



## Original Research Article

**The Inflammasomes: A confounding chapter in Periodontics**

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## ABSTRACT

This review deals with one of the most perplexing discoveries made recently in the field of medicine which is the inflammasome. They are an integral part of the innate immunity and is known to play a major role in the inflammatory process. The inflammasomes have a diverse range of functions most of which still remain to be elucidated. There are different families of inflammasomes involved in the host response to an inflammatory process. This information provides us with the possibility of new targets for modulating the host responses, thus, enabling us to respond to the bacterial challenges more efficiently in future.

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**1. Introduction**

In the last two decades, substantial progress has been made regarding the understanding of mechanisms involved in the sensing of microbial pathogens by innate immune cells that form the first line of defense. Considerable research has been conducted in connection with the pathways that translate into the signal transduction and transcription of certain antimicrobial molecules and the means by which they activate adaptive immune responses. The innate immune system recognizes the infection and initiates responses for eliminating pathogens and repairing damaged cells. One significant multiprotein complex identified in these processes is the 'inflammasome'. Despite the fact that inflammasome activation is an integral part of the innate immunity, overt inflammasome activation has the potential to give rise to many autoimmune and metabolic disorders. This necessitates the importance of understanding the

processes involved here in physiological and pathological contexts to grasp the science behind inflammasome biology.

**2. What is an Inflammasome?**

Inflammasomes are the central signalling regulators of the innate immune system that rapidly recognize and trigger the body's response to infections and foreign substances that are potentially harmful to the host. They usually consist of a sensor protein (PRR), the adaptor protein apoptosis-associated speck-like protein (ASC) containing a caspase-recruitment domain (CARD), and the pro-inflammatory caspase -1.<sup>1</sup> Following this, a vast array of physiological and pathogenic stimuli activates pro-caspase-1 that causes the cleavage of pro-inflammatory cytokines like IL-1 $\beta$  and IL-18.<sup>2</sup> These cytokines which are extremely powerful molecules with a myriad of functions are regarded as an inevitable part of the host response in inflammation and the development of periodontal diseases.

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## 2.1. Mechanism of action

Its mechanism of action is mediated by the PRR connecting to the ASC via oligomerization with its ligand followed by ASC converting pro-caspase-1 to its active form of caspase-1 with its adaptor CARD.

They are activated during inflammation by a vast array of microbial components, like evolutionarily conserved microbial structures, known as pathogen-associated molecular patterns (PAMP) from bacteria, viruses or protozoans and the various endogenous molecules generated during tissue/cell damage such as ATP, uric acid crystals, and amyloid- $\beta$  fibrils are called damage-associated molecular patterns (DAMP). These may be host derived or environmentally derived. The germline encoded pattern recognition receptors (PRR) which are found both in immune and non-immune cells then recognise these molecules. Some of these receptors assemble inflammasomes that activate cellular caspases which, thereby, induce the maturation of pro-inflammatory cytokines into their biologically active forms along with the induction of inflammation-induced programmed cell death (pyroptosis). This, provides the host cell with dual defense mechanisms. Pyroptosis is a process occurring via an activating cleavage of cytoplasmic protein gasdermin D, which forms pores in the plasma membrane. Although it had been revealed in the past that caspase-1 activation resulted in cytokine maturation and pyroptosis, the details of the pathways involved were actually explored only later in a seminal paper by Martinon et al (2002) with the discovery of the inflammasome.

Now, a tight regulation to maintain a balance in inflammasome activation is of utmost importance. Inflammasome activation is usually a two-step process except in human monocytes (Figure 1):<sup>3</sup>

1) The first step is cell priming.

- This includes the recognition of various PAMPs or DAMPs that interact with a subset of many toll-like receptors, NOD1 or NOD2, or by the cytokines, tumor necrosis factor and IL-1 $\beta$ , resulting in transcriptional upregulation of inflammasome components by activating the transcription factor nuclear factor- $\kappa$ B.

- One goal here is to upregulate inflammasome components like the sensing PRR, caspase-1 and IL-1 $\beta$  through transcription and translation.

