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Title	Historical aspects of studies on roles of the inflammasome in the pathogenesis of periodontal diseases
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Citation	Molecular Oral Microbiology, 33(3), 203-221 https://doi.org/10.1111/omi.12217
Issue Date	2018-06
Doc URL	https://hdl.handle.net/2115/74519
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Type	journal article
File Information	Mol Oral Microbiol.pdf



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Historical aspects of studies on roles of the inflammasome in the pathogenesis of periodontal diseases

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SUMMARY

The proinflammatory cytokine interleukin (IL)-1β is produced as inactive pro-IL-1β and then processed by caspase-1 to become active. In 2002, it was demonstrated that the intracellular multiprotein complex known as the inflammasome functions as a molecular platform to trigger activation of caspase-1. Inflammasomes are known to function as intracellular sensors for a broad spectrum of various pathogen- and damage-associated molecular patterns.

In 1985, it was demonstrated that *Porphyromonas gingivalis*, a representative bacterium causing chronic periodontitis, induces IL-1 production by murine peritoneal macrophages. Since then, many studies have suggested that IL-1, particularly IL-1β, plays key roles in the pathogenesis of periodontal diseases. However, the term "inflammasome" was not used until Bostanci *et al.* suggested the involvement of inflammasomes in periodontal disease in 2009. Several subsequent studies on the roles of the inflammasome in the pathogenesis of periodontal diseases have been published. Interestingly, two contradictory reports on the modulation of inflammasomes by *P. gingivalis* have been published. Some papers have described that *P. gingivalis* activates the inflammasome to produce IL-1β, whereas some stated that *P. gingivalis* inhibits inflammasome activation to subvert immune responses. Several lines of evidence have also been accumulated that the inflammasome activation is modulated by the periodontopathic bacteria other than *P. gingivalis*.

Thus, studies on the roles of inflammasomes in the pathogenesis of periodontal diseases began only 8 years ago and many pathological roles of inflammasomes remain to be clarified.

INTRODUCTION

Interleukin-1 (IL-1), a proinflammatory cytokine, consists of IL-α and IL-1β. IL-1β induces inflammatory mediator production, osteoclast formation, matrix metalloproteinase expression, and matrix-producing cell death in periodontal tissues, resulting in the destruction of alveolar bone and periodontal connective tissue [1]. Thus, IL-1β plays crucial roles in the onset and progression of periodontal disease. IL-1β is produced extracellularly after pro-IL-1β is processed by caspase-1 to become activated by the intracellular multiprotein complex known as the inflammasome. The word "inflammasome" was first used to describe platforms for inflammatory caspase activation [2]. IL-18 is also produced as pro-IL-18 and processed by caspase-1 to become activated by the inflammasome. The inflammasome is composed of the "nucleotide-binding domain leucine-rich repeat-containing receptor" (NLR), adaptor protein "apoptosis-associated speck-like protein containing a caspase-recruitment domain" (ASC), and procaspase-1 [3-5] (Fig. 1). Several types of NLRs are involved in inflammasome activation [6]. Of these inflammasomes, the NLRP3 inflammasome is the most well-studied (Fig. 1) and has been implicated in responses to a broad spectrum of pathogen- and damage-associated molecular patterns [7-23] (Table 1). We previously demonstrated that whole cells of two *Mycoplasma* species and *Streptococcus sanguinis* activate the NLRP3 inflammasome to produce IL-1β [24,25].

Periodontitis, a chronic inflammation that occurs in many adults, is a major cause of tooth loss and is characterized by chronic infection associated with Gram-negative anaerobic bacteria in the subgingival biofilm, which leads to destruction of the tissue supporting the teeth. Subgingival biofilms containing several gram-negative rods are strongly involved in the onset and progression of periodontitis. Among gram-negative

rods, *Porphyromonas gingivalis* is a representative periodontal pathogen in chronic periodontitis [26].

In this review, we focus on the historical aspects of the pathological roles of inflammasomes in periodontal diseases and modulation of inflammasome activation by *P. gingivalis* as a first step for developing a new therapeutic strategy.

INVOLVEMENT OF IL-1 IN PERIODONTAL DISEASES

The reported roles of IL-1 in the pathogenesis of periodontal diseases are summarized in Fig. 2.

