulates NLRP3 expression in human gingival epithelial cells [40] and mouse osteoblasts [41].

Contrary to these studies, *Pg* also downregulates NLRP3 expression in human gingival fibroblasts challenged with a subgingival biofilm [23] or *Pg* LPS [36]. In another study, challenge with a subgingival biofilm also decreased NLRP3 expression in human gingival fibroblasts, while the presence of *Pg* was required to mediate NLRP3 downregulation [42].

In addition, another periodontal pathogen, *Aa* reportedly also mediates NLRP3 activation in human mononuclear leukocytes [24], human osteoblastic cell lines [43], THP-1 cell lines [38,44], and murine macrophage like cell lines [45]. *Treponema denticola* (Td), a member of the red complex of bacteria, can also activate the NLRP3 inflammasome in THP-1 cells [46].

The **NLRP6 inflammasome** is involved in the recognition of microbes and intestinal homeostasis by epithelial cells during chemically induced intestinal injury [47], and it induces caspase-1 dependent processing of IL-1β by associating with ASC [48]. *Aa* infection can downregulate NLRP6 expression in human mononuclear leukocyte cells [24].

The **NLRP7 inflammasome** is linked to microbial acylated lipopeptides and *Staphylococcus aureus* infection [49]. To date, there are no published studies evaluating the role of the NLRP7 inflammasome in periodontal disease.

The **NLRP12 inflammasome**, similar to NLRP6, induces caspase-1 dependent processing of IL-1β by associating with ASC [48]. It also negatively regulates the inflammatory response by suppressing NF-κB pathways and acts as a negative regulator of TLRs and TNF-α [50]. Similar to the NLRP7 inflammasome, there are no published studies evaluating the role of the NLRP7 inflammasome in periodontal disease.

The **NLRC4 inflammasome** plays a role during inflammation by controlling IL-1β release or in NF-κB signalling [51]. It responds to bacterial flagellin and the Salmonella protein PrgJ and activates inflammasome assembly by interacting with caspase-1 [52]. Infection with *Pg* was not found to activate the NLRC4 inflammasome in human gingival epithelial cells [22] nor in the THP-1 monocytic cell line [37].

The **NLRC5 inflammasome** cooperates with the NLRP3 inflammasome. The pathogens recognised by the NLRP3 inflammasome are similar to those recognised by NLRC5. The knockdown of NLCR5 significantly diminishes caspase-1, IL-1β and IL-18 processing in response to bacterial infection, PAMPs and DAMPs in human monocytic cells [53]. The role of the NLRC5 inflammasome has not yet been evaluated in periodontal disease according to current literature.

The **PYHIN inflammasomes** contain PYHIN proteins encoded by four family gene members (IFI16, AIM2, MNDA and IFIX) instead of NLRs. AIM2 and IFI16 form the caspase-

1-activating inflammasomes. AIM2 is located in the cytosol and recognises aberrant cytoplasmic dsDNA of viral or bacterial origin. AIM2 induces cytokine maturation, release and pyroptosis, and is therefore understood to provide defense against bacterial and viral DNA [54]. AIM2 expression was significantly upregulated in CP patients compared with AgP and periodontally healthy participants [26]. In addition, gingiva of CP patients [37] and AgP patients [38] were reported to express more AIM2 compared with healthy controls. AIM2 expression was upregulated in human gingival fibroblasts in response to both supragingival and subgingival biofilms [23]. Similar to these findings, *Pg* [37] and *Aa* [38] triggered an increased AIM2 expression in THP-1 cells. However, in contrast to this finding, *Aa* did not affect AIM2 expression in human mononuclear leukocytes [24]. Consistently, treatment with a subgingival biofilm with or without *Pg* did not effect AIM2 expression in human gingival fibroblasts [42].

IFI16 is located in the nucleus of cells and it recognizes DNA from the vaccinia virus and herpes simplex virus type 1. IFI16 activates the stimulator of the interferon gene pathway to enable interferon production [55]. IFI16 also recognizes the genome of Kaposi's sarcoma–associated herpes virus (KSHV) in the nucleus of infected cells [56]. IFI16, by directly binding to AIM2, is proposed as a mediator of the AIM2 inflammasome-dependent pathway [57]. In addition, IFI16 showed anti-inflammatory effects, whereas AIM2 demonstrated pro-inflammatory effects [58]. IFI16 may play a role in periodontal disease as single nucleotide polymorphisms for both IFI16 and AIM2 have been associated with higher levels of periodontal pathogens and an increase in periodontal disease parameters [58]. (Table 1)(Figure 1)

1.3 Periodontal Pathogens and Inflammasomes

As described above, periodontal pathogens are involved in inflammasome signaling. *Pg is* a major Gram negative bacteria associated with periodontitis that creates an advantageous environment for its co-inhabitants, such as *Fusobacterium nucleatum* (*Fn*) and *Td* [59]. *Pg*

infection is also involved in inflammasome activation, through activation of TLR signalling via LPS, which subsequently activates NLRP3, pro-IL-1β and pro-IL-18 expression and induces danger signals, such as ATP and ROS. This process results in the secretion of several inflammatory cytokines [33,59]. Another Gram negative anaerobic periodontal pathogen, *Aa,* is also involved in inflammasome activation [24,43-45]. By producing virulence factors, such as LPS, leukotoxin and cytolethal distending toxin, notably *Aa* can kill human leukocytes via caspase-1 activation and IL-1β release [59]. *Td,* another periodontal pathogen, can promote *Pg*'s ability to modulate inflammasomes and enhance the colonisation of other periodontal pathogens [46]. *Fn* can also activate NF-κB and DAMP signalling to stimulate the NLRP3 inflammasome [59,60]. *Tannarella forsythia* (*Tf*), also important to periodontal disease pathogenesis, is reportedly capable of inflammasome activation [59,61].