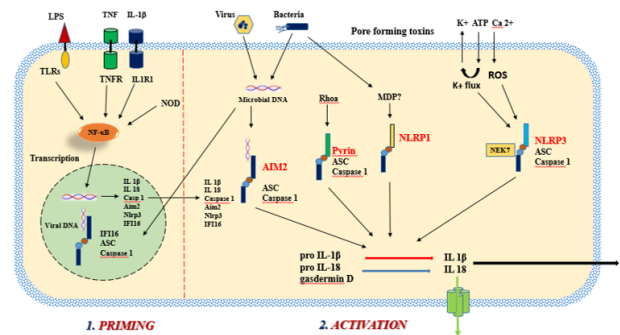
- The second goal here is the post-translational modification of the PRR and adaptor molecule ASC.

2) The second step is identifying a PAMP or DAMP specific to each inflammasome, which then induces the formation and activation of inflammasomes.

- Inflammasomes are named based on their respective PRRs that induce their activation. A number of PRR families, including a nucleotide-binding domain, leucine-rich repeat-containing proteins (NLR, also known as NOD-like receptors), absent in melanoma 2 (AIM2)-like receptors

(ALRs), toll-like receptor system (TLRs), C-type lectin receptors (CLRs) and retinoic acid-inducible gene I (Rig-I)-like receptors (RLR) have been identified.

- The earliest family of PRRs to be identified were toll-like receptors (TLRs) which were observed either on the cell surface or in intracellular compartments. RLRs and NLRs unlike TLRs and CLRs, are cytosolic proteins that recognise intracellular microbial molecules or danger signals.



**Fig. 1:** Inflammasome priming and activation. Inflammasomes should be primed before activation. Priming involves an increased expression of inflammasome components (IL1B, IL18, CAS1 AIM2, NLRP3, IFI16) induced by lipopolysaccharide or tumor necrosis factor- $\beta$  via nuclear factor- $\kappa$ B-activation. After this, each inflammasome is activated resulting in the formation of unique inflammasome complexes. This is followed by oligomerization of PRRs and subsequent recruitment of ASC to activate caspase-1. This caspase-1 then converts proIL-1 $\beta$  and proIL-18 into their mature forms (IL-1 $\beta$  and IL-18) which is then released by cells via gasdermin D-pores.

## 3. The NLR family

The pioneer family of sensor proteins that were found to assemble inflammasomes is the NOD-like receptor (or nucleotide binding domain and leucine-rich repeat containing receptor; NLR) family which is involved in the regulation of innate immune responses.<sup>4,5</sup> The NLR family was previously known as CATERPILLARs, NODs and NACHT leucine-rich repeats.<sup>4</sup>

Their members share a central nucleotide-binding domain (NBD) with most of them showing a C-terminal leucine-rich repeat (LRR) domain and a variable N-terminal domain. They are divided into four subfamilies depending upon their N-terminal domains. These four N-terminal domains include: (a) acidic transactivator domain (NLRA), (b) baculoviral inhibition of apoptosis protein repeat (BIR)-like domain (NLRB), (c) CARD and (d) pyrin domain. All NLRs except NLRP10 are categorized according to their domain structure and has a leucine-rich repeat (LRR) domain, a nucleotide binding domain (NBD), and a signalling domain.<sup>4</sup> The signalling domain either directly, through a CARD or indirectly through a PYRIN domain causes the recruitment of caspase-1. Hence,

they can be further grouped into NLRP or NLRC on the basis of whether the N terminus has a pyrin or caspase activation and recruitment domain (CARD), respectively. Certain members here like NLRP1, NLRP3, and NLRC4, have been understood as capable of forming inflammasomes while others, like NLRP6 and NLRP12, are still considered putative. Furthermore, newer families involved in forming inflammasomes such as the AIM2-like receptor (ALR) family,<sup>6</sup> and the RIG-I-like receptor (RLR) family were discovered recently.<sup>7</sup>

NOD receptors not only sense microbes and damaged cells but also promote activation of NLRP3 and NLRP1 inflammasomes. The association between NOD2 mutations and the increased susceptibility to the development of Crohn's disease, an autoinflammatory disorder of the gastrointestinal tract signified the importance of NOD2 in inflammatory disease processes. In addition, recently an association between inflammatory bowel disease and an increased risk for development of periodontitis was also reported.<sup>8</sup> Increased expression of NOD1 and NOD2 in the epithelial cells and inflammatory cells of humans in both healthy and diseased gingiva with no significant difference was reported among various periodontal conditions.<sup>9</sup> A remarkable reduction in alveolar bone resorption and osteoclastogenesis was seen in a study on mice carried out in preclinical settings that suggested the possibility of NOD2 triggering periodontal bone loss.<sup>10</sup>

