UNIVERSITY^{OF} BIRMINGHAM University of Birmingham Research at Birmingham

Inflammasomes and their regulation in periodontal disease

Aral, Kübra; Milward, Michael; Kapila, Yvonne; Berdeli, Afig; Cooper, Paul

DOI: 10.1111/jre.12733

License: Other (please specify with Rights Statement)

Document Version Peer reviewed version

Citation for published version (Harvard):

Aral, K, Milward, M, Kapila, Y, Berdeli, A & Cooper, P 2020, 'Inflammasomes and their regulation in periodontal disease: a review', *Journal of Periodontal Research*, vol. 55, no. 4, pp. 473-487. https://doi.org/10.1111/jre.12733

Link to publication on Research at Birmingham portal

Publisher Rights Statement:

This is the peer reviewed version of the following article: Aral, K, Milward, MR, Kapila, Y, Berdeli, A, Cooper, PR. Inflammasomes and their regulation in periodontal disease: A review. J Periodont Res. 2020; 00: 1–15., which has been published in final form at: https://doi.org/10.1111/jre.12733. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.

•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.

•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?) •Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

INFLAMMASOMES AND THEIR REGULATION IN PERIODONTAL DISEASE: A REVIEW

Kübra ARAL¹, Michael R MILWARD², Yvonne KAPILA³, Afig BERDELI⁴, Paul R COOPER^{5,6}

¹ Postdoctoral Researcher, Oral Biology, School of Dentistry, University of Birmingham, Birmingham, UK

² Periodontology, School of Dentistry, University of Birmingham, Birmingham, UK

³ Orofacial Sciences, The School of Dentistry, University of California San Francisco, CA, USA

⁴ Molecular Genetics, Faculty of Medicine, Ege University, Izmir, Turkey

⁵Oral Biology, School of Dentistry, University of Birmingham, Birmingham, UK

⁶Department of Oral Sciences, Faculty of Dentistry, University of Otago, PO Box 56,

Dunedin 9054, New Zealand

Corresponding Author: Dr. Kübra Aral, DDS, PhD, Oral Biology, School of Dentistry, University of Birmingham, 5 Mill Pool Way, B5 7EG, Birmingham, United Kingdom, Tel: +44 0121 466 5517, Fax number: +44 0121 625 8815, e-mail: <u>drkubraaral@gmail.com</u>, <u>k.aral@bham.ac.uk</u>

Running Title: Inflammasomes and their regulation in Periodontal Disease

Summary Sentence: Inflammasome regulators may be dysregulated in periodontal disease.

ABSTRACT

Interleukin-1ß (IL-1ß) 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-1ß 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-1 β) ¹, which is produced by various immune and tissue resident cells, including macrophages, oral fibroblasts, oral epithelial cells and osteoblasts. The release of IL-1 β is a major step in the immune response due to its ability to induce the expression of a range of other inflammatory cytokines ¹².

1.1 IL-1β and Periodontal Disease

During the pathogenesis of periodontal disease, IL-1 β activates endothelial cells and enables the adhesion of eosinophils, thereby increasing the inflammatory response. IL-1 β also regulates the destruction of the alveolar bone by promoting osteoclast formation and activity ³. Clinical studies show that IL-1 β levels are higher in the gingival crevicular fluid (GCF) of periodontitis patients compared with controls ⁴, and there is also a correlation between IL-1 β gingival tissue levels and severity of periodontal disease ⁵. The monitoring of IL1 β 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-1 β levels in GCF have reported conflicting outcomes in patients. Some studies have reported a decrease in IL-1 β levels after periodontal treatment for up to 6 months ⁷⁻¹⁰, while other studies found changes in IL-1 β levels only at 3 months ^{11,12}, and some studies found no change in IL-1 β levels ^{6,13,14}. Thus, there is only some evidence suggesting that periodontal tissue breakdown

may be controlled by regulating IL-1 β expression. In contrast, 1 month after non-surgical periodontal treatment, IL-1 β 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-1 β 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 activation²¹. 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- κ B 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-1 β 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-1 β by associating with ASC ⁴⁸. It also negatively regulates the inflammatory response by suppressing NF- κ B 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-1 β release or in NF- κ B 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-1 β 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 sarcomaassociated 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-1 β 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-1 β 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- κ B 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 Integrinβ6 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 ASC⁶³. 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-1 β 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-1 β 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-1 β 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- κ B 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- κ B 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-1 β 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-1 β 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-1 β by activating RIP-2⁷⁸. Overexpression of CARD16 can mediate NF- κ B induction and enhance TNF- α -induced NF- κ B activation through an IKK-dependent mechanism⁷⁹.