1.4 Periodontal Disease, Inflammasomes and Systemic Conditions

In the literature studies evaluating the link between periodontal disease and its systemic connection by monitoring inflammasomes and periodontal pathogens have been reported [25, 27, 30, 37, 62 , 63, 64, 65, 66,67, 68] . Indeed, the mix infection with periodontal pathogens *Pg*, *Fn*, *Td* and *Tf* caused significant upregulation of NLRP3, NLRC4 and ASC expression in aorta of Integrin-β6 knockout (Itgβ6−/−) mice [62]. *Pg* infection has been reported to cause dysregulation of the NLRP3 inflammasome response in macrophages fed on a western diet in low density lipoprotein receptor knockout (Ldlr°) mice [64]; and also upregulation of NLRP3 expression in human monocytic cells [30], THP-1 cells [37], human hepatocytes [65], mice peritoneal macrophages [66,67], human umbilical vein endothelial cells [25], bone marrow macrophages [68] and human coronary artery endothelial cells [27]. Recently, systemic effects of initial periodontal treatment were evaluated by monitoring NLRP3 inflammasome priming in peripheral blood mononuclear cells (PBMCs) in patients with chronic periodontitis. Initial periodontal treatment was found to alter the inflammasome priming status of PBMCs by downregulating IL-1β and ASC[63].

While inflammasomes may be linked with periodontal disease, a clear and exact mechanism should be elucidated in future studies evaluating inflammasome and periodontal disease interactions.

2. MODULATION OF INFLAMMASOMES IN PERIODONTAL DISEASE

Inflammasomes are a central hub for signalling and regulation of innate immunity, the part of the immune system that recognizes and triggers responses to molecules that might be harmful to the host [17]. Dysregulated inflammasome activation may cause uncontrolled IL-1β release, which can cause damage to the host and result in autoinflammatory and/or autoimmune conditions, in diseases such as familial Mediterranean fever and cyropyrin-associated periodic syndromes [69]. Notably these conditions/syndromes respond to treatment with an IL-1 receptor antagonist. In periodontal disease, 1 month after conventional non-surgical treatment, secretion and transcription of IL-1β levels were still elevated in periodontitis patients, although clinical improvements were detected [6]. Evaluation of inflammasome regulators in periodontal disease may therefore be important and warrant further study.

To date, different inflammasome regulators, including PYD-only proteins (POPs) and CARD-only proteins (COPs), TRIM family proteins (TRIMs), autophagy molecules, interferons, and microbial structures, have been described in the literature [34], and POPs, COPs and TRIMs have been evaluated in periodontal disease [70] however studies are limited. (Table 1)(Figure 1)

2.1 PYD-only proteins (POPs)

Caspase-1 activation is required for the release of IL-1β and IL-18, and can be regulated directly or within the context of inflammasomes through PYD and CARD interactions. Thus, targeting POPs and COPs as modifiers of inflammasomes may gain importance; POPs and COPs are key candidates due to their ability to control both NF-κB and inflammasome

activation. Two POPs have been well described in the literature as POP1 (PYDC1) and POP2 (PYDC2) [71]. POP1 is released primarily by monocytes, macrophages, and granulocytes and shows 88% similarity to the PYD domain of ASC. It interacts with, and sequesters, ASC from the NLRs and inhibits inflammasome activation [72]. POP1 also targets NF-κB activation by inhibiting IkappaB kinase (IKK) [71].

POP2 is expressed in primary peripheral blood leukocytes and monocytic cells and is induced by LPS and TNF-α [73] and shows similarity to NLRP2 (87%) and NLRP7 (67%) [74]. It potentially binds to NLRS and accordingly prevents inflammasome activation [74], including NLRP3 [71] and NLRP2 [75] and AIM2 [71]. POP2 also blocks TNF-α-mediated NF-κB activation at the level of p65, a function that POP1 and other PYDs appear to lack [73]. Recently a third POP, POP3, within the NLRP2P pseudogene was described and this awaits initial characterisation [76].