In 1985, the first paper regarding the functional role of IL-1 in periodontal disease was published [27]. However, this study only demonstrated that lipopolysaccharide (LPS) from *P. gingivalis* induces IL-1 production by murine peritoneal macrophages, but did not directly determine the roles of IL-1 in the pathogenesis of adult periodontal disease. The first report on the role of IL-1, particularly IL-1β, in the pathogenesis of periodontal diseases was published in 1991 [28]. The authors demonstrated that the number of cells stained with anti-human IL-1β was nearly 3-fold higher in periodontally diseased tissue compared to those in normal tissue, suggesting that IL-1β produced by cells in periodontal tissues is related to the pathological processes associated with periodontal disease. In 1992, the amount of IL-1 in crevicular fluid during human experimental gingivitis was measured and shown to increase rapidly with plaque accumulation and in advance of subsequent gingival inflammation, peaking within 7 days of the start of gingivitis [29]. Thus, the authors suggested that IL-1 is an early marker of gingival inflammatory changes. In 1995, it was demonstrated that *in vivo* administration of recombinant IL-1β accelerates silk ligature-induced alveolar bone resorption in rats [30].

In addition, several reports showed that an IL-1 genotype associated with increased IL-1 production is a strong indicator of the susceptibility to severe periodontitis in adults [31-33]. In 1997, Ishihara *et al*. demonstrated that the total amount of IL-1α, IL-1β, and the total IL-1/IL-1 receptor antagonist ratio (IL-1AI), but not the total IL-1 receptor antagonist (IL-1A), were correlated with alveolar bone loss score; a similar progressive decrease in total IL-1AI was detected in gingival tissue with periodontitis [34]. These results suggested that the levels of both crevicular IL-1 and total IL-1AI are closely associated with periodontal disease severity. In 1997, in a *Macaca fascicularis* primate model of experimental periodontitis, IL-1 and tumor necrosis factor (TNF) antagonists, their soluble receptors, inhibited the recruitment of inflammatory cells in close proximity to bone by approximately 80% and reduced osteoclast by 67% at the experimental sites compared to the levels at control sites; bone loss was reduced by 60% [35]. Thus, IL-1 and TNF, representative inflammatory cytokines, play important roles in the pathological process of periodontitis. In 1998, in the same primate model, the same group demonstrated that IL-1 and TNF antagonists inhibit the progression of inflammatory cell infiltration toward alveolar bone [36]. This research group also demonstrated that loss of connective tissue attachment and progression of periodontal disease can be retarded by antagonists to IL-1 and TNF in the same primate model [37].

Based on these reports, a previous review described the roles of IL-1 and TNF in periodontal tissue destruction [1]. These cytokines induce adhesion molecules and other mediators that facilitate and amplify the inflammatory response, stimulation of matrix metalloproteinase, and bone resorption.

INVOLVEMENT OF INFLAMMASOMES IN PERIODONTAL DISEASES

As described above, the inflammasome was first named in 2002 as a molecular platform triggering activation of caspase-1 and processing of proIL-1β and proIL-18 [2]. Therefore, we next focus on studies of the roles of the inflammasome in the pathogenesis of periodontal diseases.

In 2009, 7 years after the discovery of the inflammasome, it was first demonstrated that NLRP3 and NLRP2, but not ASC, are expressed at significantly higher levels in gingival tissues of subjects with inflammatory periodontal disease than in healthy subjects, and a positive correlation was found between NLRP3 and IL-1β or IL-18 expression levels in these tissues [38]. In addition, *in vitro* data demonstrated that *P. gingivalis* upregulates the expression of NLRP3, IL-1β, and IL-18 in a human monocytic cell line (Mono-Mac-6), but downregulates NLRP2 and ASC. Thus, the authors suggested that the NLRP3 inflammasome is involved in the pathogenesis of periodontal diseases.

In 2011, Bostanci *et al.*[39] demonstrated that supragingival and subgingival biofilms differentially regulate the gene expressions of NLRP3 and AIM2 inflammasomes and their down-stream IL-1 targets. Briefly, they found that the culture supernatant from supragingival biofilm containing 6-spieces of supragingival Zurich biofilm model[40] grown on the hydroxyapatite disc increased the expression of caspase-1, ASC, AIM2 as well as IL-1β and IL-18, but did not upregulate NLRP3 expression. However, the culture supernatant from subgingival biofilm containing 10-spieces of subgingival Zurich biofilm model[41] grown on the hydroxyapatite disc upregulated caspase-1, ASC, AIM2, IL-1β and IL-18 gene expression at lower concentrations, followed by their down-regulation at higher concentrations, which was also evident for NLRP3 expression. They suggest that upregulation of transcription of inflammasome

components by supragingival biofilms correlates with early inflammatory events in periodontal disease, whereas the downregulation of the transcription by subgingival biofilms is favorable for the survival and persistence of periodontopathic bacteria including *P. gingivalis.*

In 2012, it was demonstrated that production of prostaglandin E2 does not require the inflammasome adaptor function of ASC, but was dependent on mitogen-activated protein kinase activation and that the mitogen-activated protein kinase kinase kinase CARD domain-containing protein RIPK2 was induced by *P. gingivalis* in an ASC-dependent manner [42]. Thus, they found that the inflammasome adaptor ASC-dependent RIP2 kinase regulates reduced prostaglandin E2 production in chronic periodontitis, although they did not demonstrate the direct involvement of the inflammasome in periodontal disease.