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- κ B activation, but compared with CARD16, it significantly reduces the expression of IL-1 β 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- κ B pathway. However it can inhibit IL-1 β expression by competing with RIP-2 to prevent the oligomerization of RIP-2 and caspase-1⁸¹.

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- κ B activation, which is induced by NOD2 and IL-8 release. NOD2-S downregulates IL-1 β release by downregulating NOD2 and its adaptor molecule called RIP2⁸². 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-1 β 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-1 β 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-1 β production ⁸⁴ and is alsoassociated with familial Mediterranean fever. TRIM20 also regulate autophagy ⁸⁴.

TRIM16 is a novel pro-IL-1 β binding protein in macrophages. TRIM16 enhances IL-1 β production by interacting with pro-caspase-1 and NLRP1 to enhance innate immunity ³⁴. TRIM16 is required for the optimal secretion of IL-1 β that is triggered by lysosomal damage. It is also involved in autophagy and it has an ability to protect cells from oxidative stressinduced cytotoxicity ⁸⁵. TRIM16 is highly expressed in keratinocytes and its knockdown results in a reduction in IL-1 β secretion. However keratinocytes also express other inflammasome proteins that are responsible for pro-IL-1 β 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-1 β 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 inflammasome⁸⁹ 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 antiinflammatory 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 IFN α 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-1 β 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-1 β 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-1 β , 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-1 β 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.

ACKNOWLEDGEMENTS

This study was supported by the School of Dentistry, University of Birmingham. The corresponding author of this study (K. Aral) was supported with a scholarship by the Scientific and Technological Research Council of Turkey (TÜBİTAK), Ankara, Turkey. The authors report no conflicts of interest related to this study.

REFERENCES

- 1. Kinane DF, Lappin DF. Immune processes in periodontal disease: a review. *Ann Periodontol.* 2002;7:62-71.
- 2. Song B, Zhou T, Yang WL, Liu J, Shao LQ. Programmed cell death in periodontitis: recent advances and future perspectives. *Oral Dis.* 2017;23:609-619.
- 3. Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol*. 2003;74:391-401.
- Figueredo CMS, Ribeiro MSM, Fischer RG, Gustafsson A. Increased Interleukin-1beta Concentration in Gingival Crevicular Fluid as a Characteristic of Periodontitis. J Periodontol. 1999;70:1457-1463.
- Hou LT, Liu CM, Liu BY, Lin SJ, Liao CS, Rossomando EF. Interleukin-1beta, clinical parameters and matched cellular-histopathologic changes of biopsied gingival tissue from periodontitis patients. *J Periodontal Res.* 2003;38:247-254.
- Yoshinari N, Kawase H, Mitani A, et al. Effects of scaling and root planing on the amounts of interleukin-1 and interleukin-1 receptor antagonist and the mRNA expression of interleukin-1beta in gingival crevicular fluid and gingival tissues. J Periodontal Res. 2004;39:158-167.
- 7. Biyikoglu B, Buduneli N, Aksu K, et al. Periodontal therapy in chronic periodontitis lowers gingival crevicular fluid interleukin-1beta and DAS28 in rheumatoid arthritis patients. *Rheumatol Int.* 2013;33:2607-2616.
- Goutoudi P, Diza E, Arvanitidou M. Effect of periodontal therapy on crevicular fluid interleukin-1beta and interleukin-10 levels in chronic periodontitis. *J Dent*. 2004;32:511-520.
- Thunell DH, Tymkiw KD, Johnson GK, et al. A multiplex immunoassay demonstrates reductions in gingival crevicular fluid cytokines following initial periodontal therapy. *J Periodontal Res.* 2010;45:148-152.
- Del Peloso Ribeiro E, Bittencourt S, Sallum EA, Nociti FH, Jr., Goncalves RB, Casati MZ. Periodontal debridement as a therapeutic approach for severe chronic

periodontitis: a clinical, microbiological and immunological study. *J Clin Periodontol*. 2008;35:789-798.