We recently evaluated the role of POP1 and POP2 expression in patients with CP, AgP, G and also periodontally healthy participants. An upregulation in NLRP3 and IL-1β in all types of periodontal disease compared with healthy controls, and a downregulation in POP1 and POP2 in the gingiva of AgP patients compared to healthy controls, was detected. A decrease in the expression of POP1 was also found in CP patients, differences did not reach significance [70]. Thus, the downregulation of POP1 and POP2 in periodontal disease may promote uncontrolled inflammasome activation with a subsequent increase in IL-1β expression that contributes to periodontal tissue breakdown. (Table 1)(Figure 1)

2.2 CARD-Only Proteins (COPs)

Different COPs have been described in humans, including CARD16 (COP/Pseudo-ICE), CARD17 (INCA), and CARD18 (ICEBERG), Caspase-12s and Nod2-s; none are viral types [77]. CARD16 exhibits 97% identity to the CARD of caspase-1, which is released primarily in

placenta, lymph node, and bone marrow. It can block the oligomerization of Caspase-1 and subsequent expression of IL-1β by activating RIP-2 [78]. Overexpression of CARD16 can mediate NF-κB induction and enhance TNF-α-induced NF-κB activation through an IKK-dependent mechanism [79].

CARD17 has 81% identity to the CARD of caspase-1 and is released in brain, heart, spleen and salivary gland. The tissues releasing CARD17 also generally express caspase-1. CARD17 failed to be upregulated by LPS and TNF-α. CARD17 can not inhibit NF-κB activation, but compared with CARD16, it significantly reduces the expression of IL-1β from THP-1 cells, most likely due to its caspase-1 interaction [80].

CARD18 shows 53% identity to the CARD of caspase-1, which is detected mainly in placental tissue and in many cell lines in humans. The release of CARD18 is increased by LPS and TNF-α in THP-1 monocytes. Similar to CARD17, it does not activate the NF-κB pathway. However it can inhibit IL-1β expression by competing with RIP-2 to prevent the oligomerization of RIP-2 and caspase-1 [81].

NOD2-S is expressed in the human colon and is up-regulated by the anti-inflammatory cytokine, IL-10. Overexpression of NOD2-S leads to NF-κB activation, which is induced by NOD2 and IL-8 release. NOD2-S downregulates IL-1β release by downregulating NOD2 and its adaptor molecule called RIP2 [82]. Caspase-12 has a dominant-negative suppressive effect on caspase-1, thereby mediating susceptibility to bacterial infection [34].

We previously compared the expression of CARD16 and CARD18 in the gingiva of patients with CP, AgP, and G with periodontally healthy patients. We identified an overexpression of NLRP3 and IL-1β and a downregulation of CARD18 in all types of periodontal disease compared with healthy controls. A downregulation in CARD16 expression was also found for all periodontal disease groups, although this was not statistically significant [70]. Thus, the downregulation of CARD16, CARD18, and POPs in periodontal disease may

contribute to uncontrolled inflammasome activation and subsequent activation of IL-1β expression that contributes to periodontal tissue breakdown. (Table 1)(Figure 1)

2.3 TRIM family proteins (TRIMs)

Inflammasome mediated IL-1 responses are also modulated by the TRIM family of proteins. TRIM proteins are associated with several physiological processes, including cell proliferation, signal transduction, transcription, DNA repair and pluripotency [83].

TRIM20 (MEFV) recognizes inflammasome components, including NLRP3, CASP1 and NLRP1, and it sequesters inflammasomes. It supresses caspase-1 activation and IL-1β production [84] and is alsoassociated with familial Mediterranean fever. TRIM20 also regulate autophagy [84].

TRIM16 is a novel pro-IL-1β binding protein in macrophages. TRIM16 enhances IL-1β production by interacting with pro-caspase-1 and NLRP1 to enhance innate immunity [34]. TRIM16 is required for the optimal secretion of IL-1β that is triggered by lysosomal damage. It is also involved in autophagy and it has an ability to protect cells from oxidative stress-induced cytotoxicity [85]. TRIM16 is highly expressed in keratinocytes and its knockdown results in a reduction in IL-1β secretion. However keratinocytes also express other inflammasome proteins that are responsible for pro-IL-1β maturation and its subsequent secretion [86].

TRIM30, another member of the TRIM family of proteins, can supress the activation of the NLRP3 inflammasome in response to different stimuli, including ATP, by inhibiting reactive oxygen species (ROS) production in macrophages [87]. TRIM30 is also a negative regulator of TLR signalling [34].

The expression of TRIM16 and TRIM20 was also evaluated in our previous study. Similar to POPs and COPs, TRIM20 was also shown to be involved in periodontal disease. TRIM20 was downregulated in CP, AgP and G patients compared with the periodontally

healthy group. TRIM16 was also downregulated in all periodontal disease groups, although this was not statistically significant [70]. Thus, the overexpression of NLRP3 and IL-1β may result from the downregulation of TRIMs that promote periodontal tissue breakdown [70]. (Table 1)(Figure 1)

2.4 Type I Interferon

Type I Interferons (IFNs), including IFN-α and IFN-β, are produced during infection as part of the host response against viruses, bacteria, parasites and fungi. Their production is induced in response to PRRs. Activation of IFN-α and IFN-β is induced by the IFN-regulatory factor (IRF) family of transcription factors (in most cases IRF3 and IRF7) [88]. Type I (IFN) signalling is important in inflammasome activation by modulating the AIM2 inflammasome [89] and also reducing the amount of intracellular pro-IL-1β and/or inhibiting caspase-1 activation [90]. This decrease in pro-IL-1β is related to the ability of type I IFNs to promote anti-inflammatory cytokine IL-10 production that utilises STAT3 for inhibiting pro-IL-1β and pro-IL-1α. Type I IFNs also use a transcription factor, STAT1, to reduce caspase-1, which was initially understood to be specific for NLRP1 and NLRP3 inflammasomes, however its exact mechanism of action is unclear [34].

