# Possible role of inflammasomes in the pathogenesis of periodontal diseases

**Nada Hashim, Rasha Babiker, Riham Mohammed, Bakri Gismalla, Mohammed Mustahsen Rehman**

## Abstract

Dr. Jurg Tschopp created the word "inflammasome" in 2002. Inflammasome activation and its function in disease processes have been the subject of significant investigation over the last 15 years. Four important inflammasomes have been identified: NLRP1, NLRP3, NLRC4, and AIM2. When these inflammasomes are activated, they process and secrete inflammatory cytokines such as IL-1b and IL-18, as well as cause pyroptosis, an inflammatory form of cell death. In this review, we will look at how these inflammasomes have been connected to Periodontitis pathogenesis.

**Keywords: Inflammasome, NLRP3, periodontal disease, periodontal pathogen, IL-1β.**

## Background

Periodontitis is a complex, multifactorial inflammatory disease influenced by genetic and environmental factors, resulting in a destructive inflammatory response against the ubiquitous microbial biofilm (1).

The condition is characterized by the progressive loss of tooth-supporting structures and has been connected to dysbiotic plaque biofilms. Periodontitis is the sixth most prevalent disease in the world, accounting for 11.2 percent of all cases, and it is the leading cause of tooth loss worldwide (2-4).

It is more than simply a localized oral disease; it also impacts an individual's systemic health and is a risk factor for several systemic disorders (5,6).

As a result, understanding the causes of periodontitis onset and progression is crucial for developing effective treatment and preventative interventions.

## Inflammasomes

The body's initial line of defense against infections is the innate immune response. Pathogens, such as bacteria and viruses, are recognized by pattern recognition receptors in the innate immune system (PRR). There are five types of PRRs that can detect a wide range of microbial components, known as pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP), which are host cell components generated during inflammation or derived from the environment (7).

Many PRRs are present on various cells, such as epithelial, endothelial, and cells of the adaptive immune system, despite the fact that innate immunity cells are the ones that express PRRs more frequently. When a PRR is activated by its ligand, subsequent signaling cascades are triggered, which have a variety of effects. One of these effects is the activation of innate immune cells. Another is the production of cytokines and chemokines, which attract immune cells to the site of infection (7).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2023 doi:10.20944/preprints202301.0416.v1

Inflammasome activation is an important element of the innate immune system route that activates a family of cysteine proteases known as inflammatory caspases. These caspases regulate the immunological response by cleaving certain cellular substrates (8,9).
The inflammasome is a multimeric assembly of procaspase-1 and a member of the NLR family, either directly through CARD-CARD couplings or indirectly through the adaptor protein ASC. The procaspase-1 zymogen is activated by autoproteolysis once this complex is formed, which could process pro- IL-1β.and pro-IL-18.
Frequently, danger signals are required to activate inflammasomes. Pro-inflammatory cytokines and, more intriguingly, an inflammatory form of programmed cell death known as pyroptosis is produced when inflammasomes are activated (10).
Pyroptosis is a bacterial clearance mechanism generated by the innate immune system that is used to characterize the remarkable pro-inflammatory process of cell death (9) .
Because it is a process of cellular self-destruction driven by caspases, it was first confused with traditional apoptosis. However, the processes, characteristics, and results of pyroptosis differ significantly from those of apoptosis, with the most notable difference being the inflammatory reactions. Furthermore, pyroptosis occurs quickly and is followed by the release of a plethora of pro-inflammatory molecules (11).
Several significant signaling pathways are involved in the processes of pyroptosis, and each of them activates the downstream of GSDMD.
Interleukins-1 **β** (IL-1 **β**) and -18 (IL-18), two cytoplasmic molecules, are finally able to escape through the holes caused by GSDMD and cause a significant inflammatory response.RANKL (receptor activator of p-NF-B ligand) is one of several genes that can be expressed as a result of activated IL-1. Determining the presence of pyroptosis may therefore be determined by signals which include Caspase 1 activation, GSDMD cleavage, IL-1, and IL-18 maturation and release (8). (Figure 1).
Four key inflammasomes have been best characterized, namely NLRP1, NLRP3, NLRC4, and AIM2 (12).
NLRP1 is known for being the first protein to form an inflammasome, as well as its probable function in host innate immunity and inflammatory disorders.The NLRP3 gene on human chromosome 1 encodes NLRP3, which is mostly expressed in macrophages (7,13).
Inflammasome assembly is distinctive in that it is triggered by both external and endogenous stimuli. Each protein senses a different set of activation signals, yet some of them may overlap. Unlike the AIM2 and NLRC4 inflammasomes, which are activated solely by particular PAMPs, dsDNA, and bacterial proteins, respectively, NLRP3 is activated by a wide range of signals, including PAMPs, DAMPs, and bacterial toxins (14).
Activation of these inflammasomes leads to the processing and secretion of inflammatory cytokines, including IL-1β and IL-18, These cytokines, which are incredibly potent molecules with a wide range of actions, are viewed as an essential component of the host response in inflammation and the development of periodontal diseases (15,16).