- Aral K, Aral CA, Kapila Y. Six-month clinical outcomes of non-surgical periodontal treatment with antibiotics on apoptosis markers in aggressive periodontitis. *Oral Dis.* 2019.
- 12. Al-Shammari KF, Giannobile WV, Aldredge WA, et al. Effect of non-surgical periodontal therapy on C-telopeptide pyridinoline cross-links (ICTP) and interleukin-1 levels. *J Periodontol.* 2001;72:1045-1051.
- Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Levels of interleukin-1 beta, -8, and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *J Periodontol*. 2000;71:1535-1545.
- Rosalem W, Rescala B, Teles RP, Fischer RG, Gustafsson A, Figueredo CM. Effect of non-surgical treatment on chronic and aggressive periodontitis: clinical, immunologic, and microbiologic findings. *J Periodontol.* 2011;82:979-989.
- Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140:805-820.
- 16. Bauernfeind F, Ablasser A, Bartok E, et al. Inflammasomes: current understanding and open questions. *Cell Mol Life Sci.* 2011;68:765-783.
- Lamkanfi M, Dixit VM. In Retrospect: The inflammasome turns 15. Nature. 2017;548:534-535.
- 18. Schroder K, Tschopp J. The inflammasomes. *Cell*. 2010;140:821-832.
- Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*. 2002;10:417-426.
- 20. Indramohan M, Stehlik C, Dorfleutner A. COPs and POPs Patrol Inflammasome Activation. *J Mol Biol.* 2018;430:153-173.

- 21. Boyden ED, Dietrich WF. Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat Genet.* 2006;38:240-244.
- Yilmaz O, Sater AA, Yao L, Koutouzis T, Pettengill M, Ojcius DM. ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by Porphyromonas gingivalis. *Cell Microbiol.* 2010;12:188-198.
- Bostanci N, Meier A, Guggenheim B, Belibasakis GN. Regulation of NLRP3 and AIM2 inflammasome gene expression levels in gingival fibroblasts by oral biofilms. *Cell Immunol.* 2011;270:88-93.
- Belibasakis GN, Johansson A. Aggregatibacter actinomycetemcomitans targets NLRP3 and NLRP6 inflammasome expression in human mononuclear leukocytes. *Cytokine*. 2012;59:124-130.
- Huck O, Elkaim R, Davideau JL, Tenenbaum H. Porphyromonas gingivalis-impaired innate immune response via NLRP3 proteolysis in endothelial cells. *Innate Immun*. 2015;21:65-72.
- Xue F, Shu R, Xie Y. The expression of NLRP3, NLRP1 and AIM2 in the gingival tissue of periodontitis patients: RT-PCR study and immunohistochemistry. *Arch Oral Biol.* 2015;60:948-958.
- 27. Champaiboon C, Poolgesorn M, Wisitrasameewong W, Sa-Ard-Iam N, Rerkyen P, Mahanonda R. Differential inflammasome activation by Porphyromonas gingivalis and cholesterol crystals in human macrophages and coronary artery endothelial cells. *Atherosclerosis.* 2014;235:38-44.
- 28. Lich JD, Ting JP. Monarch-1/PYPAF7 and other CATERPILLER (CLR, NOD, NLR) proteins with negative regulatory functions. *Microbes Infect*. 2007;9:672-676.
- 29. Minkiewicz J, de Rivero Vaccari JP, Keane RW. Human astrocytes express a novel NLRP2 inflammasome. *Glia.* 2013;61:1113-1121.
- 30. Bostanci N, Emingil G, Saygan B, et al. Expression and regulation of the NALP3 inflammasome complex in periodontal diseases. *Clin Exp Immunol.* 2009;157:415-422.