Type-1 interferons are reportedly involved in the pathogenesis of periodontal disease [91,92]. Expression of IFNα was higher in the gingival tissues of periodontitis patients compared with gingivitis patients [93]. In addition, IFN-α levels were higher in the serum of periodontitis patients compared with healthy controls, and periodontal treatment reduced IFN-α levels compared with non-diseased controls [91]. Furthermore, IFN-β was induced by the outer membrane protein of *Treponema lecithinolyticum,* which is associated with periodontitis, in THP-1 cells [94]. *Pg* was linked to RANKL expression and, subsequently, alveolar bone loss in experimental periodontitis [92]. The intolerance of human gingival fibroblasts to LPS was

enhanced by pretreatment with IFN-β [95]. Ntably, hoowever, to date, no study has evaluated the role of type I IFN as an inflammasome regulator in periodontal disease.

2.5 Type II Interferons

Type II IFNs (including IFN-γ) is a significant cytokine in signalling T helper type 1 and $CD8^+$ T cell responses and it plays a role as a feedback regulator of inflammasome responses. It acts as an inhibitor of IL-1β production in monocytes and macrophages infected by *M. tuberclosis*. [96]. However, there is limited knowledge regarding the relationship between IFN-γ and the inflammasome.

The role of IFN-γ in periodontal disease was previously evaluated. IFN-γ levels were increased in the GCF [97,98] and saliva [99] from periodontitis patients compared with healthy controls, however there were no differences in the serum levels between these patients [100]. Interestingly, higher IFN-γ levels in GCF were associated with a significantly greater risk for progression of periodontitis in HIV+ patients [101]. Furthermore, infection with *Pg* caused increased IFN-γ secretion in periodontal ligament-derived mesenchymal stem cells (PDLSCs) [102] and human keratinocytes [103]. However, to date, no study has evaluated the role of IFN-γ in inflammasome activation in periodontal disease.

2.6 Autophagy

Autophagy, an intracellular degradation process, is involved in several different components of the immune response, including antigen presentation, cell death and cytokine secretion. Autophagocytosis-deficient mice overproduce IL-1β and IL-18 when challenged with LPS and other PAMPs; initially this was thought to originate from enhanced caspase-1 activation [104]. This overexpression of caspase-1 emerged when autophagy-deficient cells failed to remove damaged mitochondria that generated excessive ROS, which triggered NLRP3 activation [105].

Furthermore, autophagy can sequester pro-IL-1β, which limits the substrate for caspase-1 activation [106].

The link between periodontal disease and autophagy has been evaluated in recent reviews. These reviews found evidence that autophagy is involved in periodontal disease, namely that periodontal disease promotes excessive ROS formation that induces dysregulated autophagy [107]. However, the role of autophagy in periodontal disease is still unclear and the mechanisms involved have not been identified [105]. To date, there have been no studies investigating the autophagy-inflammasome relationship in periodontal disease.

2.7 Other Modulators

Modulation of inflammasome activation by a variety of microbes have been reported in the literature. For example, Myxoma virus protein M013 and Shope fibroma virus protein (SFV-gp013L) can inhibit inflammasome activation by binding ASC [108]. Some poxviruses also reduce inflammasomes by inhibiting caspase-1.

Effector and memory T cells, which target NLRP1 and NLRP3 inflammasomes, were involved in inhibiting caspase-1 and IL-1β in macrophages and dendritic cells via TNF ligands, including CD40L and RANKL, which are expressed on activated T cells [34]. In addition apoptosis regulators, Bcl-2 and Bcl-XL, also supress caspase-1 by binding NLRP1 [109].

Although these modulatory mechanism of inflammasomes have been proposed to play a potential role in periodontal disease, further studies are needed to elucidate the exact molecular mechanisms modulated.

3. CONCLUSION AND FUTURE PERSPECTIVES

In summary, periodontal disease may be modulated with the aid of inflammasome regulators, such as agents that target COP, POP, and TRIM family proteins, which are normally inhibited

during periodontal disease. Current protocols focus on neutralizing the circulating cytokines, however targeting the inflammasomes directly may diminish cytokine production, thereby offering a potential new therapeutic target in treating inflammasome-related diseases. This offers the possibility of modulating the excessive inflammatory response seen in periodontal disease by the use of inflammasome regulators, for example agents that target COP, POP, and TRIM family proteins, which are normally inhibited in the healthy periodontium.

In this review, a broad perspective has been provided relating to inflammasome-periodontal disease association by including studies focusing on several periodontal pathogens including *Pg, Fn, Aa* and *Td,* as well as on the possible modulatory mechanisms that may be involved. However, this review is focused and is limited to the studies which are primarily derived from single molecule analyses, subsequently a broader picture at all times needs to be considered. In addition, although inflammasomes appear to play a potential role in periodontal disease pathogenesis, corroboration of these findings to validate periodontal disease and inflammasome associations.

