Role of *Porphyromonas gingivalis* in the Downregulation of NLRp3 Inflammasome in Periodontitis

Neelam Gavali, Amit Chaudhari, Pramod Waghmare, Yogesh Khadtare, Pooja Shendge, Shubhangi Wadgounkar[1]

Departments of Periodontology and [1]General Pathology and Microbiology, Bharati Vidyapeeth (Deemed to be) University Dental College and Hospital, Pune, Maharashtra, India

Abstract

The innate immune response is the body's first line of defense against pathogens. The innate immune system recognizes pathogens, including bacteria and viruses by engagement of the germline-encoded pattern recognition receptors (PRRs). There are five families of PRRs which are able to sense vast families of microbial components, referred to as pathogen-associated molecular patterns and damage-associated molecular patterns (DAMPs), they are host cell components produced during inflammation or environmentally derived. Although PRRs are predominately expressed by innate immune cells, many of the PRRs are also found on other cells including epithelial, endothelial, and cells of the adaptive immune system. PRR engagement by its ligand induces downstream signaling cascades that induce multiple effects, including activation of innate immune cells and cytokine/chemokine production for the recruitment of immune cells to the site of infection or tissue damage. There are multiple inflammasomes that are formed, which are named for their sensor PRR that induces its activation. It is still not clear how many sensors are capable of forming inflammasomes, with strong literature support for over 10 different inflammasomes, including NLRP1, NLRP3, NLRP6, NLRP12, pyrin, NAIP/NLRC4, RIG-I AIM2, IFI16, NLRC3, and NLP6 5,7 which are recently reviewed. The study of periodontal disease thus represents an excellent model to study the role of inflammasomes due to the abundance of Microbe Associated Molecular Patterns (MAMP) and DAMPs and the elevated proportion of macrophages in the tissue microenvironment.

Keywords: *Inflammasome, NLRP3 inflammasome, Porphyromonas gingivalis*

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Introduction

Inflammasomes are widely recognized to be activated in myeloid cells, including monocytes, macrophages, dendritic cells, and neutrophils, they can also be activated in keratinocytes, gingival and dermal fibroblasts, and mucosal epithelial cells. In response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), some pattern recognition receptors (PRRs) assemble inflammasomes for the activation of cellular caspases that, in turn, induce the maturation of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-18 together with the induction of inflammation-induced programmed cell death (pyroptotic) (Julie T. Marchesan 2020).[1-8]

Recognition of mature IL-1 β and IL-18 by their receptors has pleiotropic actions, which also includes

a. Recruitment of neutrophils and other innate immune cells

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Address for correspondence: Dr. Neelam Gavali, Department of Periodontology, Bharati Vidyapeeth (Deemed to be) University Dental College and Hospital, Pune, Maharashtra, India.
E-mail: drneelamkakde@gmail.com

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b. Activation of B-cells and antibody production and
c. Differentiation of T-cells.

Historical Background

In 2002, a landmark article by Martinon *et al*. described for the first time an overexpression or cell-free system, the existence of a molecular platform comprised caspase-1, caspase-5, (apoptosis-associated speck [ASC]-like protein containing a caspase activation and recruitment domain [CARD]), and NLRP1 (NLR family, pyrin domain containing,[9] 1-a complex now known as the NLRP1) inflammasome. In 2003, Mariathasan *et al*. provided genetic evidence to demonstrate the importance of inflammasomes in the endogenous system. They generated mice deficient in ASC or NLRC4 (NLR family, CARD domain containing and found that macrophages lacking these components have an impaired ability to activate caspase-1 and induce pro-IL-1 β processing in response to infection by *Salmonella enterica* species.[10] The sensor that mediates LPS and ATP-induced inflammasome activation was later identified to be NLRP3 by the same group in 2006.

The review by Chavarría-Smith and Vance (2015) describes comprehensively the immunobiology of the NLRP1 inflammasome.[11] They provide detailed discussions on the different models by which NLRP1 is activated upon sensing lethal toxin and muramyl dipeptide, whether cleavage of NLRP1 itself is sufficient for its activation, and how the host and pathogen regulate NLRP1 responses.

What is an Inflammasome?