- 31. Kanneganti TD, Ozoren N, Body-Malapel M, et al. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature*. 2006;440:233-236.
- 32. Duncan JA, Gao X, Huang MT, et al. Neisseria gonorrhoeae activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J Immunol.* 2009;182:6460-6469.
- 33. Shibata K. Historical aspects of studies on roles of the inflammasome in the pathogenesis of periodontal diseases. *Mol Oral Microbiol.* 2018;33:203-211.
- Rathinam VA, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. *Nat Immunol.* 2012;13:333-342.
- 35. Jin C, Flavell RA. Molecular mechanism of NLRP3 inflammasome activation. *J Clin Immunol.* 2010;30:628-631.
- Cheng R, Liu W, Zhang R, Feng Y, Bhowmick NA, Hu T. Porphyromonas gingivalis-Derived Lipopolysaccharide Combines Hypoxia to Induce Caspase-1 Activation in Periodontitis. *Front Cell Infect Microbiol.* 2017;7:474.
- Park E, Na HS, Song YR, Shin SY, Kim YM, Chung J. Activation of NLRP3 and AIM2 inflammasomes by Porphyromonas gingivalis infection. *Infect Immun.* 2014;82:112-123.
- Kim S, Park MH, Song YR, Na HS, Chung J. Aggregatibacter actinomycetemcomitans-Induced AIM2 Inflammasome Activation Is Suppressed by Xylitol in Differentiated THP-1 Macrophages. *J Periodontol.* 2016;87:e116-126.
- Isaza-Guzman DM, Medina-Piedrahita VM, Gutierrez-Henao C, Tobon-Arroyave SI. Salivary Levels of NLRP3 Inflammasome-Related Proteins as Potential Biomarkers of Periodontal Clinical Status. *J Periodontol.* 2017;88:1329-1338.
- Guo W, Wang P, Liu Z, Yang P, Ye P. The activation of pyrin domain-containing-3 inflammasome depends on lipopolysaccharide from Porphyromonas gingivalis and extracellular adenosine triphosphate in cultured oral epithelial cells. *BMC Oral Health*. 2015;15:133.

- Yoshida K, Okamura H, Hiroshima Y, et al. PKR induces the expression of NLRP3 by regulating the NF-kappaB pathway in Porphyromonas gingivalis-infected osteoblasts. *Exp Cell Res.* 2017;354:57-64.
- 42. Belibasakis GN, Guggenheim B, Bostanci N. Down-regulation of NLRP3 inflammasome in gingival fibroblasts by subgingival biofilms: involvement of Porphyromonas gingivalis. *Innate Immun.* 2013;19:3-9.
- Zhao P, Liu J, Pan C, Pan Y. NLRP3 inflammasome is required for apoptosis of Aggregatibacter actinomycetemcomitans-infected human osteoblastic MG63 cells. *Acta Histochem.* 2014;116:1119-1124.
- 44. Shenker BJ, Ojcius DM, Walker LP, Zekavat A, Scuron MD, Boesze-Battaglia K. Aggregatibacter actinomycetemcomitans cytolethal distending toxin activates the NLRP3 inflammasome in human macrophages, leading to the release of proinflammatory cytokines. *Infect Immun.* 2015;83:1487-1496.
- 45. Okinaga T, Ariyoshi W, Nishihara T. Aggregatibacter actinomycetemcomitans Invasion Induces Interleukin-1beta Production Through Reactive Oxygen Species and Cathepsin B. J Interferon Cytokine Res. 2015;35:431-440.
- Jun HK, Lee SH, Lee HR, Choi BK. Integrin alpha5beta1 activates the NLRP3 inflammasome by direct interaction with a bacterial surface protein. *Immunity*. 2012;36:755-768.
- 47. Wang P, Zhu S, Yang L, et al. Nlrp6 regulates intestinal antiviral innate immunity. *Science*. 2015;350:826-830.
- 48. Wang L, Manji GA, Grenier JM, et al. PYPAF7, a novel PYRIN-containing Apaf1-like protein that regulates activation of NF-kappa B and caspase-1-dependent cytokine processing. *J Biol Chem.* 2002;277:29874-29880.
- Radian AD, Khare S, Chu LH, Dorfleutner A, Stehlik C. ATP binding by NLRP7 is required for inflammasome activation in response to bacterial lipopeptides. *Mol Immunol.* 2015;67:294-302.