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Table 1. Studies in the literature evaluating inflammasomes and their regulation

Author and Year	Study Type	Activator Signal	Evaluated Cell	Groups	Evaluated Parameters	Results
Aral et al. 2019 [70]	Human	-	Human gingiva	CP, AgP and G patients and periodontally healthy participants	ASC, Caspase-1, IL-1β,IL-18, NLRP3, NLRP2, AIM2, POP1, POP2, CARD16, CARD18, TRIM16, TRIM20	NLRP3 and IL-1β were upregulated in the G, CP and AgP groups compared with group H. AIM2 was downregulated in the CP group compared with the H, G, and AgP groups. TRIM20, TRIM16 and CARD18 were downregulated in the G, CP and AgP groups compared with the H group. POP1 and POP2 were downregulated in the CP and AgP, and AgP and G groups, respectively.
Garcia-Hernandez et al. 2018 [110]	Human	-	Human gingiva and GCF	CP Patients with or without DM Periodontally and Systemically Healthy participants	CASP1, NLRP3, ASC, IL-1β, IL-18	CP patients with uncontrolled DM were expressed more NLRP3, ASC, Caspase-1 and IL-1β, IL-18 compared to CP and H groups.
Isaza-Guzman et al. 2017 [39]	Human	-	Human saliva	Saliva collected from AgP, CP and H participants	Caspase-1, IL-1β, ASC, NLRP3	NLRP3, ASC, and IL-1β were higher but caspase-1 levels were similar in CP and AgP groups compared to healthy participants. NLRP3 was higher in AgP than CP.
Xue et al 2015 [26]	Human	-	Human gingival tissue	Gingival tissues collected from CP, Agp and H participants.	NLRP3, NLRP1, AIM2	NLRP3 expression was higher in CP and AgP patients than periodontally healthy controls. AIM2 was expressed higher in CP group than group H. No differences were found for NLRP1 in groups.
Basic et al. 2017 [111]	In vitro	Hydrogen sulfide (H2S) for 24 h	human peripheral blood mononuclear cells THP1 monocytic cell	Cells were treated NaHS (1 mM) for 24 h	IL-1β, IL-18, NLRP3	PBMCs and THP1 treated by NaHS produced more IL-1ß and IL-18. THP1 cells deficient from ASC and NLRP3, did not produce more IL-1ß and IL-18 when exposed to NaHS than unexposed control cells. The NLRP3 inflammasome is essential for NaHS induced IL-1ß and IL-18 secretion in monocytes.

Belibasakis et al. 2012 [24]	In vitro	Aa for 3 h	Human Mononuclear Leukocytes	Infected by Aa (10 MOI)	NLRP1, NLRP2, NLRP3, NLRP6, AIM2	NLRP3 and IL-1β were upregulated, NLRP6 and ASC were downregulated but IL-18, Caspase1, NLRP1, NLRP2 and AIM2 were not effected by Aa.
Belibasakis et al. 2013 [42]	In vitro	Subgingival biofilm for 6 h with and without Pg	Human gingival fibroblasts	Subgingival biofilm model with or without Pg and control	NLRP3, AIM2, IL-1β, ASC, Caspase-1	NLRP3 and IL-1β were reduced without affected AIM2, ASC and Caspase-1 by subgingival biofilm including Pg. However, the lack of Pg prevented the downregulation of NLRP3 and IL-1β expression without influencing ASC, AIM2, ASC and Caspase-1.
Bostanci et al. 2011 [23]	In vitro	Supragingival biofilm for 6 h or Subgingival biofilm for 6 h	Human gingival fibroblasts	Supragingival biofilm (0,3,30,300 μg/ml) Subgingival biofilm (0,3,30,300 μg/ml)	Caspase-1, ASC, AIM2, IL-1β, IL-18, NLRP1, NLRP2, NLRP3	NLRP1 was similar, NLRP2 was not expressed. Caspase-1, ASC, AIM2, IL-1β and IL-18 were upregulated but NLRP3 was not influenced by supragingival biofilm. Caspase-1, ASC, AIM2, IL-1β and IL-18 were upregulated at lower but all these+NLRP3 downregulated at higher concentrations.
Bui et al. 2016 [60]	In vitro	Fn for 0, 2, 4, 6, 8 h	Human gingival epithelial cells	Infected by Fn (MOI of 25, 50, or 100)	Caspase-1, IL-1β, ASC, NLRP3	Fn promoted Caspase-1, IL-1β gene transcription and ASC at 25,50, 100 MOI for 8 h and at 100 MOI for 2,4,6,8 h. Knockdown of NLRP3 inhibited caspase-1 activation and IL-1β secretion at 100 MOI of Fn for 8 h.
Cecil et al. 2017 [61]	In vitro + in vivo	Outer membrane vesicles (OVMs) of Pg, Td, Tf for 6 h	THP1 cells Mice	Cells were treated with OVMs from Pg, Td, Tf 10, 50, 100 MOI or E.coli, Pg, Td, and Tf LPS (100 μg/mL) for 6 h.	TNFα, IL-8, IL-10, IL-1β, ASC	Pg, Td, and Tf OMVs activated inflammasomes in THP-1 monocytes and macrophages and induced TNFα, IL-8, and IL-1β cytokine secretion. (Pg also induced IL-10) Pg OMVs activated macrophage inflammasomes in vivo with 80% of macrophages exhibiting inflammasome complex formation. OMV-induced IL-1β secretion was dependent on the inflammasome component ASC but was only attenuated by the absence of Caspase 1.