The innate immune response is the body's first line of defense against pathogens. A key function of the innate immune system is inflammasome activation. The term "inflammasome" was coined by the late Jurg Tschopp and his research team in 2002. After infection or cellular stress, inflammasomes are assembled, activated, and involved in host defense and in the pathophysiology of diseases. Inflammasomes follow canonical or noncanonical pathways.[10] A typical functional canonical inflammasome complex consists of

1. A nucleotide-binding leucine-rich repeat (NLR) protein,
2. An adaptor molecule apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC)
3. And procaspase-1.

Caspase-1-dependent process is termed as "canonical" inflammasome activation [Figure 1].

A "noncanonical" inflammasome activation has been described as a pathway that is dependent on caspase-11 (in mice) or caspase-4/5 (in humans).

Definitions

- Polymerization is any process in which relatively small molecules called monomers combine chemically to produce a very large chain-like or network molecules called a polymer
- Oligomerization is a chemical process that converts monomers to macromolecular components through a finite degree of polymerization
- Transcription is the process where the genetic information on a DNA strand is transferred into an RNA strand by a series of polymerization reactions catalyzed by enzymes called DNA-dependent RNA polymerase
- The translation is the process of protein synthesis where the information on RNA is expressed in the form of polypeptide chains
- Cytokines are polypeptide substances produced by activated lymphocytes (lymphokines) and activated monocytes (monokines).

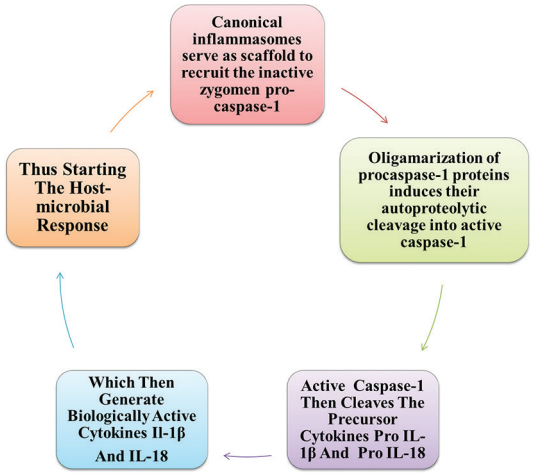

Figure 1: pathway of Inflammasomes Mariathasan (2004)

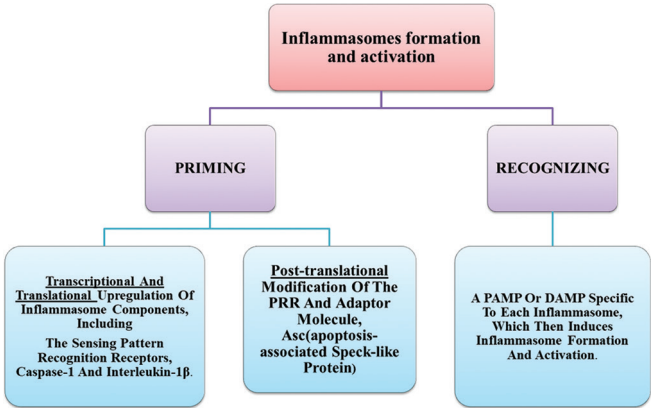

Figure 2: inflammasomes Activation & Formation Marchesan (2000)

Role of Inflammasomes in Periodontal Diseases

The maturation of IL-1 β and its subsequent secretion are dependent on an oligomeric assembly of a multiprotein complex called the inflammasome. IL-1 β levels are expressed higher in the serum, GCF, saliva, and gingival tissue of periodontitis patients, and this cytokine is a potential marker in the management of the disease. IL-1 can also enhance the expression of the receptor activator of nuclear factor-kappa B ligand (RANKL) on osteoblasts. RANKL is an osteoclastogenic factor that upregulates alveolar bone loss. Thus, the unbalanced production of pro- and anti-inflammatory cytokines induces severe damage in the periodontal tissue. IL-1, IL-8, and tumor necrosis factor-α are produced by fibroblasts and promote neutrophil chemotaxis in the inflamed periodontal site. Cytokines are soluble mediators produced by resident cells (epithelial and fibroblasts) and phagocytes in the early chronic phases of periodontal inflammation and by T- and B- lymphocytes in established and advanced lesions in the periodontium [Figure 3].[1,7]

Role of *Porphyromonas gingivalis* in Periodontitis

Porphyromonas gingivalis is a Gram-negative oral anaerobe that is involved in the pathogenesis of periodontitis, an inflammatory disease that destroys the tissues supporting the tooth which eventually may lead to tooth loss. Among the over 500 bacterial species living in the oral cavity, a bacterial complex named "red complex" and composed of *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia* has been strongly associated with advanced periodontal lesions (C. Bodet 2007).