- 50. Williams KL, Lich JD, Duncan JA, et al. The CATERPILLER protein monarch-1 is an antagonist of toll-like receptor-, tumor necrosis factor alpha-, and Mycobacterium tuberculosis-induced pro-inflammatory signals. *J Biol Chem.* 2005;280:39914-39924.
- 51. Gong YN, Shao F. Sensing bacterial infections by NAIP receptors in NLRC4 inflammasome activation. *Protein Cell*. 2012;3:98-105.
- 52. Miao EA, Mao DP, Yudkovsky N, et al. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci U S A*. 2010;107:3076-3080.
- 53. Davis BK, Roberts RA, Huang MT, et al. Cutting edge: NLRC5-dependent activation of the inflammasome. *J Immunol.* 2011;186:1333-1337.
- 54. Wang B, Yin Q. AIM2 inflammasome activation and regulation: A structural perspective. *J Struct Biol.* 2017;200:279-282.
- 55. Unterholzner L, Keating SE, Baran M, et al. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 2010;11:997-1004.
- 56. Kerur N, Veettil MV, Sharma-Walia N, et al. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. *Cell Host Microbe*. 2011;9:363-375.
- 57. Veeranki S, Duan X, Panchanathan R, Liu H, Choubey D. IFI16 protein mediates the anti-inflammatory actions of the type-I interferons through suppression of activation of caspase-1 by inflammasomes. *PLoS One*. 2011;6:e27040.
- 58. Marchesan JT, Jiao Y, Moss K, et al. Common Polymorphisms in IFI16 and AIM2 Genes Are Associated With Periodontal Disease. *J Periodontol.* 2017;88:663-672.
- Lv K, Wang G, Shen C, Zhang X, Yao H. Role and mechanism of the nod-like receptor family pyrin domain-containing 3 inflammasome in oral disease. *Arch Oral Biol.* 2019;97:1-11.
- 60. Bui FQ, Johnson L, Roberts J, et al. Fusobacterium nucleatum infection of gingival epithelial cells leads to NLRP3 inflammasome-dependent secretion of IL-1beta and the danger signals ASC and HMGB1. *Cell Microbiol.* 2016;18:970-981.

- Cecil JD, O'Brien-Simpson NM, Lenzo JC, et al. Outer Membrane Vesicles Prime and Activate Macrophage Inflammasomes and Cytokine Secretion In Vitro and In Vivo. *Front Immunol.* 2017;8:1017.
- 62. Velsko IM, Chukkapalli SS, Rivera-Kweh MF, et al. Periodontal pathogens invade gingiva and aortic adventitia and elicit inflammasome activation in alphavbeta6 integrin-deficient mice. *Infect Immun.* 2015;83:4582-4593.
- 63. Higuchi K Ziamuddin SM, Yamashita Y, Ozaki Y, Yoshimura A. Initial periodontal treatment affects nucleotide-binding domain leucine-rich repeat-containing protein 3 inflammasome priming in peripheral blood mononuclear cells. *Archives of Oral Biology*. 2019;110.
- 64. Brown PM, Kennedy DJ, Morton RE, Febbraio M. CD36/SR-B2-TLR2 Dependent Pathways Enhance Porphyromonas gingivalis Mediated Atherosclerosis in the Ldlr KO Mouse Model. *PLoS One*. 2015;10:e0125126.
- Furusho H, Miyauchi M, Hyogo H, et al. Dental infection of Porphyromonas gingivalis exacerbates high fat diet-induced steatohepatitis in mice. *J Gastroenterol*. 2013;48:1259-1270.
- Yamaguchi Y, Kurita-Ochiai T, Kobayashi R, Suzuki T, Ando T. Activation of the NLRP3 inflammasome in Porphyromonas gingivalis-accelerated atherosclerosis. *Pathog Dis.* 2015;73.
- Yamaguchi Y, Kurita-Ochiai T, Kobayashi R, Suzuki T, Ando T. Regulation of the NLRP3 inflammasome in Porphyromonas gingivalis-accelerated periodontal disease. *Inflamm Res.* 2017;66:59-65.
- 68. Fleetwood AJ, Lee MKS, Singleton W, et al. Metabolic Remodeling, Inflammasome Activation, and Pyroptosis in Macrophages Stimulated by Porphyromonas gingivalis and Its Outer Membrane Vesicles. *Front Cell Infect Microbiol.* 2017;7:351.
- 69. Ting JP, Kastner DL, Hoffman HM. CATERPILLERs, pyrin and hereditary immunological disorders. *Nat Rev Immunol.* 2006;6:183-195.