In 2014, novel findings suggested a strong correlation between the NLRP3 inflammasome and severe periodontitis [43]. They demonstrated that blocking the functions of CD24, a negative regulator of inflammation in periodontal tissues [44-47], by its antibody-enhanced expression of NLRP3 together with the co-activators ASC and caspase-1, resulting in burst release of activated IL-18, which plays important roles in inflammation. Additionally, subjects with mild chronic periodontitis showed increased titers of serum antibodies that were auto-reactive with CD24 compared with in subjects with severe periodontitis. Thus, the negative regulator CD24 upregulates NLRP3 in periodontal disease, strongly suggesting that the NLRP3 inflammasome plays pathological roles in periodontal diseases.

In 2015, it was demonstrated that expression of NLRP3 and IL-1β in the oral gingival epithelium of patients with chronic periodontitis and/or type 2 diabetes mellitus

was significantly upregulated compared to in control individuals, and simultaneous stimulation with LPS and high glucose contributed to significant upregulation of NLRP3 expression compared to stimulation with LPS or high glucose alone [48]. These results suggested that for patients with type 2 diabetes mellitus and chronic periodontitis, a hyperglycemic status may exacerbate the inflammation state of gingival tissue by activating the NLRP3 inflammasome pathway.

In 2015, it was demonstrated that the intensity of NLRP3 expression was significantly higher in chronic periodontitis or generalized aggressive periodontitis than in healthy tissue, and a more significant difference was observed in the periodontal epithelium layer [49]. In addition, they showed that NLRP1 was minimally expressed in healthy and periodontitis gingival tissues, whereas absent in melanoma 2 (AIM2) was expressed at a higher level in the chronic periodontitis group than in other subjects. NLRP1 and AIM2 are also known to mediate inflammasome assembly against microbial infections [9,10].

In 2017, the study was carried out to compare salivary levels of NLRP3, ASC, caspase-1 and IL-1β from patients with aggressive periodontitis (AgP) or chronic periodontitis (CP) and periodontally healthy controls (HC) as well as elucidate its association with periodontal clinical status[50]. The study indicates the possibility that salivary levels of NLRP3, ASC, and IL-1β, but not caspase-1, act as strong/independent indicators of amount and extent of periodontal breakdown in both CP and AgP.

MODULATION OF THE INFLAMMASOME BY THE PERIDONTOPATHIC BACTERIA *P. gingivalis*

There are several papers which describe modulation of inflammasomes by

periodontopathic bacteria such as *Aggregatibacter actinomycetemcomitans*[51-54], *Fusobacterium nucleatum*[55] other than *P. gingivalis*. In this review, we focus on involvement of the representative periodontopathic bacterium *P. gingivalis* in inflammasome modulation. There have been two contradictory reports on modulation of the inflammasomes by *P. gingivalis*, a representative periodontopathic bacteria. Some papers demonstrated that *P. gingivalis* activates the inflammasome, which plays pathological roles in periodontal diseases [56-67] (Table 2). In contrast, some papers demonstrated that *P. gingivalis* inhibits inflammasomes to subvert immune responses [68-71] (Table 3). Olsen and Yilmaz[72] have already published the review which discusses in-depth on several molecular mechanisms by which *P. gingivalis* modulates innate immunity by limiting the activation of the NLRP3 inflammasome. In addition, Almeida-da-Silva *et al.*[73] have published the review which mainly describes modulation of the ability of *P. gingivalis* to evade the immune responses by purinerigic signaling known as the second signal for the NLRP3 inflammasome activation. Therefore, this review focuses on historical flow of studies on modulation of inflammasomes by *P. gingivalis*.

1. ACTIVATION OF INFLAMMASOMES

As described above, in 1985, *P. gingivalis* LPS was shown to induce IL-1 production by using murine peritoneal macrophages [27]. At that time, however, it was not known that the intracellular sensor inflammasome regulates the production of IL-1β. In addition, it was demonstrated that ASC, one component of the inflammasome complex, is involved in inducing cytokines such as IL-1β in response to *P. gingivalis* infection via a caspase-1-dependent pathway [56], although the term “inflammasome” was not used.