P. gingivalis can locally invade periodontal tissues and evade the host defense mechanisms. In doing so, it utilizes a panel of virulence factors that cause dysregulation of the innate immune and inflammatory responses (N. Bostanci 2012).

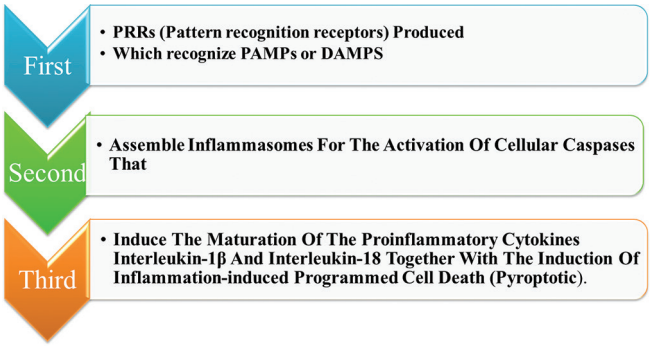

Figure 3: maturation Process of Cytokines By Inflammasomes Mariathasan *et al* 2004[10]

Importance of NLRP3 Inflammasome

The innate immune response is the body's first line of defense against pathogens. The innate immune system recognizes pathogens, including bacteria and viruses, by engaging the germline encoded with PRR. There are five families of PRRs that are able to sense a vast array of microbial components, referred

1. PAMP
2. DAMP.

PAMP and DAMP are host cell components produced during inflammation or environmentally derived factors [Figure 2].[2,3]

The suppression of the innate immune response by *P. gingivalis* in human macrophages could facilitate the maintenance of the chronic state of infection during periodontal diseases. Insufficient inflammation can lead to persistent infection of pathogens and excessive inflammation can cause chronic or systemic inflammatory diseases.[4] Therefore, it is important that the host balances inflammasome activation. Because inflammasome activation is highly inflammatory, it is tightly regulated to prevent aberrant activation. With the exception of human monocytes, inflammasome activation is a two-step process.

The cell must respond to two sequential signals for inflammasome formation and activation to occur [Figure 4].[1,2]

Inflammasomes are widely recognized to be activated in myeloid cells, including monocytes, macrophages, dendritic cells, and neutrophils, they can also be activated in keratinocytes, gingival and dermal fibroblasts, and mucosal epithelial cells. Deregulated inflammasome activation may cause uncontrolled IL-1 β release which can cause damage to the host and result

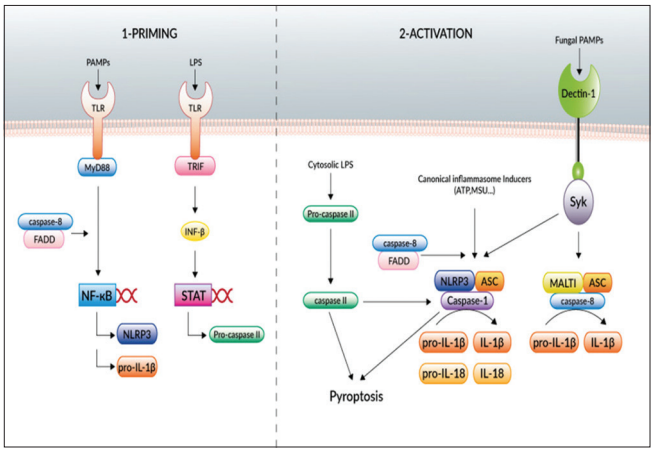

Figure 4: priming and Activation Process of release of NLRP3 inflammasome (Trends in Biochemical Science, Yuan He 2016)

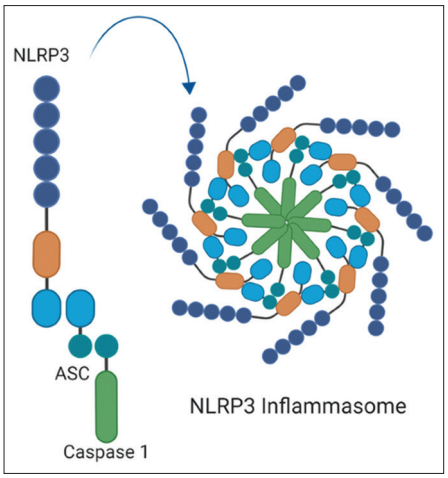

Figure 5: structure of NLRP3 Inflammasomes (Max Silvis 2021)

in autoinflammatory and/or autoimmune conditions, in diseases such as familial Mediterranean fever and cryopyrin-associated periodic syndromes (Ting JP 2006).