**The role of inflammasomes in the pathogenesis of periodontitis**

The balance between subgingival bacteria and the host's inflammatory response is disrupted in periodontitis. The bacteria that cause periodontitis increase the expression of genes involved in apoptosis, and immune and inflammatory responses.
The dysregulation of inflammatory cytokines, both pro-inflammatory and anti-inflammatory, is one of the molecular mechanisms that causes the destruction of periodontal tissues. It has long been established that cytokines are crucial for maintaining tissue homeostasis, controlling

immunological responses, and communication between cells. During inflammatory reactions, LPS from Gram-negative bacteria triggers the production of pro-inflammatory cytokines. Alveolar bone loss has consistently been associated with higher cytokine levels (17).
In the pathogenesis of periodontal disease, interleukin-1 β (IL-1 β) is a prominent pro-inflammatory cytokine that is generated by host cells and induces inflammation of the periodontal tissues. Pro-IL-1 β is transformed into its physiologically active form by the activation of inflammasomes, a crucial host defense system regulator. When compared to control sites, the administration of an IL-1 inhibitor caused a 50% reduction in radiographic bone loss, suggesting that IL-1 β may have a potential role in the development of periodontal disease (18,19).

High levels of inflammation are linked to inflammasome triggering; as a result, it needs to be tightly controlled to avoid abnormal activation. By properly activating inflammasomes, the host regulates the degree of inflammation under normal, healthy circumstances (20).
Numerous earlier studies have emphasized the significance of the inflammasome's proper activation in the etiology of periodontal disease. As a homeostatic checkpoint, inflammasomes control the degree of inflammation in both health and disease. Having a better understanding of the various methods that can be used to control an excessive inflammatory response at the cellular and molecular level may help to develop treatments and preventative measures that are more successful in treating periodontal disease and the systemic diseases that are linked to it (21-23).

**Inflammasomes and Periodontal Pathogens**

*Porphyromonas gingivalis*, the keystone pathogen, crucially accounts for the change from symbiosis to dysbiosis and periodontal damage (22).
It was shown that activating the NLRP3 inflammasome is an essential recognition route for preserving homeostasis in periodontal tissues. They may work by differentiating between commensal and pathogenic strains and launching distinctly diverse immune responses during the microbial invasion.
Furthermore, activation of NLRP3 inflammasomes during periodontal infection with the periodontal pathogen, *P. gingivalis*, was found to be associated with enhanced Caspase-1 activation and release of mature IL-1, and ultimately, increased cytotoxicity (22).
The red complex, composed of P. gingivalis, T. denticola, and T. forsythia, was found to activate caspase-1 and caspase-4, resulting in cell death and the release of endogenous danger molecules that may play critical roles in the pathogenesis and progression of periodontitis by augmenting immune and inflammatory responses (24).

**Clinical relevance of inflammasomes with periodontal disease**

Autoinflammatory disorders are linked to inappropriate caspase-1 activation, which is brought on by inflammasome mutations (25).
Additionally, caspase-1 has a role in the development of a number of disorders, including periodontal disease, which is characterized by inflammation and cell death. Therefore, Caspase-1 deficiency or inhibition may prevent tissue inflammation, which is one of the symptoms of periodontal disease. As a result, caspase-1 represents a possible therapeutic target that may be affected by certain pharmacological inhibitors (26).
It must be emphasized that caspase-1 is also a component of the immune system and is therefore needed for defense against virulence agents. The understanding of inflammatory caspases in health and disease can also be improved by research to discover and characterize new caspase substrates.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2023 doi:10.20944/preprints202301.0416.v1

The NLRP3 inflammasome appears to be the one responsible for caspase-1 activation. As a result, caspase-1 is a critical modulator of inflammasome function, and its activity directly indicates NLRP3 activation. Emerging data showed that the NLRP3 inflammasome may respond to a diverse set of bacterial ligands, including LPS, bacterial RNA, and peptidoglycans (PAMPs or DAMPs), and that it plays an important role in periodontal disease (23,27).

