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Review

The Role of Inflammasome NLPR3 in the Development and Therapy of Periodontitis

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Abstract

Periodontitis is a chronic inflammatory disease that affects tooth-supporting tissues and even leads to tooth loss. NLRP3 inflammasomes play a critical role in periodontitis pathogenesis. Aberrant activation or overexpression of NLRP3 inflammasomes in cellular players, including osteoclasts, osteoblasts, periodontal ligament fibroblasts, and leukocytes often contributes to cellular dysfunction and environment abnormality, thus resulting in the disorganization of ligament and alveolar bone. In this review, we mainly focus on the negative regulation of NLRP3 inflammasome in periodontitis and highlight the importance of NLRP3 inflammasome as a candidate therapeutic target in periodontitis treatment. Then we elucidate the development status of NLRP3 inflammasome inhibitors and show their application potential for treating periodontitis. In summary, this review reveals the recent progress and perspectives of NLRP3 inflammasome and the therapeutic potential of NLRP3 inflammasome inhibitors in periodontitis.

Key words: Periodontitis, NLPR3 Inflammasome, Periodontal Ligament Cell, Osteoclast, Osteoblast, Leukocyte

Introduction

Periodontitis is a chronic inflammatory condition caused by plaque-associated bacteria that cause an inflammatory reaction in the tooth supporting tissues. Periodontitis is the sixth most common disease in the world, affecting around 743 million people and having a high prevalence of 11.2 percent [1, 2]. Periodontitis has become the most common cause of tooth loss all over the world [3]. In addition, growing data suggests that periodontitis may be a risk factor for a variety of systemic diseases, such as cardiovascular diseases (CVD) [4], diabetes [5], Alzheimer's disease [6], rheumatoid arthritis [7], adverse pregnancy outcomes [8], and cancer [9]. Periodontitis is not just a localized oral disease but also influences systemic health of individuals. Therefore, there is an urgent need to understand the mechanisms of periodontitis pathogenesis, which is essential for developing effective therapies and preventive approaches against periodontitis [1].

A great deal of evidence shows that both environmental and genetic factors contribute to periodontitis pathogenesis [10-12]. Plaque biofilm is the initiating factor of periodontitis [13-15]. The pathogenesis of periodontitis involves the complex interaction of multiple cell types, such as epithelial cells [16-18], immune cells [19-21], osteoclasts, osteoblasts, and periodontal ligament fibroblasts. It is worth noting that several activated proinflammatory transcription factors [11, 22, 23], inflammatory cytokines [24], and tissue-destructive molecules [25, 26] provide a network of signals to regulate the intracellular signaling which are vital for the pathological changes of periodontitis tissues (Figure 1). But unfortunately, the definition and diagnostic criteria of periodontitis have not been unified. Thus, based on the changes of inflammation related proteins, there have been an increasing number of

studies searching for periodontitis biomarkers [10, 27, 28].

In recent years, increasing evidences confirmed that a complex of nucleotide-binding oligomerization domain-like receptor (NLR) complexes named "inflammasome" functions in periodontium immune response [29, 30]. Inflammasomes are the master regulators of the innate immune system in chronic diseases, and they take part in controlling and limiting invading microbes [31]. Appropriate inflammasome-mediated inflammation and cell death are conductive to reversing the adverse effects to promote tissue regeneration. Conversely, overexpression and excessive activation of the inflammasome often leads to uncontrolled inflammation, cytokine storm, tissue damage, and autoinflammatory and autoimmune diseases [32, 33]. Similar to other early inflammatory related protein of periodontitis, growing evidences have shown elevated inflammasome levels in saliva and serum in patients with periodontitis, which correlate positively with the severity of periodontitis [27, 34-37]. Therefore, in this review, we mainly focus on the negative regulation of inflammasome and illustrate the importance of inflammasome as a potential therapeutic target in periodontitis therapy.