#### 4. NLRP1 inflammasome

The first ever NLR described to form an inflammasome complex was NLRP1. It is encoded by a single gene in humans that contains a PYD (pyrin) domain, FIIND (function-to-find domain), and CARD domain.<sup>11,12</sup> Recent research has shown that NLRP1 signalling is induced by the peptidoglycan component muramyl dipeptide (MDP) of bacterial cell walls and anthrax lethal factor (LF) of the *Bacillus anthracis* lethal toxin (LeTx).<sup>12,13</sup> The toxin consists of protective antigen (PA) and lethal factor (LF). These two proteins create pores in the host cell membrane and subsequently activates NLRP1 $\beta$ <sup>14</sup> causing inflammasome assembly.

It was believed earlier that the human NLRP1 comprised of NLRP1, ASC, caspase-1, and caspase-5. Processing of caspase-5 is stimulated by the interaction between NLRP1 and caspase-5 but the adaptor protein ASC is required to mediate the interaction and to subsequently process caspase-1.<sup>11</sup> Despite all the observations made, the specific triggers of human NLRP1 and murine NLRP1a/c still remain to be discovered. It is even possible that they may have different roles in mouse and man as some reports showed that ATP can bind to NLRP1 thus activating it.<sup>11</sup> ATP binding was inhibited by the anti-apoptotic proteins BCL-2 and BCL-xl through inhibition of NLRP1 inflammasome activation.<sup>5</sup>

Interestingly, a role for NOD2 (NLRC2) in the NLRP1 inflammasome assembly in response to both MDP and anthrax lethal toxin was suggested.<sup>15</sup> NOD2 is a sensor for MDP and initiates activation of NF- $\kappa$ B and mitogen activated protein kinase (MAPK) via an adaptor protein RIP2 (receptor-interacting-serine/threonine-protein kinase 2).<sup>16</sup> In a cell-free system, NLRP1 can activate caspase-1 in response to MDP but to detect both MDP and LeTx in vivo, NOD2 is required. NOD2 seemed to generate signals necessary for caspase activation, pro-IL1 $\beta$  expression and secretion of mature IL-1 $\beta$ .<sup>15,17</sup> However, further research is required to comprehend the exact function of NLRP1 and NOD2 in response to anthrax lethal toxin and MDP. The risk for developing several autoimmune diseases, such as generalized vitiligo, vitiligo-associated type I diabetes, and Addison's disease<sup>18,19</sup> rheumatoid arthritis, systemic sclerosis, and Crohn's disease were attributed to single nucleotide polymorphisms in the human genomic NLRP1 region.<sup>20</sup> The participation of NLRP1a in pyroptosis of hematopoietic cells were also observed due to mutations resulting in hyperactive inflammasomes and IL-1 $\beta$  release.<sup>21</sup> However, the specific stimuli involved in regulation of NLRP1a inflammasome are elusive and only further research in the area can define the molecular mechanisms behind it.

Decreased NLRP1 expression was observed in the samples of healthy, chronic and aggressive periodontitis gingiva with more frequent expression in the epithelium and connective tissue of individuals with aggressive periodontitis. Most studies attempting to explore its role have concentrated on autoinflammatory diseases, with little information on it in the context of periodontal diseases.

#### 5. NLRP3 inflammasome

NLRP3 is undoubtedly the most extensively studied NLR. Its expression is low in myeloid cells and Toll-like receptor agonists, such as lipopolysaccharide (LPS), and inflammatory cytokines, such as TNF- $\alpha$  transcriptionally induces it.<sup>5</sup> The involvement of NLRP3 has been detected in a considerable number of inflammatory diseases like diabetes mellitus, obesity and atherosclerosis. As we understand, NLRP3 activation is basically a two-step process and three models pertaining to its activation were put forward which include: the ion flux model, the reactive oxygen species (ROS) model, and the lysosome rupture model.<sup>22</sup> In the ion flux model, there were changes in cytosolic levels of K<sup>+</sup>, Ca<sup>2+</sup>, and H<sup>+</sup>. Extracellular ATP induces rapid K<sup>+</sup> efflux via activation of the ATP-gated ion channel P2X7;<sup>23</sup> Nigericin creates a K<sup>+</sup> pore in the cell membrane; the influenza M2 protein triggers export of H<sup>+</sup> ions from the Golgi complex into the cytosol; and high concentrations of extracellular Ca<sup>2+</sup>, increase cytosolic Ca<sup>2+</sup>, and cAMP (cyclic adenosine monophosphate).<sup>22</sup>