Many studies have revealed that the NLRP3 inflammasome plays an important role in periodontal disease caused by Porphyromonas gingivalis (P. gingivalis) and might be a viable preventative and therapeutic target. Furthermore, the salivary levels of NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and IL-1 might be utilized to determine the degree and severity of periodontal breakdown in periodontitis (28,29).

In gingival tissues, there was a positive link between the expression levels of NLRP3 and IL-1, according to a clinical investigation, which found that patients with periodontal disease had considerably greater levels of NLRP3 expression than controls. Another clinical trial revealed that controls had significantly lower levels of NLRP3 and IL-1 in saliva samples from a periodontitis group (30-32).

These findings lend evidence that the NLRP3 inflammasome has a role in the etiology of periodontal disease when taken as a whole. Additionally, many NLRP3 inflammasome-related conditions including RA, diabetes and Alzheimer's disease are thought to be at risk as a result of periodontal disease. IL-1 and NLRP3 inflammasome activation may therefore contribute to chronic inflammation and/or infections in periodontal disease hastening the onset of these conditions (33-35).

Interestingly, elevated NLRP3 inflammasome activation has been seen in type 2 diabetes patients, and metformin has been demonstrated to contribute to inflammasome activation control. As a result, it is proposed that metformin might be a possible therapeutic drug to target NLRP3 (29).

Furthermore, a poor alveolar bone condition in aged mice was related to macrophage caspase-1 activation and higher levels of IL-1, both of which are predominantly regulated by the NLRP3 inflammasome, in periodontal ligament and serum. As a result, the administration of MCC950, which greatly suppresses the NLRP3 inflammasome, significantly reduced alveolar bone loss in aged mice (36).

Many recent improvements have been made in understanding the critical role of the NLRP3 inflammasome in periapical inflammation. As a result, the NLRP3 inflammasome may be a suitable target for anti-inflammatory therapies (37,38). As a result, inhibitors of the NLRP3 inflammasome signaling pathway may also be used as potential therapeutic targets in the future (39).

## Conclusion

There are several families of inflammasomes, each with a unique set of functions, the majority of which are currently unknown. Inflammasomes play an important role in the inflammatory process and are an essential component of innate immunity, which opens up the prospect of novel targets for modulating host responses and enabling an effective response to bacterial challenges in the future.

Several inflammasome components are now found in greater concentrations in saliva, gingival crevicular fluid, and periodontal tissues. It is uncertain if the molecular processes that cause abnormal inflammasome activation in chronic conditions also impact the periodontal host response. There is a significant push for more research into the involvement of inflammasome pathways as treatment targets for periodontal disease.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2023 doi:10.20944/preprints202301.0416.v1

**Outlook**

Innate immunology has advanced significantly with the identification of inflammasomes as protein platforms that regulate the processing of IL-1 and IL-18. It is important to note that *P. gingivalis*-treated THP-1 cells with NLRP3-, Caspase-1-, and Caspase-4-siRNA knockdown were linked to reduced production of IL-1 periodontitis and alveolar bone resorption, which is essential for the dysregulated immuno-inflammatory response in periodontal pathogenesis.

The focus of inflammasome research is predicted to progressively shift away from the in vitro and in vivo studies using small animal models that dominated the first ten years of the field's existence and toward the role of altered inflammasome function in complex periodontal disease. This could open the door for a therapeutic intervention that targets inflammasome-regulated pathways that are implicated in the development of periodontal disease, together with a massive effort to uncover and develop small-molecule approaches to the specific suppression of inflammasome activation.

**References**

1- Julie, T. Marchesan. Inflammasomes as contributors to periodontal disease. J Periodontol. 2020,91, S6–S11.
2- Peres, MA.; Macpherson, LMD.; Weyant, RJ.; Daly, B.; Venturelli, R.; Mathur, MR., *et al*. Oral diseases: a global public health challenge. *Lancet.* 2019,394,249-60
3- Kinane, DF.; Stathopoulou, PG.; Papapanou, PN. Periodontal diseases. *Nat Rev Dis Primers.* 2017,3,17038.
4- Acharya, A.; VanWormer, JJ.; Waring, SC.; Miller, AW.; Fuehrer, JT.; Nycz, GR. Regional epidemiologic assessment of prevalent periodontitis using an electronic health record system. Am J Epidemiol. 2013,177,700-7.
5- Genco, RJ.; Borgnakke, WS. Diabetes as a potential risk for periodontitis: association studies. *Periodontol 2000.* 2020,83,40-5.
6- Kamer, AR.; Craig, RG.; Niederman, R.; Fortea, J.; de Leon, MJ. Periodontal disease as a possible cause for Alzheimer's disease. *Periodontol 2000.* 2020,83,242-71
7- Marchesan JT, Girnary MS, Moss K, Monaghan ET, Egnatz GJ, Jiao Y, Zhang S, Beck J, Swanson, KV. Role of inflammasomes in the pathogenesis of periodontal disease and therapeutics. Periodontol 2000. 2020, 82,93-114.
8- Feng, Y.; Huang, X. Methodology for Comprehensive Detection of Pyroptosis. Methods Mol. Biol. 2021, 2255, 149–157.
9- Miao, E.A.; Leaf, I.A.; Treuting, P.M.; Mao, D.P.; Dors, M.; Sarkar, A.; Warren, S.E.; Wewers, M.D.; Aderem, A. Caspase-1-Induced Pyroptosis Is an Innate Immune Effector Mechanism against Intracellular Bacteria. Nat. Immunol. 2010, 11, 1136–1142.
10- 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.
11- Yu, C.; Zhang, C.; Kuang, Z.; Zheng, Q. The Role of NLRP3 Inflammasome Activities in Bone Diseases and Vascular Calcification. *Inflammation* 2021,*44*, 434–449.