- Aral K BE, Cooper PR, Milward MR, Kapila Y, Berdeli A, Aral CA, Karadede B. Differential expression of inflammasome regulatory transcipts in periodontal disease *Journal of Periodontology*. 2019.
- Le HT, Harton JA. Pyrin- and CARD-only Proteins as Regulators of NLR Functions. Front Immunol. 2013;4:275.
- 72. Stehlik C, Krajewska M, Welsh K, Krajewski S, Godzik A, Reed JC. The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclearfactor-kappa B and pro-caspase-1 regulation. *Biochem J.* 2003;373:101-113.
- 73. Atianand MK, Harton JA. Uncoupling of Pyrin-only protein 2 (POP2)-mediated dual regulation of NF-kappaB and the inflammasome. *J Biol Chem.* 2011;286:40536-40547.
- 74. Bedoya F, Sandler LL, Harton JA. Pyrin-only protein 2 modulates NF-kappaB and disrupts ASC:CLR interactions. *J Immunol.* 2007;178:3837-3845.
- 75. Dorfleutner A, Bryan NB, Talbott SJ, et al. Cellular pyrin domain-only protein 2 is a candidate regulator of inflammasome activation. *Infect Immun.* 2007;75:1484-1492.
- 76. Atianand MK, Fuchs T, Harton JA. Recent evolution of the NF-kappaB and inflammasome regulating protein POP2 in primates. *BMC Evol Biol.* 2011;11:56.
- 77. Kersse K, Vanden Berghe T, Lamkanfi M, Vandenabeele P. A phylogenetic and functional overview of inflammatory caspases and caspase-1-related CARD-only proteins. *Biochem Soc Trans.* 2007;35:1508-1511.
- 78. Lee SH, Stehlik C, Reed JC. Cop, a caspase recruitment domain-containing protein and inhibitor of caspase-1 activation processing. *J Biol Chem.* 2001;276:34495-34500.
- 79. Druilhe A, Srinivasula SM, Razmara M, Ahmad M, Alnemri ES. Regulation of IL-1beta generation by Pseudo-ICE and ICEBERG, two dominant negative caspase recruitment domain proteins. *Cell Death Differ*. 2001;8:649-657.
- Lamkanfi M, Denecker G, Kalai M, et al. INCA, a novel human caspase recruitment domain protein that inhibits interleukin-1beta generation. J Biol Chem. 2004;279:51729-51738.

- 81. Humke EW, Shriver SK, Starovasnik MA, Fairbrother WJ, Dixit VM. ICEBERG: a novel inhibitor of interleukin-1beta generation. *Cell*. 2000;103:99-111.
- Rosenstiel P, Huse K, Till A, et al. A short isoform of NOD2/CARD15, NOD2-S, is an endogenous inhibitor of NOD2/receptor-interacting protein kinase 2-induced signaling pathways. *Proc Natl Acad Sci U S A*. 2006;103:3280-3285.
- 83. Ozato K, Shin DM, Chang TH, Morse HC, 3rd. TRIM family proteins and their emerging roles in innate immunity. *Nat Rev Immunol.* 2008;8:849-860.
- 84. Kimura T, Jain A, Choi SW, Mandell MA, Johansen T, Deretic V. TRIM-directed selective autophagy regulates immune activation. *Autophagy*. 2017;13:989-990.
- Jena KK, Kolapalli SP, Mehto S, et al. TRIM16 controls assembly and degradation of protein aggregates by modulating the p62-NRF2 axis and autophagy. *EMBO J*. 2018;37.
- 86. Munding C, Keller M, Niklaus G, et al. The estrogen-responsive B box protein: a novel enhancer of interleukin-1beta secretion. *Cell Death Differ*. 2006;13:1938-1949.
- Hu Y, Mao K, Zeng Y, et al. Tripartite-motif protein 30 negatively regulates NLRP3 inflammasome activation by modulating reactive oxygen species production. J Immunol. 2010;185:7699-7705.
- 88. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol.* 2015;15:87-103.
- 89. Fernandes-Alnemri T, Yu JW, Juliana C, et al. The AIM2 inflammasome is critical for innate immunity to Francisella tularensis. *Nat Immunol.* 2010;11:385-393.
- 90. Guarda G, Braun M, Staehli F, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity*. 2011;34:213-223.
- Wright HJ, Matthews JB, Chapple IL, Ling-Mountford N, Cooper PR. Periodontitis associates with a type 1 IFN signature in peripheral blood neutrophils. *J Immunol*. 2008;181:5775-5784.