Notably, these conditions responded to the treatment with an IL-1 receptor antagonist. In periodontal disease, 1 month after nonsurgical treatment, secretion of IL-1 levels was still elevated in periodontitis patients, although clinical improvements were seen (Yoshinari N 2006).

NLRP3 Inflammasome

The nucleotide-binding oligomerization domain-like receptor (NLR) inflammasomes are intracellular PRRs that detect PAMPs [Figure 5].

The NLRP3 inflammasome, in particular, consists of three components:

- The NLRP3 "sensor,"
- The caspase-1 "effector" and the
- Apoptotic speck protein containing a C-terminal caspase recruitment domain (ASC) "adaptor" that links the former two molecules.

NLRP3 is activated by cell stresses and bacteria or viruses.

Recent clinical evidence demonstrates that the expression of this inflammasome is higher in periodontally diseased tissues compared with healthy ones.

The inflammasome suppression by *P. gingivalis* occurs at the level of the second signal. These results are supported by the finding that *P. gingivalis* also can repress the secretion of IL-18, another cytokine that is known to be processed by the inflammasome.[6] The induction of IL-1 and cell death also is suppressed in primary human macrophages, further supporting the findings that *P. gingivalis* acts at the level of the second signal. The ability of *P. gingivalis* to stimulate first signal activation without second signal activation is unique among pathogenic bacteria.[13]

In addition, this selective first signal activation could explain why *P. gingivalis* has the ability to stimulate IL-1 β and cell death in monocytic cells (Taxman D 2011) but not macrophages. Immature monocytes are able to provide an endogenous signal that negates the requirement for second signal activation. Although *P. gingivalis* produces abundant gingival proteases, a similar mechanism for enzymatic modulation of bacterial immune-stimulatory components has not been determined to date.

Periodontal diseases may be modulated with the aid of inflammasome regulators which are normally inhibited during periodontal disease by microorganisms. The 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 disease.[8]

Belibasakis *et al*. in studied the downregulation of NLRP3 inflammasome in gingival fibroblasts by subgingival biofilms with the involvement of *P. gingivalis*. Subgingival biofilms with and without Pg were collected and human gingival fibroblasts were evaluated. Parameters studied were NLRP3, IL-1 β, ASC, and caspase-1. The study showed that NLRP3 and IL-1 β were reduced by subgingival biofilm including Pg. However, the lack of Pg prevented the downregulation of NLRP3 and IL-1 β expression.[3]

Xue *et al*. in 2015 studied the expression of NLRP3 and NLRP1 in the gingival tissue of periodontitis patients by reverse transcription-polymerase chain reaction method and immunohistochemistry. Gingival tissue samples were collected from chronic periodontitis patients, evaluated parameters were NLRP3 and NLRP1.[12,13] The study concluded that NLRP3 expression was higher in patients suffering from chronic periodontitis. Dan Zhao *et al*. in (2016) studied the activation of NLRP1 and NLRP3 inflammasomes which contributed to cyclic stretch-induced pyroptosis and release of IL-1 β in human periodontal ligament cells.[5] This study concluded that as inflammasomes have been reported to be involved in both programmed cell death and inflammation; further studies are required to elucidate the exact roles and signaling pathways of inflammasomes in stretch-induced periodontal inflammation and aggressive periodontitis than healthy patients.

Conclusion

In addition to the removal of damaged cells, inflammasomes are also involved in cell repair, metabolism, and proliferation. Various molecules believed to be involved in the maintenance of cellular homeostasis have been demonstrated to act as critical regulators of inflammasome function and vice versa. Newly uncovered functions for the inflammasome in cell metabolism and proliferation require further investigation as they play an important role in the initiation of cytokine production. An increase in cytokine production further leads to the initiation and aggravation of the periodontal disease. Evaluation of inflammasome regulators in periodontal disease is important and further studies are needed.

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Conflicts of interest

There are no conflicts of interest.

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