12- Strowig, T.; Henao-Mejia, J.; Elinav, E. *et al.* Inflammasomes in health and disease. *Nature 2012,* 481, 278–286.
13- Zhang, Y.; Dong, Z.;Song, W. NLRP3 inflammasome as a novel therapeutic target for Alzheimer's disease. Signal Transduct Target Ther. 2020, 5, 37.
14- Strowig, T.; Henao-Mejia, J.; Elinav, E.; Flavell, R. Inflammasomes in health and disease. NATURE 2012, 481,278-286.
15- Schroder, K.; Tschopp, J. The Inflammasomes. Cell 2010, 140,821-32.
16- SM, Man T-D Kanneganti. Converging roles of caspases in inflammasome activation, cell death and innate immunity at Rev Immunol.2016,17,7-21.
17- Ben-Sasson, S.Z.; Hu-Li, J.; Quiel, J.; Cauchetaux, S.; Ratner, M.; Shapira, I.; Dinarello, C.A.; Paul, W.E. IL-1 acts on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc. Natl. Acad. Sci. USA* 2009, *106*,7119–7124.
18- Aral, K.; Milward, MR.; Kapila, Y.; Berdeli, A.; Cooper, PR. Inflammasomes and their regulation in periodontal disease: A review. J Periodontal Res. 2020,55,473-487.
19- T. W. , Oates.; D.; T, Graves.; D. L., Cochran. Clinical, radiographic and biochemical assessment of IL-1/TNF-α antagonist inhibition of bone loss in experimental periodontitis Clin Periodontol 2002, 29,137-43.
20- Delaleu N and Bickel M: Interleukin-1 beta and interleukin-18: Regulation and activity in local inflammation. Periodontol 2000. 2004; 35:42–52.
21- Rana, A.; Kaur, A.; Faraz, F.; Tandon, S."Targeting inflammasomes: A possible therapeutic approach for periodontal disease (Review)". World Academy of Sciences Journal 2021,3,50.
22- Ding, PH.; Yang, MX.; Wang, NN. ; Jin, LJ.; Dong, Y.; Cai, X.; Chen, LL. *Porphyromonas gingivalis*-Induced NLRP3 Inflammasome Activation and Its Downstream Interleukin-1β Release Depend on Caspase-4. Front. Microbiol. 2020, 11, 1881.
23- Lu, W.L.; Song, D.Z.; Yue, J.L.; Wang, T.T.; Zhou, X.D.; Zhang, P.; Zhang, L.; Huang, D.M. NLRP3 Inflammasome May Regulate Inflammatory Response of Human Periodontal Ligament Fibroblasts in an Apoptosis-Associated Speck-like Protein Containing a CARD (ASC)-Dependent Manner. *Int. Endod. J.* 2017, *50*, 967–975.
24- Juna, KH.; Junga JY.; Choia, BK. Treponema denticola, Porphyromonas gingivalis, and Tannerella forsythia induce cell death and release of endogenous danger signals. Archives of Oral Biology 2017, 73, 72–78
25- Simon, A.; van der Meer, J.W.M. Pathogenesis of Familial Periodic Fever Syndromes or Hereditary Autoinflammatory Syndromes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2007, *292*, R86–R98.
26- Bergsbaken, T.; Fink, S.L.; Cookson, B.T. Pyroptosis: Host Cell Death and Inflammation. *Nat. Rev. Microbiol.* 2009, *7*,99–109.
27- O'Brien, M.; Moehring, D.; Muñoz-Planillo, R.; Núñez, G.; Callaway, J.; Ting, J.; Scurria, M.; Ugo, T.; Bernad, L.; Cali, J.; et al. A Bioluminescent Caspase-1 Activity Assay Rapidly Monitors Inflammasome Activation in Cells. *J. Immunol. Methods* 2017, *447*,1–13.
28- Yamaguchi, Y.; Kurita-Ochiai, T.; Kobayashi, R. *et al.* Regulation of the NLRP3 inflammasome in *Porphyromonas gingivalis*-accelerated periodontal disease. *Inflamm. Res.2017,* 66,59–65.