The generation of reactive oxygen species (ROS) by many NLRP3-activating stimuli, like adenosine triphosphate (ATP), alum, uric acid, and Nigericin<sup>1</sup> has been observed in NLRP3 activation although its exact function here is disputable. Some studies described an alternative role for ROS in inflammasome activation where oxidized mitochondrial DNA released from dysfunctional mitochondria can directly activate NLRP3.<sup>24</sup> Recently, NIMA(never in mitosis gene a)-related kinase 7 (NEK7), a serine-threonine kinase was also observed to induce NLRP3 activation.<sup>25,26</sup> There is still no clarity regarding NEK7 binding and NLRP3 oligomerization, although it is required for ASC polymerization.<sup>27</sup> Another recently observed regulator of NLRP3 activation was PKD (protein kinase D).<sup>28</sup>

The non-canonical pathway of NLRP3 activation was more complex<sup>29</sup> than the canonical pathway that activates NLRP3 directly. The noncanonical pathway involves activation of caspase-11 by intracellular LPS<sup>30</sup> but the exact mechanism here still remains to be understood. Nevertheless, currently there is no agreement on a standard mechanism explaining NLRP3 activation due to the diverse range of stimuli involved here. Not many clinical studies have explored the relation of NLRP3 with periodontal disease. A four to five fold increase in NLRP3 mRNA expression was reported in gingival tissues of individuals with chronic periodontitis with almost a seven fold increase in case of aggressive periodontitis.<sup>31,32</sup> Immunohistochemistry revealed that the increased expression was more pronounced in the epithelial layer, thus suggesting the role of NLRP3 in epithelium as part of the host's innate immunity in the gingival sulcus and tissues.<sup>31</sup> Even inflammation confined to gingiva or that which has not reached the bone increased NLRP3 levels.<sup>32</sup> Salivary samples of individuals with chronic and aggressive periodontitis also reported remarkably increased expressions of NLRP3.<sup>33</sup> Moreover, a positive correlation between mRNA expressions of NLRP3 and IL1 $\beta$  and IL18 in gingival tissues was reported.<sup>32</sup> Nevertheless, no study has described a genetic background for NLRP3 with regard to periodontal disease as yet.

## 6. NLRP6

As opposed to the case of NLRP3, non-hematopoietic cells like epithelial cells and goblet cells in the intestine show an increased expression of NLRP6,<sup>34</sup> although there are some who claim that its expression is mainly observed in hematopoietic cells.<sup>35</sup> It has been shown to cause strong proinflammatory effects in NLRP6-deficient mice in response to bacterial challenge due to negative regulation of NF- $\kappa$ B and MAPK signalling pathways.<sup>36</sup> Macrophages infected with *Listeria monocytogenes*, *E. coli*, and *Salmonella* also demonstrated similar processes.<sup>35</sup> With this background, the role of NLRP6 in caspase-1 activation at

mucosal sites was believed to be of utmost importance.<sup>34</sup> It was demonstrated that subsequent IL-18 release promoted the production of antimicrobial peptides which act against microbial dysbiosis.<sup>37</sup> The identity of the ligand activating NLRP6 remained elusive until recently taurine, a microbial metabolite was described.<sup>38</sup> Mucosal immunity in the gut has been attributed to the production of a specific metabolite profile enriched in taurine by the commensal non-pathogenic microbiota that binds to NLRP6.<sup>38</sup> All these data imply its key role in gut homeostasis and associated disorders.

Furthermore, in the context of viral infection, it was demonstrated that systemic infections with encephalomyocarditis and norovirus caused NLRP6 to affect viral loads at mucosal sites specifically in the gastrointestinal tract.<sup>39</sup> It was also reported that oral infections with the same viruses needed NLRP6-mediated defense to survive.<sup>40</sup> However, adequate research has not been conducted to establish its importance at nonmucosal sites.