Activation of the inflammasome by *P. gingivalis* was reported in a study showing that treatment of gingival epithelial cells infected with *P. gingivalis* induced expression of the IL-1β gene and intracellular accumulation of IL-1β protein, whereas IL-1β was not secreted unless infected cells were subsequently stimulated with ATP, as well as that knockdown of NLRP3 by siRNA attenuated the ability of ATP to induce IL-1β secretion in infected cells [57] (Table 2). Similar findings have been reported in another study [58]. They also demonstrated that activation of the NLRP3 inflammasome does not rely on *P. gingivalis* infection unless stimulated by *P. gingivalis* LPS and/or extracellular ATP. Thus, these findings suggest that the NLRP3 inflammasome is an important mediator of the inflammatory response in the gingival epithelium.

Several lines of evidence [59-61] support the finding that periodontitis is associated pathologically with atherosclerotic vascular disease, although the details remain unclear. It was recently found that macrophages primed with *P. gingivalis* LPS and then treated with cholesterol crystals released IL-1β [62].

In 2014, it was demonstrated that expression levels of inflammasome components in gingival tissues from patients with chronic periodontitis are much higher than in those from healthy controls and that *P. gingivalis* induces the activation of NLRP3 and AIM2 inflammasome via TLR2 and TLR4 signaling, leading to IL-1β secretion and pyroptic cell death [63]. In addition, studies suggested that *P. gingivalis*-induced NLRP3 inflammasome activation depends on ATP release, K^+ efflux, and cathepsin B.

In 2015, it was reported that *P. gingivalis* can activate the NLRP3 inflammasome, in which the pathogenic factors gingipain and fimbriae play important roles, suggesting that the NLRP3 inflammasome plays a critical role in periodontal disease and atherosclerosis induced by *P. gingivalis* challenge through sustained inflammation [64]. In

2017, the authors of the previous study also demonstrated that oral injection of *P. gingivalis* to wild-type mice, but not to NLRP3-deficient mice, significantly increased alveolar bone loss and gingival gene expression of pro-IL-1β and proIL-18 [65]. In addition, they showed that *P. gingivalis* upregulated production of IL-1β and IL-18 by peritoneal macrophages from wild-type mice, but not from NLRP3-deficient mice.

In 2017, Yoshida *et al.*[74] have reported that P. gingivalis infection activated the double-stranded RNA-dependent kinase (PKR) in osteoblasts, which in turn upregulated NLRP3 expression through activation of NF-κB. Fleetwood *et al.*[75] have reported that treatment of murine bone marrow-derived macrophages (BMMs) with live *P. gingivalis*, heat-killed *P. gingivalis* and outer membrane vesicles (OMVs) upregulate the expression of NLRP3, procaspase-1 and pro-IL-1β and that treatment with OMVs, and to a lesser extent heat-inactivated OMVs induces cleavage of procaspase-1 and pro-IL-1β into their mature forms as well as inducing IL-1β and IL-18, whereas treatment with live *P. gingivalis* and heat-killed *P. gingivalis* do not. That is, they suggest that OMVs play more important roles in the activation of the NLRP3 inflammasome than *P. gingivalis* cells. In addition, Cecil et al.[76] have also reported that OMVs of *P. gingivalis*, *Treponema denticola* and *Tannerella forthia* activate inflammasomes in a human monocytic THP-1 cells and its subsets to induce IL-1β secretion and ASC speck formation and NLRP3 and/or AIM2 inflammasome(s) in murine BMMs to induce IL-1β secretion.

There is growing evidence of a relationship between diabetes and inflammasome activation [66]. Recently, infection by *P. gingivalis*, a major bacterial species in periodontal disease, was recognized as a common complication of diabetes. It has been demonstrated that human gingival fibroblasts infected with *P. gingivalis* grown in BHI

medium containing high glucose showed increased expression of IL-1β and NLRP3 [67].

Thus, these publications suggest that activation of the inflammasome by *P. ginigivalis* leads to IL-1β production and plays important roles in the pathogenicity of diabetes and atherosclerotic vascular disease as well as periodontal diseases.

2. INHIBITION OF INFLAMMASOME ACTIVATION

Several lines of evidence suggest that *P. gingivalis* inhibits inflammasome activation, leading to evasion of the host immune response (Table 3).

In 2012, it was reported that *P. gingivalis* suppresses inflammasome activation by another periodontal bacterium such as *F. nucleatum*, but not by ATP or nigericin [68]. Based on the finding that *P. gingivalis* limits both the number of cells taking up beads and number of beads taken up for bead-positive cells, they speculated that the mechanism by which *P. gingivalis* inhibits inflammasome activation contributes to suppression of bacterial endocytosis.