To date, several inflammasomes have been described. NLRP3, as the most studied inflammasome, is activated by the infected pathogens and releasing of endogenous danger signals and then drives pathological inflammation in periodontitis [38-40]. In this review, we describe the recent progress and our current understanding of NLRP3 inflammasome pathogenesis in periodontitis. With the goal to provide information for future study and clinical practice, we focus on the molecular mechanisms that activate and regulate excessive NLRP3 inflammasome, then we explore NLRP3 inflammasome activation and its physiopathological consequences in periodontitis. Finally, we also discuss the recently identified NLRP3 inflammasome inhibitors to provide insights into therapeutic strategies for treating periodontitis mediated by NLRP3 inflammasome.

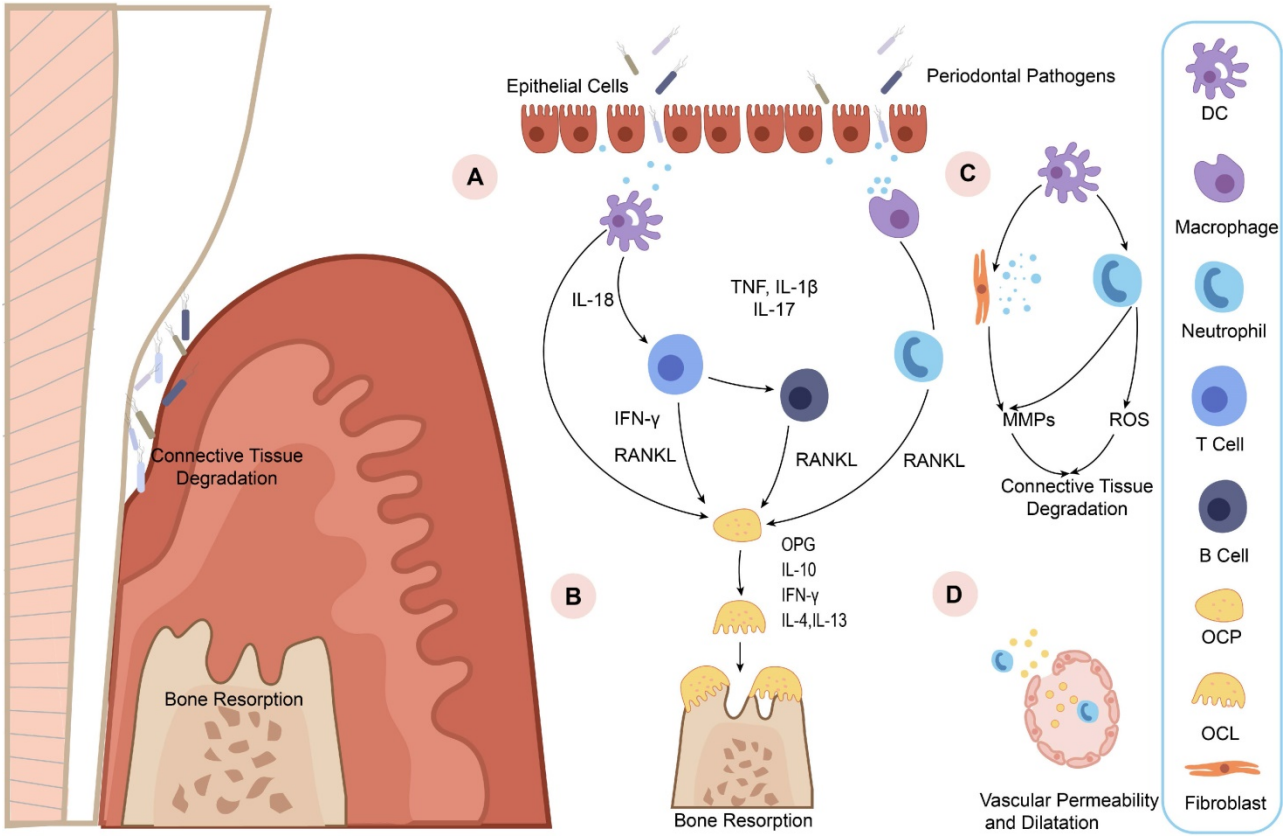

Figure 1. Diagrammatic representation of periodontitis pathogenesis. A. Increasing epithelial permeability leads to the invasion of pathogens, triggering immune cells to detect lipopolysaccharide (LPS) in the pathogens and pro-inflammatory cytokine production. Then the pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1β (IL-1β), interleukin-17 (IL-17), and IL-18 may activate neutrophils and osteoblasts to express RANKL and drive osteoclast maturation. **B.** OPG is an osteoclastogenesis inhibitor that acts as a soluble RANKL decoy receptor under inflammatory microenvironment. The imbalance of RANKL and OPG directly stimulates osteoclastogenesis. IL-10, IFN-γ, IL-4, and IL-13 then block osteoporosis by inhibiting osteoclastogenesis. **C.** Immune cells release MMPs and reactive oxygen species (ROS) to destruct and disorganize the extracellular matrix (ECM) in the periodontal tissue. **D.** Increased vascular permeability allows pro-inflammatory mediators and antimicrobial peptides to enter the bloodstream and causes inflammation in distal areas.