## 7. NLRP12

NLRP12 is similar to NLRP6 in many ways. NLRP12 also has shown to be involved in the regulation of NF- $\kappa$ B and MAPK pathways to maintain intestinal homeostasis.<sup>41</sup> In addition, it was observed that after *Yersinia pestis* infection, IL-18 and IL-1 $\beta$  production was regulated by this inflammasome and that NLRP12-deficient mice were more vulnerable to bacterial challenge.<sup>42</sup> Nevertheless, the specific triggers for NLRP12 activation and the precise mechanisms involved still remains unknown as is the case with NLRP6.

## 8. NLRC4 inflammasome

NLRC4 (also known as IPAF, Card12) is found to be involved in infections with various gram-negative bacteria. It contains an N-terminal CARD, a central NBD, and a C-terminal LRR domain.<sup>5</sup> NLRC4 activation, like other NLRs involves the deletion of the LRR (leucine rich repeat) domain. Sometimes CARD – CARD interactions were observed in the association of NLRC4 specifically with the CARD domain of pro-caspase-1.<sup>43</sup> However, it is noteworthy that there are other studies demonstrating that NLRC4-mediated caspase-1 activation needs the functional involvement of ASC. Hence, the requirement of ASC here is disputable. It maybe that ASC stabilizes or facilitates caspase-1 recruitment to NLRC4. Alternatively, NLRC4 may also recruit ASC via PYD–PYD interaction by cooperating with a PYD-containing NLRP protein to activate caspase-1.<sup>5</sup>

NLRC4 recognizes the following components of pathogenic bacteria:<sup>44</sup>

1. Flagellin, the building block of their locomotion apparatus, and
2. Proteins from the type III and IV bacterial secretion systems known to inject virulence factors into the host cell.

The flagella which is a structure on the bacterial cell wall that allows bacterial motility contain polymers of the protein flagellin which are its structural compounds. Flagellin is an apt example of a PAMP as it is an evolutionarily conserved element that aids in bacterial motility. NLRC4-deficient macrophages show a significant reduction in caspase-1 activation, release of IL-1 $\beta$ , and pyroptosis following infection with the gram-negative *Salmonella typhimurium*,<sup>45</sup> *Legionella pneumophila*,<sup>46</sup> *Pseudomonas aeruginosa*, and *Shigella flexneri*.<sup>45</sup> These bacteria share either a type III (T3SS) or type IV secretion system. An impaired ability of *S. typhimurium* and *L. pneumophila* strains deficient in flagellin to activate caspase-1 in macrophages were initially observed.<sup>46</sup> Even the delivery of purified flagellin into the host cell activated caspase-1 in an NLRC4-dependent manner. Notably, caspase-1 activation by NLRC4 is independent of TLR5.<sup>47</sup> These results led many to hypothesize that flagellin is the primary PAMP activating NLRC4. However, it was observed that only flagellin from certain bacteria activated NLRC4. Even if delivered to the host cytosol, flagellin of *E. coli* does not activate inflammasomes.<sup>48</sup> On the other hand, the flagellated gram-positive *Listeria monocytogenes*, was shown to trigger NLRC4 activation, even though they lack a secretion system. This provides flagellin access to the cytosol and thus NLRC4 and *Listeria* escapes the phagolysosome to multiply in the cytosol.<sup>49</sup> On the contrary, the nonflagellated *S. flexneri* was shown to activate caspase-1 in an NLRC4-dependent manner.<sup>5</sup>

It was demonstrated recently that phosphorylation of NLRC4 was necessary for inflammasome assembly.<sup>22</sup> Surprisingly, intraperitoneal delivery of flagellin in mouse activated NLRC4 which induced host mortality within 30 minutes. It is quite interesting to note that the inflammasome-dependent response here is determined by the production of eicosanoids and not IL-1 $\beta$  or IL-18. This is followed by a rapid loss of vascular fluid and mortality.<sup>50</sup> The mechanism governing this response is currently unknown but it highlights its relevance in cellular responses.