In 2013, it was reported that 10 species from subgingival biofilms, including *P. gingivalis*, reduce NLRP3 and IL-1β levels but do not affect AIM2 expression in human gingival fibroblasts and that exclusion of *P. gingivalis* from the biofilm partially rescued NLRP3 and IL-1β expression [69]. These results suggest that subgingival biofilms down-regulate NLRP3 and IL-1β expression, partly because of the existence of *P. gingivalis*. Therefore, these dampened host innate immune responses may favor the survival and persistence of associated biofilm species in periodontal tissues.

A recent study found that treatment of endothelial cells with live cells, but not with heat-killed cells, of *P. gingivalis* induced proteolysis of NLRP3, but this proteolysis was not observed after ATP pre-treatment and/or *P. gingivalis*-LPS stimulation [70].

Additionally, the levels of secreted IL-1β significantly increased after ATP pre-treatment and/or *P. gingivalis*-LPS stimulation, but not after *P. gingivalis* infection. These data demonstrate that *P. gingivalis* and its LPS differentially controlled the NLRP3 inflammasome pathway in endothelial cells, suggesting a novel potential mechanism developed by *P. gingivalis* to reduce IL-1β secretion and escape the host immune response.

As described above, *P. gingivalis* stimulates pro-IL-1β synthesis but not mature IL-1β secretion, unless the P2X7 receptor is activated by extracellular ATP [57]. An additional study demonstrated that fimbriae dampens $P2X_7$-dependent IL-1β secretion [71]. Briefly, the authors showed that when bone marrow-derived macrophages from wild-type or $P2X_7$-deficient mice were infected with *P. gingivalis* 381 or the isogenic fimbria-deficient (DPG3) strain with or without subsequent ATP stimulation, DPG3 induced higher IL-1β secretion after ATP stimulation compared to 381 in wild-type bone marrow-derived macrophages, but not in $P2X_7$-deficient cells.

Recently, it was demonstrated that a nucleoside-diphosphate kinase of *P. gingivalis* inhibits caspase-1 activation and IL-1β secretion in gingival epithelial cells by downregulating the ATP-$P2X_7$ signaling pathway and reducing the release of the high mobility group box 1 protein, a pro-inflammatory danger signal [77].

Thus, these publications suggest that *P. ginigivalis* inhibits inflammasome activation to subvert host immune responses.

SUMMARY AND CONCLUSIONS

IL-1β is produced as biologically inactive pro-IL-1β and then processed by caspase-1,

also known as IL-1β-converting enzyme, to active IL-1β [78]. In 2002, it was first demonstrated that the intracellular multiprotein complex known as the inflammasome functions as a molecular platform that triggers activation of caspase-1 [2]. Inflammasomes are intracellular sensors that drive host immune responses. However, excessive inflammasome activity can be harmful because gain-of-function mutations in inflammasome components are associated with autoimmune and autoinflammatory disorders [3,4,79].

In 1985, LPS from *P. gingivalis*, a representative bacterium in chronic periodontitis, was reported to induce IL-1 production by murine peritoneal macrophage [27]. After this report, many studies suggested that IL-1, particularly IL-1β, plays key roles in the pathogenesis of periodontal diseases. Taxman *et al.* demonstrated that ASC, one component of the inflammasome complex, is involved in inducing cytokines such as IL-1β by *P. gingivalis* via a caspase-1-dependent pathway [56]. However, they did not use the term "inflammasome," regardless of the discovery of inflammasome. In 2009, 7 years after the discovery of inflammasomes, Bostanci *et al.* first demonstrated that inflammasome activation plays important roles in periodontal disease [38]. Since then, several studies on the roles of the inflammasome in the pathogenesis of periodontal diseases have been published. Of these reports, there are two contradictory reports regarding the modulation of inflammasomes by *P. gingivalis*, a representative bacterium in chronic periodontitis. Some papers described that *P. gingivalis* activates the inflammasome, whereas some papers described that *P. gingivalis* inhibits inflammasome activation. The discrepancy between studies may be attributed to the different cell types used in the studies or to differences between a live single-type bacterial infection and biofilm infection. In addition, many pathogenic bacteria such as

Yersinia species [80], *Legionella pneumophila* [81], *Pseudomonas aeruginosa* [82], and *Mycobacterium tuberculosis* [83] inhibit inflammasome activation [3,23], although the mechanisms differ from each other.

Inflammasomes are intracellular sensors that drive host immune responses to maintain homeostasis of host cells and tissues. Thus, the activity of *P. gingivalis* in inhibiting inflammasome activation is more strongly associated with chronic inflammation such as adult chronic periodontitis. Inhibition of inflammasome activation by *P. ginigivalis* may allow other bacteria in the subgingival dental plaque as well as the bacterium to survive for longer periods of time in the gingival tissue and contribute to periodontal diseases.