29- Tan, Y.; Chen, J.; Jiang, Y.; Chen, X.; Li, J.; Chen, B.; Gao, J. The anti-periodontitis action of metformin via targeting NLRP3 inflammasome. Archives of Oral Biology 2020,114, 104692.
30- Murakami, T.; Takahata, Y.; Hata, K.; Nishimura, R. Role of interleukin-1 and inflammasomes in oral disease. *J. Oral Biosci.* 2020,*62*, 242–248.
31- Bostanci, N.; Emingil, G.; Saygan, B.; Turkoglu, O.; Atilla, G.; Curtis, M.A.; Belibasakis, G.N. Expression, and regulation of the NALP3 inflammasome complex in periodontal diseases. *Clin. Exp. Immunol.* 2009,*157*,415–422.
32- Isaza-Guzmán, D.M.; Medina-Piedrahíta, V.M.; Gutiérrez-Henao, C.; Tobón-Arroyave, S.I. Salivary Levels of NLRP3 Inflammasome-Related Proteins as Potential Biomarkers of Periodontal Clinical Status. *J. Periodontol.* 2017, *88*, 1329–1338.
33- Detert, J.; Pischon, N.; Burmester, G.R.; Buttgereit, F. The association between rheumatoid arthritis and periodontal disease. *Arthritis Res. Ther.* 2010,*12*, 218.
34- Mealey, B.L.; Ocampo, G.L. Diabetes mellitus and periodontal disease. *Periodontol. 2000* 2007, *44*, 127–153.
35- Singhrao, S.K.; Harding, A.; Poole, S.; Kesavalu, L.; Crean, S. Porphyromonas gingivalis Periodontal Infection and Its Putative Links with Alzheimer's Disease. *Mediat. Inflamm.* 2015, *2015*,137357.
36- Zang, Y.; Song, JH.; Oh, SH.; Kim, JW.; Lee, MN.; Piao, X.; Yang, JW.; Kim, OS.; Kim, TS.; Kim, SH.; Koh, JT. Targeting NLRP3 Inflammasome Reduces Age-Related Experimental Alveolar Bone Loss. 2020,99,1287-1295.
37- Galler, KM.; Weber, M.; Korkmaz, Y.; Widbiller, M.; Feuerer , M. Inflammatory Response Mechanisms of the Dentine-Pulp Complex and the Periapical Tissues. Int J Mol Sci 2021, 22,1480.
38- Jang, JH.; Shin, HW.; Lee, JM.; Lee, HW.; Kim, EC.; Park, SH. An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. Mediators Inflamm. 2015, 2015,794143.
39- Isaza-Guzmán, D.M.; Medina-Piedrahíta, V.M.; Gutiérrez-Henao, C.; Tobón-Arroyave, S.I. Salivary levels of NLRP3 inflammasome-related proteins as potential biomarkers of periodontal clinical status. *J. Periodontol.* 2017, *88*,1329–1338.
40- Fernanda, R G.; Rocha Andrea E.; Delitto Joao, A.; Souza, CD.; Laura, A.; González-Maldonado, G.; Wallet SM.; and Junior, CS. Relevance of Caspase-1 and Nlrp3 Inflammasome on Inflammatory Bone Resorption in A Murine Model of Periodontitis. Scientific reports 2020,10, 7823.
41- Zhao1, D.; Wu ,Y.; Zhuang ,J.; Xu, C.; Zhang, F. Activation of NLRP1 and NLRP3 inflammasomes contributed to cyclic stretch-induced pyroptosis and release of IL-1β in human periodontal ligament cells. Oncotarget 2016, 7, 68292 – 68302.
42- Hou, L.;Ye, Y.; Gou, H.; Tang, H.; Zhou, Y.; Xu, X.; Xu, Y. A20 inhibits periodontal bone resorption and NLRP3-mediated M1 macrophage polarization. Exp Cell Res. 2022, 418,113264.

 doi:10.20944/preprints202301.0416.v1