Champaiboon et al. 2014 [27]	In vitro	1-Pg LPS or E.coli LPS for 24h 2-cholesterol crystals or ATP for 24h	primary human monocyte-derived macrophages & coronary artery endothelial cells (CAECs)	Macrophages infected with 5 μg/ml Pg LPS or E.coli LPS then CC (2000 μg/ml) or ATP (5nM) CAECs infected with 50 μg/ml Pg LPS or E.coli LPS then CC (500 to 2000 μg/ml) or ATP (5nM)	IL-1β, NLRP3, IL-8	Macrophages infected with both Pg and ATP or CC caused more IL-1B production compared to Pg alone. Pg infection promoted IL-8 release and NLRP3 expression in CAECs.
Cheng et al. 2017 [36]	In vitro + In vivo	Pg LPS or E.coli LPS up to 4 h	Human gingival fibroblasts Mice gingival fibroblasts	Gingival samples of CP and H participants and also mice gingiva with periodontitis Cells infected with (1 μg/mL) E.coli LPS under normal and hypoxic conditions	Caspase-1, IL-1β, NLRP3	NLRP3, caspase-1 and IL-1β expression upregulated in gingiva of CP patients. *Pg* LPS down-regulated NLRP3, pro-IL-1β and IL-1β. However, under moderate hypoxia (2% O_2), *P. gingivalis* LPS enhanced NLRP3 expression, cleaved-caspase-1, and mature IL-1β. *E.coli* LPS was able to promote mature IL-1β in both normoxic and hypoxic condition.
Fleetwood et al. 2017 [68]	In vitro	Outer membrane vesicles (OVMs) of Pg, for 2 h	Bone marrow macrophages	Infected Pg, Heat-killed Pg, OMVs or heat-inactivated OMVs at 10,25,100 MOI for 2 h.	TNFα, IL-6, IL-10, IL-1β, IFNβ, NO	OVMs promoted cytokine release more than Pg. Pg and heat-killed Pg induced cytokine release similarly. All treatments promoted NLRP3, pro-caspase-1, and pro-IL-1β but only OVMs cause the release of IL-1β.
Furusho et al. 2013 [65]	In vitro	Pg for 6 h	Human hepatocytes	Hepatocytes induced with or without Pg (1 μg/ml)	IL-1β, IL-6, IL-8, TNF-α, NLRP3, Casp1, TLR2, TLR4	Pg upregulated NLRP3 and Casp-1 mRNA, as well as IL-IL-1β, IL-6, IL-8 and TNF-α in steatotic hepatocytes. Pg from dental origin promoted liver disease progression through activation of inflammasomes and TLR pathways. Eliminating Pg may be significant on the management of liver diseases.
Guo et al. 2015 [40]	In vitro	Pg for 2 or 4 h ATP for 3 h	The epithelial cell line (H413)	H413 cells infected either with Pg for 2 or 4 h (100 MOI) or Pg LPS (1 μg/ml) With or without treated 5nM ATP for 3 h before Pg infections	NLRP3, ASC, Caspase-1, IL-1β, IL-18	Pg infection had a greater capacity to induce IL-1β secretion than Pg LPS but vice versa for IL-18. In the absence of ATP, infection with Pg did not activate NLRP3, ASC and caspase-1 until Pg LPS stimulation and or ATP.
Huck et al. 2015 [25]	In vitro	Pg for 6 h And Pg LPS for 24 h 5mM ATP for 2 h	Human umbilical vein ECs	Infected by Pg 200 MOI and stimulated with Pg LPS (1 μg/ml) for 24h and treated	NLRP1, NLRP3, IL-1β	Pg or Pg-LPS treated with or without ATP did not induce NLRP1 and IL-1β but increased NLRP3 expression. Pg did not induce IL-1β