As described above, studies on the roles of inflammasomes in the pathogenesis of periodontal diseases began only 8 years ago. In the oral cavity, there are teeth embedded in gingival tissue, which are mainly made of phosphate and calcium, and sometimes several artificial dental prostheses to restore intraoral defects, including resins, ceramics and metals such as titanium, cobalt and chromium, which is extremely different from nasal, stomach and gut cavities. More Recently, interestingly, it was found that titanium ions also stimulate inflammasome activation[84]. Thus, many unknown pathological roles of inflammasomes in various oral diseases including periodontal diseases remain to be clarified.

CONFLICT OF INTEREST

The author has no financial conflicts of interest.

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Table 1. Activators of the NLRP3 inflammasome

Activators	References
Silica, asbestos	Davis, BK (2011)
Cholesterol crystal	Duewell, P (2010)
Various bacterial toxins	Vladimer GI (2013); Franchi L (2012)
Bacterial ATP and K+ efflux	Franchi L (2007); Petrilli V (2007)
Oxidized mitochondorial DNA	Shimada K (2012)
ROS from mitochondorial	Zhou R (2011)
Guanylate-binding protein 5	Shenoy AR (2012)
dsRNA-dependent kinase	Lu B (2012)
Cathepsin B	Davis, BK (2011)
Bacterial mRNA	Sander LE (2011)
Blocking of ribosomal function	Vyleta ML (2012)
Muramyl dipeptide	Martinon F (2004); Marina-Garcia N (2008)
Bacterial RNA, PolyI:C, LPS, lipopeptide, R848	Kanneganti TD (2006)
mycoplasmas	Sugiyama M (2016)

Table 2. Activation of inflammasomes by *P. gingivalis*

Type of Inflammasome	Signal 1	Signal 2	Target cell	References
NLRP3	Pg infection	ATP	Human primary GEC	Yilmaz O (2010)
NLRP3/AIM2	Supragingival biofilm	ND	Human GF	Bostanci N (2011)
ND	Pg LPS	Cholesterol crystal	Human primary HMDM	Champaiboon C (2014)
NLRP3, AIM2	Pg infection	ND	A human monocytic cell line, THP-1	Park E (2014)
NLRP3	Pg infection	Pg LPS and/or ATP	A human GEC line, H413	Guo W (2015)
NLRP3	Pg infection	ND	Mouse PM	Yamaguchi Y (2017)
NLRP3	Pg infection	ND	Mouse osteoblast	Yoshida K (2017)
NLRP3	OMVs of Pg	ND	Mouse BMM	Fleetwood AJ (2017) & Cecil JD (2017)
NLRP3/AIM2	OMVs of Pg	ND	Mouse BMM	Cecil JD (2017)
?	OMVs of Pg, Td, Tf	ND	A human monocytic cell line, THP-1	Cecil JD (2017)

ND: not described, Pg: *P. gingivalis*, Td: *Treponema denticola*, Tf: *Tannella forthia*, HMDM: human monocyte-derived macrophage, GF: gingival fibroblasts, GEC: gingival epithelial cell, PM: peritoneal macrophages, OMVs: outermembrane vesicles, BMM: bone marrow-derived macrophage

Table 3. Inhibition of the inflammasome activation by *P. gingivalis*

Type of Inflammasome	Mechanism	Active entity	Target cell	references
NLRP3/AIM2	Subgingival biofilm	ND	Human GF	Bostanci N (2011)
NLRP3	Suppression of other bacterial endocytosis	ND	Mouse BMDM	Taxman DJ (2012)
NLRP3	ND	ND	Primary human GF	Belibasakis GN (2013)
NLRP3	Proteolysis of NLRP3	ND	Human UVEC	Huck O (2015)
NLRP3	ND	Fimbriae	Mouse BMDM	Morandini AC (2014)
ND	Downregulation of ATP/$P2X_7$-signaling and release of HMGB1	Nucleotide-diphosphate kinase	Human GEC	Johnson L (2015)

ND: not described, BMDM: bone marrow-derived macrophage, GF: gingival fibroblasts, UVEC: umbilical vein endothelial cells, GEC: gingival epithelial cell, HMGB1: high mobility group box

FIGURE LEGENDS

Fig. 1. Production of biologically active IL-1β

IL-1β is produced as pro-IL-1β in the cytosol, which is biologically inactive, through activation of nuclear factor-κB by Toll-like receptor-mediated signaling. Biologically active IL-1β is produced after processing by caspase-1, which is also processed from pro-caspase-1 by activation of the intracellular sensor inflammasome. The inflammasome is an intracellular multiprotein complex comprising "nucleotide-binding domain leucine-rich repeat-containing receptor," the adaptor protein "apoptosis-associated speck-like protein containing a caspase-recruitment domain," and procaspase-1, which is formed by signals mediated by extracellular ATP, pathogen-associated molecular patterns, danger-associated molecular patterns or K^+ efflux.