- 92. Mizraji G, Nassar M, Segev H, et al. Porphyromonas gingivalis Promotes Unrestrained Type I Interferon Production by Dysregulating TAM Signaling via MYD88 Degradation. *Cell Rep.* 2017;18:419-431.
- 93. Kajita K, Honda T, Amanuma R, et al. Quantitative messenger RNA expression of Tolllike receptors and interferon-alpha1 in gingivitis and periodontitis. *Oral Microbiol Immunol.* 2007;22:398-402.
- 94. Lee SH, Kim JS, Jun HK, Lee HR, Lee D, Choi BK. The major outer membrane protein of a periodontopathogen induces IFN-beta and IFN-stimulated genes in monocytes via lipid raft and TANK-binding kinase 1/IFN regulatory factor-3. *J Immunol*. 2009;182:5823-5835.
- Zaric SS, Coulter WA, Shelburne CE, et al. Altered Toll-like receptor 2-mediated endotoxin tolerance is related to diminished interferon beta production. *J Biol Chem.* 2011;286:29492-29500.
- 96. Mayer-Barber KD, Andrade BB, Barber DL, et al. Innate and adaptive interferons suppress IL-1alpha and IL-1beta production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. *Immunity*. 2011;35:1023-1034.
- 97. Dutzan N, Vernal R, Hernandez M, et al. Levels of interferon-gamma and transcription factor T-bet in progressive periodontal lesions in patients with chronic periodontitis. J Periodontol. 2009;80:290-296.
- 98. Papathanasiou E, Teles F, Griffin T, et al. Gingival crevicular fluid levels of interferongamma, but not interleukin-4 or -33 or thymic stromal lymphopoietin, are increased in inflamed sites in patients with periodontal disease. *J Periodontal Res.* 2014;49:55-61.
- 99. Isaza-Guzman DM, Cardona-Velez N, Gaviria-Correa DE, Martinez-Pabon MC, Castano-Granada MC, Tobon-Arroyave SI. Association study between salivary levels of interferon (IFN)-gamma, interleukin (IL)-17, IL-21, and IL-22 with chronic periodontitis. *Arch Oral Biol.* 2015;60:91-99.
- 100. Cheng WC, Saleh F, Abuaisha Karim B, Hughes FJ, Taams LS. Comparative analysis of immune cell subsets in peripheral blood from patients with periodontal disease and healthy controls. *Clin Exp Immunol.* 2018;194:380-390.