Inflammasome in periodontitis

Pattern recognition receptor (PRR) is related to the activation of host innate immune response and adaptive immunity to periodontal pathogens. Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), and retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) are members of the PRRs [41]. PRRs can be activated in the host by recognizing molecules released by pathogens or damaged cells. These molecules are called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [42]. The inflammasome formation requires the PRRs that play a crucial role in innate immunity. The etiology of numerous inflammatory illnesses, including periodontitis, is due to improper inflammasome activation. The inflammasome is a multi-protein complex, consisting of a PRR, an active form of caspase-1, and an adaptor protein or apoptosis-related speck-like protein containing a caspase activation and recruitment domain (CARD) (ASC) [43]. Various types of inflammasomes have been identified, including Nod-like receptor pyrin domain-containing protein (NLRP1, NLRP2, NLRP3, NLRP6, NLRP12), NLR containing a CARD 4 (NLRC4), NLRC5, PYHINS, and absent in melanoma 2 (AIM2) [44]. Structurally, these family members share similar domain architectures (Figure 2). Existing evidences show that periodontitis is connected to these inflammasomes.

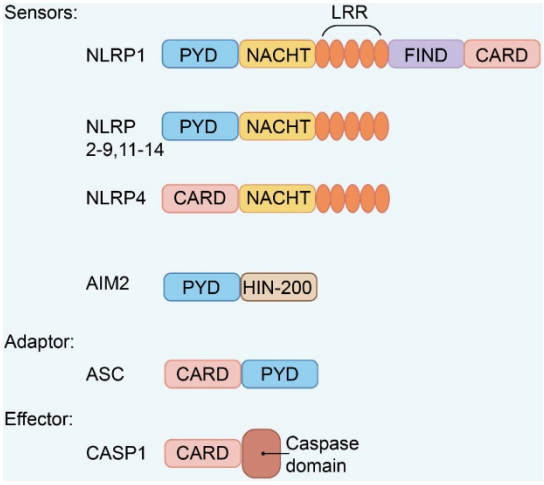

Figure 2. Domain architecture of representative inflammasome. Inflammasome family members with similar domain architectures including sensors, adaptor ASC, and effector CASP1.