				with or without 5nM ATP for 2 h		secretion, but Pg-LPS stimulation promoted IL-1β production potentiated by ATP.
Jun et al. 2012 [46]	In vitro	Td surface protein or LPS for 1 to 6 h. ATP for 30 min	THP-1 cells	Td surface protein for 1 to 6 h. Then incubated with 2.5 nM ATP for 30 min.	NLRP3, NF-κB	Td activated the NLRP3 inflammasome, ATP release and K+ efflux.
Kollgaard et al. 2017 [112]	In vitro	Pg LPS E.coli PLS TLR agonist Arg-gingipain 2- cholesterol crystal for 20 h	Peripheral blood mononuclear cells	Infected by (10.0 μg/mL) Pg LPS (0.01 or 1.0 μg/mL) E.Coli LPS or (0.1 or 1.0 μg/mL) or TLR2 or (210 nM) Arg-gingipain agonist with or without 2 mg/mL CHC for 20 h	IL-1β, IL-6, IL-10, and TNF-α	CHCs enhanced IL-1β secretion induced by Pg LPS and IL-1β secretion induced by Pg. This enhancement was abrogated by the CHCs, via stimulation of NLRP3 inflammasomes, acted in synergy with Pg to promote monocyte secretion of pro-atherogenic cytokines.
Okinaga et al. 2015 [45]	In vitro	Aa for up to 9 h	murine macrophage-like cell line (RAW264) Bone marrow cells THP-1 cells U937 cells	Infected with Aa (50 MOI) for up to 9 h	NLRP3, IL-1β	Pro-IL-1β and mature IL-1β and NLRP3 expression and IL-1β release were promoted by Aa in cells. Aa induced IL-1β secretion independent of caspase-1 and NLRP3 activation in RAW 264 cells.
Shenker et al. 2015 [44]	In vitro	Aa cytolethal distending toxin for 2 to 4 h	THP-1 cell lines	Cells infected with (0 to 500 ng/ml) for 2 to 4 h	NLRP3, IL-1β, IL-18, caspase-1, ASC.	Aa Cdt caused activation and production and the release of caspase-1 and also IL-1β and IL-18 release in THP-1 cells. NLRP3 and ASC were required for Cdt-induced caspase-1 activation and cytokine release.
Yamaguchi et al. 2017 [67]	In vitro + In vivo	Pg for 3 w orally Pg infection for 16h	Peritoneal macrophage cells	NLRP3 knockout mice injected challenged with Pg five times a week for 3 w Peritoneal macrophage cells challenged with Pg 10 MOI for 16 h	Caspase-1, IL-1β, IL-18, IL-6, NLRP3 pro-IL-1β, pro-IL-18, RANKL, OPG	Pg did not affect bone loss in NLRP3-KO mice. Pg activated immune response by the NLRP3 inflammasome.
Yilmaz et al. 2010 [22]	In vitro	Pg or *E.coli* LPS for 6 h and/or ATP for 3 h	Human primary gingival epithelial cells	Infected with Pg 100 MOI for 5 hr or infected with E.coli LPS (2 μg/ml for 6 h with or without 5mM ATP for 3 h	NLRP1,NLRC4, NLRP3, IL-1β	Pg induced expression and the accumulation of IL-1β. However, not secreted in lack of ATP.
Yoshida et al. 2017 [41]	In vitro	SNAP-Pg for 0-24 h	The mouse osteoblastic cell line MC3T3-E1	Infected with or without SNAP Pg (100 MOI) for 0-24 h	NLRP3, NF-κB	NLRP3 increased and reached maximum expression 6 h post infection. The level of NLRP3 protein was increased by SNAP-Pg infection from 6 to 12 h. SNAP-Pg increased

						NLRP3 expression through the NF-κB signalling pathway.
Zhao et al. 2014 [43]	In vitro	Aa for 2 h	Human osteoblastic MG63 cells	MG63 cells infected by Aa of 500, 1000 and 2500 MOI	NLRP3, IL-1β, IL-18, ASC, Caspase1	Aa upregulated NLPR3, ASC and Caspase-1 expression, and the production of IL-1β and IL-18.
Kim et al 2016 [38]	In vitro+ Human	Aa for 3,6,24 h	THP-1 cells (human monocytic cell line) Human gingiva	Infected by Aa for 10, 50, 100 MOI and then pre-treated with or without xylitol (3%) for 30min	IL-1β, AIM2, NLRP3, and ASC	Aa promoted IL-1β production in THP-1 cells with a MOI dependent manner. Gingival AIM2, NLRP3, and ASC expression were higher in AgP patients than healthy controls. AIM2 and ASC protein production were increased by Aa. Aa induced IL-1β production by activation of the AIM2 inflammasome. Xylitol inhibited ASC and AIM2 proteins and formation of ASC puncta.
Kuo et al. 2016 [113]	In vitro+ Human	High glucose or Normal glucose treated Pg High glucose or Normal glucose treated Fn for 6 and 12 h 2-Fimbria of Pg	Human gingival fibroblasts	Gingival biopsies from CP with or without Type II DM Infected with Hg-Pg or Ng-Pg for 6 h and 12 h (5,20,50 MOI)	NLRP3, IL-1β, pro-IL-1β, caspase-1, pro-caspase-1	Hg-Pg and Hg-Fn caused upregulation and expression of IL-1β. HGF infection with HGPg induced MOI-dependent NLRP3 gene expression. HGPg infection not only induced NLRP3 upregulation, also enhanced active caspase-1 and mature IL-1β expression in HGFs. NLRP3 inflammasome mediated IL-1β production upon HGPg infection.
Park et al. 2014 [37]	In vitro+ Human	Pg for 6 or 24 h	THP-1 cell line Human GCF and gingival tissue	Infected with Pg for 10,50,100 MOI for 6 or 24 h.	NLRC4, NLRP3, AIM2, ASC, pro-caspase-1, pro-IL-1β, TNF-α, IL-1β,	NLRP3, AIM2, caspase-1 were higher in gingiva of CP patients. IL-1β and TNF-α were also higher in GCF of CP patients. Pg activated caspase-1 and IL-1β expression. Pg increased the expression of NLRP3 and AIM2 but did not affect the expression of ASC and NLRC4. KO of ASC, NLRP3, AIM2 inhibited IL-1β secretion through inhibiting caspase-1 activation without affecting pro-caspase-1 expression.
Bi et al. 2018 [114]	In vivo	-	Human gingival epithelial cells treated with or	Mice with or without β6 integrin-KO mice	Bone loss, NLRP3, AIM2, Caspase-1, IL-1β, IL-6, IL-	ITGβ6-KO mice with experimental periodontitis showed more bone loss. AIM2 inflammasome, CCL5 and IL-10 was downregulated