PYD: pyrin domain, CARD: caspase recruitment domain

Fig. 2. Effects of IL-1 on periodontal tissues that have been published so far.

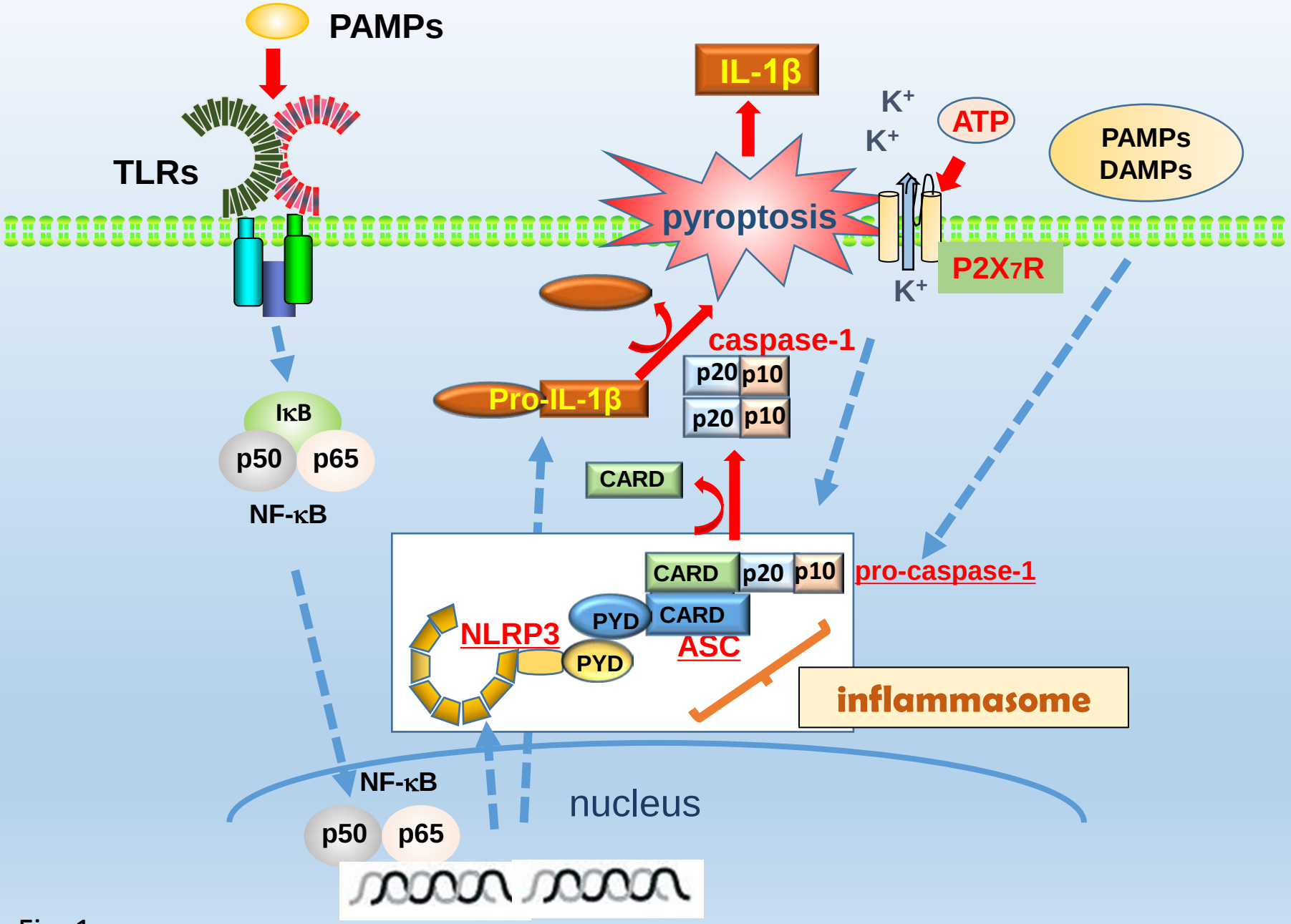

PAMPs
TLRs
IL-1β
K+
K+
ATP
PAMPs
DAMPs
pyroptosis
P2X7R
K+
caspase-1
p20
p10
p20
p10
Pro-IL-1β
IκB
p50
p65
NF-κB
CARD
CARD
p20
p10
pro-caspase-1
PYD
CARD
NLRP3
ASC
PYD
inflammasome
NF-κB
p50
p65
nucleus