- 101. Alpagot T, Font K, Lee A. Longitudinal evaluation of GCF IFN-gamma levels and periodontal status in HIV+ patients. *J Clin Periodontol*. 2003;30:944-948.
- 102. Misawa MYO, Silverio Ruiz KG, Nociti FH, Jr., et al. Periodontal ligament-derived mesenchymal stem cells modulate neutrophil responses via paracrine mechanisms. *J Periodontol.* 2019.
- Kim WH, An HJ, Kim JY, et al. Anti-Inflammatory Effect of Melittin on Porphyromonas Gingivalis LPS-Stimulated Human Keratinocytes. *Molecules*. 2018;23.
- Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature*. 2008;456:264-268.
- 105. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011;469:221-225.
- 106. Harris J, Hartman M, Roche C, et al. Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. *J Biol Chem.* 2011;286:9587-9597.
- Jiang M, Li Z, Zhu G. The role of autophagy in the pathogenesis of periodontal disease. Oral Dis. 2019.
- 108. Rahman MM, Mohamed MR, Kim M, Smallwood S, McFadden G. Co-regulation of NF-kappaB and inflammasome-mediated inflammatory responses by myxoma virus pyrin domain-containing protein M013. *PLoS Pathog.* 2009;5:e1000635.
- 109. Saleh M, Mathison JC, Wolinski MK, et al. Enhanced bacterial clearance and sepsis resistance in caspase-12-deficient mice. *Nature*. 2006;440:1064-1068.
- 110. Garcia-Hernandez AL, Munoz-Saavedra AE, Gonzalez-Alva P, et al. Upregulation of proteins of the NLRP3 inflammasome in patients with periodontitis and uncontrolled type 2 diabetes. *Oral Dis.* 2019;25:596-608.
- 111. Basic A, Alizadehgharib S, Dahlen G, Dahlgren U. Hydrogen sulfide exposure induces NLRP3 inflammasome-dependent IL-1beta and IL-18 secretion in human mononuclear leukocytes in vitro. *Clin Exp Dent Res.* 2017;3:115-120.

- 112. Kollgaard T, Enevold C, Bendtzen K, et al. Cholesterol crystals enhance TLR2- and TLR4-mediated pro-inflammatory cytokine responses of monocytes to the proatherogenic oral bacterium Porphyromonas gingivalis. *PLoS One*. 2017;12:e0172773.
- 113. Kuo HC, Chang LC, Chen TC, et al. Sterol Regulatory Element-Binding Protein-1c Regulates Inflammasome Activation in Gingival Fibroblasts Infected with High-Glucose-Treated Porphyromonas gingivalis. *Front Cell Infect Microbiol.* 2016;6:195.
- 114. Bi J, Dai J, Koivisto L, et al. Inflammasome and cytokine expression profiling in experimental periodontitis in the integrin beta6 null mouse. *Cytokine*. 2018.
- 115. Yoneda T, Tomofuji T, Ekuni D, et al. Anti-aging effects of co-enzyme Q10 on periodontal tissues. *J Dent Res.* 2013;92:735-739.
- 116. Taxman DJ, Lei Y, Zhang S, Holley-Guthrie E, Offenbacher S, Ting JP. ASCdependent RIP2 kinase regulates reduced PGE2 production in chronic periodontitis. J Dent Res. 2012;91:877-882.

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-1β and IL-18. THP1 cells deficient from ASC and NLRP3, did not produce more IL-1β and IL-18 when exposed to NaHS than unexposed control cells. The NLRP3 inflammasome is essential for NaHS induced IL-1β and IL-18 secretion in monocytes.

Belibasakis et al. 2012 24	In vitro	Aa for 3 h	Human Mononuclea r 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-1 β 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-1 β 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 TNF α , IL-8, and IL-1 β 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-1 β secretion was dependent on the inflammasome component ASC but was only attenuated by the absence of Caspase 1.

Champaibo on 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. <i>Pg</i> LPS down-regulated NLRP3, pro-IL-1β and IL-1β. However, under moderate hypoxia (2% O ₂), <i>P.</i> <i>gingivalis</i> LPS enhanced NLRP3 expression, cleaved- caspase-1, and mature IL-1β. <i>E.coli</i> 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-1 β , 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-1 β 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	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-1 β secretion induced by Pg LPS and IL-1 β 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 <i>E.coli</i> 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,NLR C4, 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-1 β and TNF- α were also higher in GCF of CP patients. Pg activated caspase-1 and IL-1 β 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-1 β 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-1 β , 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	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-1 β -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-/-)	<mark>NLRP3,</mark> NLRC4, ASC	significant upregulation of NLRP3, NLRC4 and ASC expression in aorta of Integrin-β6 knockout (Itgβ6-/-) mice
Yiguchi et al. 2019 ⁶³	<mark>Invitro+</mark> 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.