			without ITGβ6 KO		10, IL-18, IL-17ra, CCL2, CCL3,CCL5, RANKL, OPG	but OPG was significantly upregulated in ITGβ6-KO mice compared to wild type ones. NLRP3 upregulated but AIM2 and Caspase-1 downregulated in gingival epithelial cells with ITGβ6 KO.
Yamaguchi et al. 2015 [66]	In vivo	Pg wild type or Gingipain-null mutant FimA-deficient mutant for 3 w	peritoneal macrophages, and gingival or aorta	Mice fed up with Pg or Gingipain-null mutant of FimA-deficient mutant 5 times a week for 3 weeks	NLRP3, pro IL-1β, pro IL-18, pro caspase-1	Pg increased gingival or aortic gene expression NLRP3, pro-IL-1β, pro-IL-18 and pro-caspase-1. The NLRP3 inflammasome may have a major role in periodontal disease and atherosclerosis induced by Pg through sustained inflammation.
Yoneda et al. 2013 [115]	In vivo	-	Rat gingiva and Serum	Rats with normal and older age treated with or without coQ10	NLRP3, Caspase-1, ASC, NF-κB, IL-1β	Coq10 decreased the NLRP3, Caspase-1, ASC, NF-KB, IL-1β expression in gingiva. Antioxidative effects of CoQ10 may prevent inflammatory reactions by inactivated NLRP3 inflammasome.
Bostanci et al. 2009 [30]	Invitro+ Human	Pg for 6 h	Human monocytic cell line (Mono-mac-6)	Gingival Biopsies from CP, AgP, G and H participants Pg (0, 0.78, 1.5, 3.2, 6.4 μg/ml)	NLRP2, NLRP3, AIM2, IL-1β, IL-18	Gingival mRNA levels of NLRP3 and NLRP2 found higher in all disease groups compared to healthy group. ASC found similar between groups. NLRP3, IL-1β and IL-18 were positively correlated in diseased tissues. Pg upregulated NLRP3 but downregulated NLRP2 and ASC.
Guo et al. 2014 [27]	Invitro+ Human	Pg for 3 h	Human gingival Epithelium (H413)	Gingival Biopsies from CP and H participants H413 cells infected Pg (100 MOI)	NLRP3, ASC, NLRP2, IL-1β, IL-18, NOD1, NLRP1	NLRP3 expression was detected in oral epithelium strongly, in sulcular and junctional epithelium moderately but in pocket epithelium weekly in both healthy and CP tissues. NLRP3 in sulcular and junctional epithelium was significantly higher in CP patients than H patients. Infection with Pg caused an upregulation in NLRP3 and NLRP1 but a downregulation in IL-18. No differences were found for 1β, IL-18, NLRP2, NOD1, ASC.

Taxman et al. 2012 [116]	Invitro+ Human	Pg for 18-22 h	THP1 monocytic cells	Gingival Biopsies from CP and H participants Pg (10 MOI) ShASC1 (>%90), ShASC2(>%70), ShControl	ASC, COX2, PGE2, RIPK2	ASC regulates *COX-2* expression and PGE2 production in THP1 monocytic cells infected by *Pg*. Production of PGE2 was IL-1β-independent and did not require the inflammasome adaptor function of ASC, but was dependent on MAPK activation. A potential role for ASC in modulating RIPK2 expression both in vitro and in vivo.
Velsko et al. 2015 [62]	In vitro + In vivo	Pg, Fn, Td and Tf for 24 weeks	Integrin β6 knockout (Itgβ6−/−) mice and Wild type (WT) mice	Aorta and gingival biopsies from mice (Itgβ6−/−)	NLRP3, NLRC4, ASC	significant upregulation of NLRP3, NLRC4 and ASC expression in aorta of Integrin-β6 knockout (Itgβ6−/−) mice
Yiguchi et al. 2019 [63]	Invitro+ Human	Silica crystals	Chronic periodontitis patients	Peripheral blood samples before and after initial periodontal treatment	NLRP3, ASC, IL-1β, caspase-1	Initial periodontal treatment decreased IL-1β and ASC expression without influencing NLRP3 and caspase-1 levels in PBMCs. Silica crystals triggered IL-1β release decreased in low BOP% group but increased in high BOP% group after initial periodontal treatment.
Brown et al. 2015 [64]	In vivo+ In vitro	Pg	Western diet fed low density lipoprotein receptor knockout (Ldlr°) mice	Macrophages of (Ldlr°) mice	NLRP3	Pg dysregulated the NLRP3 inflammasome in Ldlr° mice

FIGURE LEGENDS

Figure 1: Inflammasome and its effects mechanism. The different stimulatory molecules are shown externally to the cell and the many different intracellular signalling cascades activated by them are shown which result in inflammasome induction. For abbreviation definitions, see the main text body.