## 9. ALR family

The ALR family has four members in humans out of which IFI16 (Interferon Gamma Inducible Protein 16) and AIM2 (absent in melanoma 2) are the only inflammasome-forming members. While AIM2 identifies only double-stranded DNA, IFI16 is able to identify both single-stranded and double-stranded DNA of various viruses and

intracellular bacteria. They are characterized by an N-terminal PYD and a C-terminal hematopoietic interferon-inducible nuclear protein with 200-amino acid repeat (HIN200) domain. In AIM2, the PYD is capable of interacting with the PYD of ASC, unlike other ALRs.<sup>39</sup> This specific interaction recognises AIM2 as the only member of the family capable of forming an inflammasome. The human ALR,  $\gamma$ -IFN-inducible protein 16, is considered a putative inflammasome.<sup>39</sup>

## 10. AIM2-Like Receptors

AIM2 (absent in melanoma 2) was originally identified as a  $\gamma$ -IFN-inducible protein in a tumor suppressor screen. Later on, it was shown to detect nucleic acids that played a role in assembling inflammasomes, especially double-stranded DNA which could either be of bacterial/viral origin or self DNA from apoptotic cells.<sup>28,51</sup> AIM2 and the ALRs belong to the PYHIN family, which comprises of proteins that have a PYRIN domain and a hematopoietic IFN-inducible nuclear protein with 200-amino acids (HIN-200) domain.<sup>6</sup> Structural analysis also attributed an autoinhibitory role to the HIN200 domain due to its interaction with the PYD of AIM2 in the absence of a ligand.

Although AIM2 binds to both viral and bacterial DNA, there is a remarkable difference in the mechanisms involved in each case. Type I IFN signalling is involved in AIM2 activation induced by bacteria such as *Francisella*, but not viruses like mouse cytomegalovirus.<sup>52</sup> Be that as it may, not all bacteria and viruses activate AIM2 inflammasome. Recent studies point towards certain bacteria, such as *Mycobacterium* and *Legionella*, that can reduce dsDNA release and thereby avoid detection by AIM2.<sup>53</sup> Recently a link between the pathology associated with chemotherapeutic drug therapy and AIM2 without affecting their anti-cancer properties was observed.<sup>54</sup> Various autoinflammatory diseases, such as psoriasis, systemic lupus erythematosus, and abdominal aortic aneurysm<sup>55</sup> have been associated with AIM2. Therefore, all these observations raise the possibility of AIM2 being a therapeutic target for these autoimmune disorders in future.

Another member of the HIN200 family, p202, lacks the N-terminal PYD and may negatively regulate AIM2 inflammasome.<sup>56</sup> In addition to AIM2, humans have three more ALRs, IFI16, IFIX (interferon-inducible protein X), and MNDA (myeloid cell nuclear differentiation antigen), whereas mice express an extended range of ALRs consisting of at least 13 members.<sup>57</sup> Although most of these are poorly characterized, many murine ALRs were reported to have triggered IL-1 $\beta$  production, suggesting their role in inflammasome assembly.<sup>57</sup>

## 11. IFI16 and RIG-I-Like Receptors

The ability of human IFI16 to form an inflammasome in response to Kaposi's sarcoma-associated herpes virus infection, and activation of IFI16 in quiescent CD4+ T cells during HIV infection was found to trigger extensive pyroptosis of T cells. Hence, it is observed to be a significant contributor of depletion of CD4+ T cells during progression to AIDS.<sup>58</sup> Some of IFI16's are even capable of modulating AIM2 either through its upregulation or down regulation.<sup>59</sup> Finally, the RIG-I like receptor (RLR) family member RIG-I, which is best known to stimulate type I IFN production on recognition of viral RNA, was also found to be capable of forming an inflammasome.<sup>22</sup> However, it still remains to be clear as to what exactly determines if RIG-I should form an inflammasome or if it should just trigger type I IFN production. Although the expression of IFI16 was higher in inflammatory bowel diseases like Crohn's disease and ulcerative colitis, the exact function of IFI16 and AIM2 in periodontal disease pathogenesis remains elusive. In periodontitis models, its variations in tissue expression were observed depending on periodontal disease status, limited tissue characterization and correlation of specific gene variants.<sup>60</sup> AIM2 levels were almost two times higher in the lamina propria and epithelium of chronic periodontitis and aggressive periodontitis cases.<sup>31</sup>

Current research indicates an upregulation of IFI16 and AIM2 in diseased tissues which may reflect a disruption of the epithelial barrier function. Any variants of these proteins seemed to affect their expression or function thus, predisposing an individual to periodontal disease by bringing about changes in the oral microbial composition.