Fig. 1

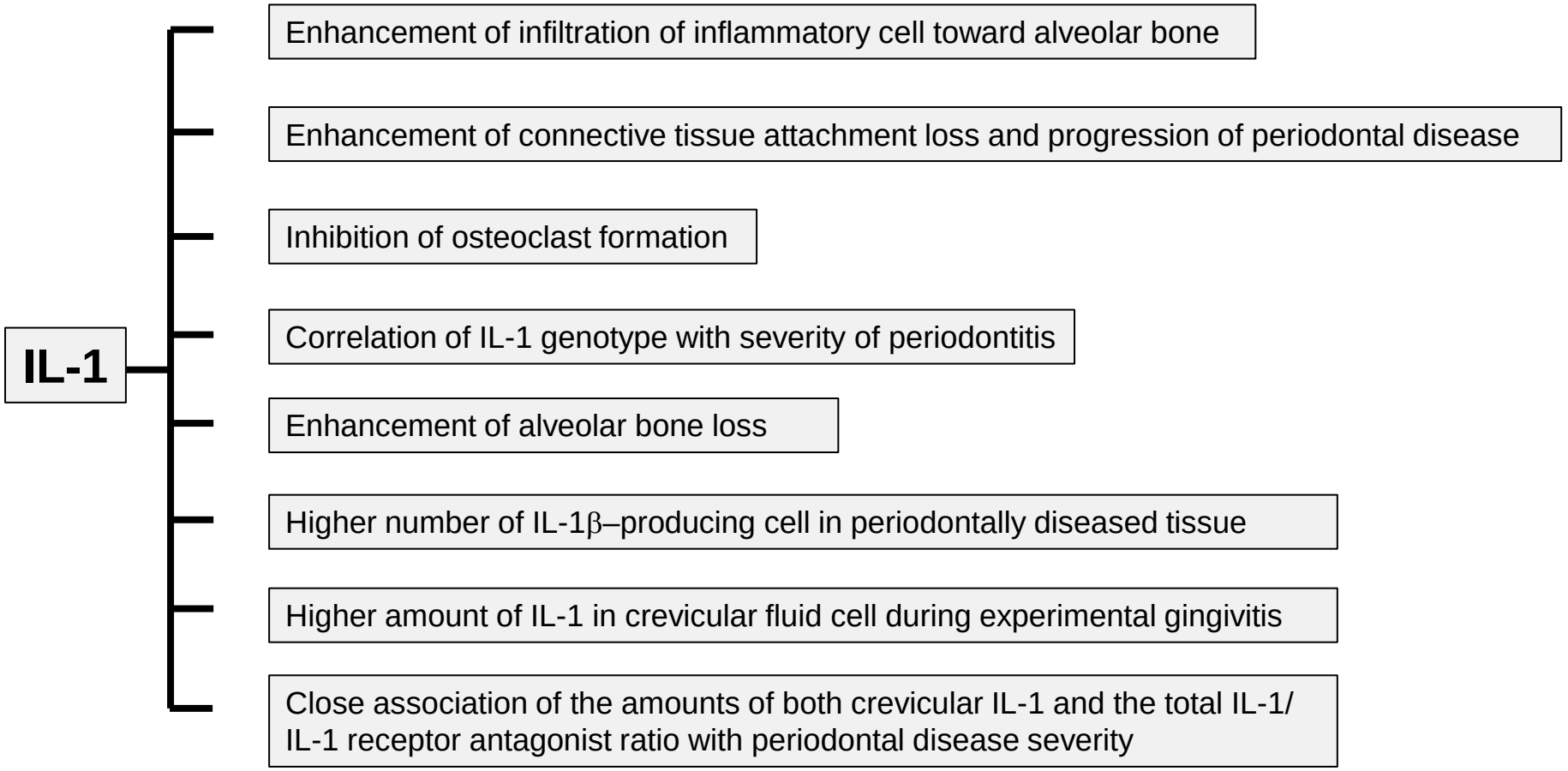

IL-1
Enhancement of infiltration of inflammatory cell toward alveolar bone
Enhancement of connective tissue attachment loss and progression of periodontal disease
Inhibition of osteoclast formation
Correlation of IL-1 genotype with severity of periodontitis
Enhancement of alveolar bone loss
Higher number of IL-1β–producing cell in periodontally diseased tissue
Higher amount of IL-1 in crevicular fluid cell during experimental gingivitis
Close association of the amounts of both crevicular IL-1 and the total IL-1/ IL-1 receptor antagonist ratio with periodontal disease severity

Fig. 2