## 12. Therapeutics targeting the inflammasome

The host response has been long established as a major factor in contributing to periodontal destruction through inflammation and disruption of tissue homeostasis. Most of the treatment approaches to improve periodontal status of an individual focused on antimicrobial therapy rather than modulation of host response. An uncontrolled inflammasome activity results in pro-inflammatory effects contributing to the development of many diseases. Therefore, approaches involving inhibitors/antagonists targeting inflammasome components, their activation status and cytokine production are important in treating periodontal diseases.

### 12.1. Inhibiting the upstream intracellular signaling pathways

1. Many upstream signals like ion flux, lysosomal disruption, mitochondrial damage and ROS can cause NLRP3 activation. A drug called Allopurinol is considered a standard treatment of hyperuricemia associated with treatment of gout (a form of arthritis

that is NLRP3 inflammasome dependent)<sup>61</sup> and kidney stones.<sup>62</sup>

2. The therapeutic drug SS-31 selectively targets cardiolipin, a phospholipid in the inner mitochondrial membrane.<sup>63</sup> This prevents excessive ROS production. However, no studies have evaluated the effects of these drugs in periodontal diseases in a preclinical or clinical setting so far.

### 12.2. Blocking inflammasome components

1. IL-1 $\beta$  and IL-18 release is preceded by caspase-1 activation. Three drugs targeting caspase-1, Emricasan, VX-740 and VX-765, have been tested in humans.<sup>64</sup> The micro-computed tomography results here showed that caspase-1 inhibition caused a 50% reduction of alveolar bone loss than normal.
2. Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase, best known for its important role during B cell development. During NLRP3 activation, it communicates with NLRP3 and ASC.<sup>65</sup> The BTK inhibitor ibrutinib (PCI-32765, Imbruvica) is a FDA-approved drug for the treatment of various B cell cancers. Recently, another BTK inhibitor, acalabrutinib (ACP-196), has shown to prevent alveolar bone loss<sup>66</sup> indicating its possible role in treating periodontal diseases.
3. Recently, it was observed that interleukin-1 $\beta$  levels reduced to almost half in experimental periodontitis<sup>67</sup> during colchicine treatment.

### 12.3. Inhibiting inflammasome-mediated cytokines

1. Interleukin-1 $\beta$  is the most potent pro-inflammatory cytokine released by activation of inflammasomes and is implicated in many inflammasome-associated diseases and plays a critical role in the periodontal disease progression.<sup>68</sup> eg: monoclonal antibody targeting IL-1 $\beta$  (canakinumab), by a modified IL-1 $\beta$  receptor antagonist (anakinra) or by a soluble decoy receptor (rilonacept). A combination of IL-1 inhibitor and TNF $\alpha$  inhibitor in a study, demonstrated a 50% reduction in the levels of radiographic bone loss compared to the control sites<sup>69</sup> suggesting that both IL-1 $\beta$  and TNF- $\alpha$  may act as potential targets for treating periodontal disease.
2. GSK1070806 is an anti-IL-18 monoclonal antibody. It was studied in a diabetic population where no improvements in glucose control were observed.<sup>70</sup> Nevertheless, further research is required to evaluate these therapeutics targeting IL-18 in the context of periodontal diseases.

### 13. Conclusion

This is a brief review explaining the functions and activation mechanisms of inflammasomes illustrating their increasingly significant roles in infectious, autoimmune, and autoinflammatory diseases. While some of them have been established for years, further research is paramount to elucidate many of the unique post-translational regulatory mechanisms that modulate their activation, repression and susceptibility to various infections. The available evidence indicate that various inflammasome components are expressed in higher levels in saliva, gingival crevicular fluid and periodontal tissues. In light of the observations made so far, it is possible that individuals with autoinflammatory diseases may have certain unknown biological pathways that may lead to periodontal disease. Furthermore, the pathways involved in the regulation of each of these inflammasomes may present new targets for drugs, thereby resulting in minimal pathological effects in many diseases.

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### 15. Conflict of Interest

The authors declare they have no conflict of interest.

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