NLRP1 is one of the first discovered inflammasomes, but how it is activated remains unclear, especially in periodontitis. NLRP1 contains CARD and pyrin domain (PYD) and mediates intracellular signaling processes including caspase-1 (CASP1) activation [45, 46]. The expression level of NLRP1 inflammasome has been evaluated in chronic periodontitis (CP) and aggressive periodontitis (AgP) [47]. Yilmaz et al. [48] reported that there was no difference in NLRP1 in *Porphyromonas gingivalis* (*P. gingivalis*) of human gingival epithelial cells. In AgP, NLRP1 showed a low expression level in the gingival tissues and expressed more frequently in the epithelium and connective tissues. These evidences suggest that the function of NLRP1 in periodontal disease remains unclear and needs further investigation. The AIM2 inflammasome has been reported in a variety of periodontitis investigations, and AIM2 has been identified as a susceptibility gene for periodontitis in a genome-wide association study (GWAS) with expression quantitative trait loci data [47, 49]. AIM2 has been demonstrated to be expressed in periodontitis gingival tissue, including gingivitis, CP, and AgP [47, 50]. The NLRP3 inflammasome is by far the best-studied and largest multimeric protein complex among these inflammasomes. The role of NLRP3 in periodontal disease has been extensively reported within the recent research. In what follows, we focus on the activation of NLRP3 inflammasome, the role of NLRP3 inflammasome in periodontitis pathogenesis and the therapeutic potential of NLRP3 inflammasome inhibitors in treating periodontitis.

NLRP3 inflammasome

The activation of NLRP3 inflammasome

NLRP3, a member of the NLR family of intracellular receptors, is a sensor that detects external pathogens and danger signals, triggering the formation and activation of the NLRP3 inflammasome. The adaptor (ASC) and effector (CASP1) are also contained in the NLRP3 inflammasome [51]. The NLRP3 inflammasome is activated by two distinct signals: a priming signal and an activation signal (Figure 3) [52].

Priming step

Priming serves at least two steps. The first step is to upregulate NLRP3, pro-IL-1, and caspase 1 expression and activate the NF-B signaling cascades by recognizing PAMPs, DAMPs, or LPS that engage PRRs like TLRs.

The second step is to cause NLRP3 to undergo PTMs. PTMs stabilize NLRP3 in an auto-suppressed inactive conformation before stimulation. For NLRP3, diverse kinds of PTMs have been identified, including ubiquitylation, phosphorylation, and SUMO. The PYD domain of NLRP3 can be phosphorylated. NLRP3 activation is inhibited by phosphorylation at Ser3 [53] and Tyr861 [54], whereas phosphorylation of

Ser198 by JUN N-terminal kinase 1 (JNK1) (also known as MAPK8) enhances NLRP3 activation [55]. Protein kinase D (PKD) [56] phosphorylates NLRP3 to promotes NLRP3 activation, whereas PKA inhibits NLRP3 activation [57]. Phosphorylation of Ser295 reduces NLRP3 ATPase activity and prevents NLRP3 activation [56]. It is not clear why NLRP3 is phosphorylated at the same site by PKA and PKD but has the opposite effects [56, 58]. Further studies are needed to clarify the phosphorylation of NLRP3.

By modulating the rate of NLRP3 breakdown, NLRP3 is deubiquitylated following priming and activation. NLRP3 Trp73 is recognized by Fbox/LRR-repeat protein 2 (FBXL2), which targets it for ubiquitylation and proteasomal destruction [59]. TLR stimulation increases F-box only protein 3 (FBXO3) expression, which degrades FBXL2. E3 ubiquitin ligase TRIM31 and membrane-associated RING finger protein 7 (MARCH7) triggered by TLR and IL-1R activation cause Lys48-linked ubiquitylation and degradation of NLRP3 [60, 61]. The leucine-rich-repeat domain (LRR domain) of NLRP3 is deubiquitylated and homo-oligomerized by activated BRCC3 in response to priming signals [62]. The protein E3 SUMO protein ligase MUL1 sumoylates NLRP3 in resting cells, inhibiting NLRP3 activation [63]. SENP6 and SENP7 desumoylate NLRP3 after activation, promoting inflammasome activation [63]. In conclusion, these results show how this dynamic landscape of PTMs delicately controls NLRP3 inflammasome activity. This crosstalk among the three different PTMs highlights the complex control of NLRP3 activation by the post-translational regulation.

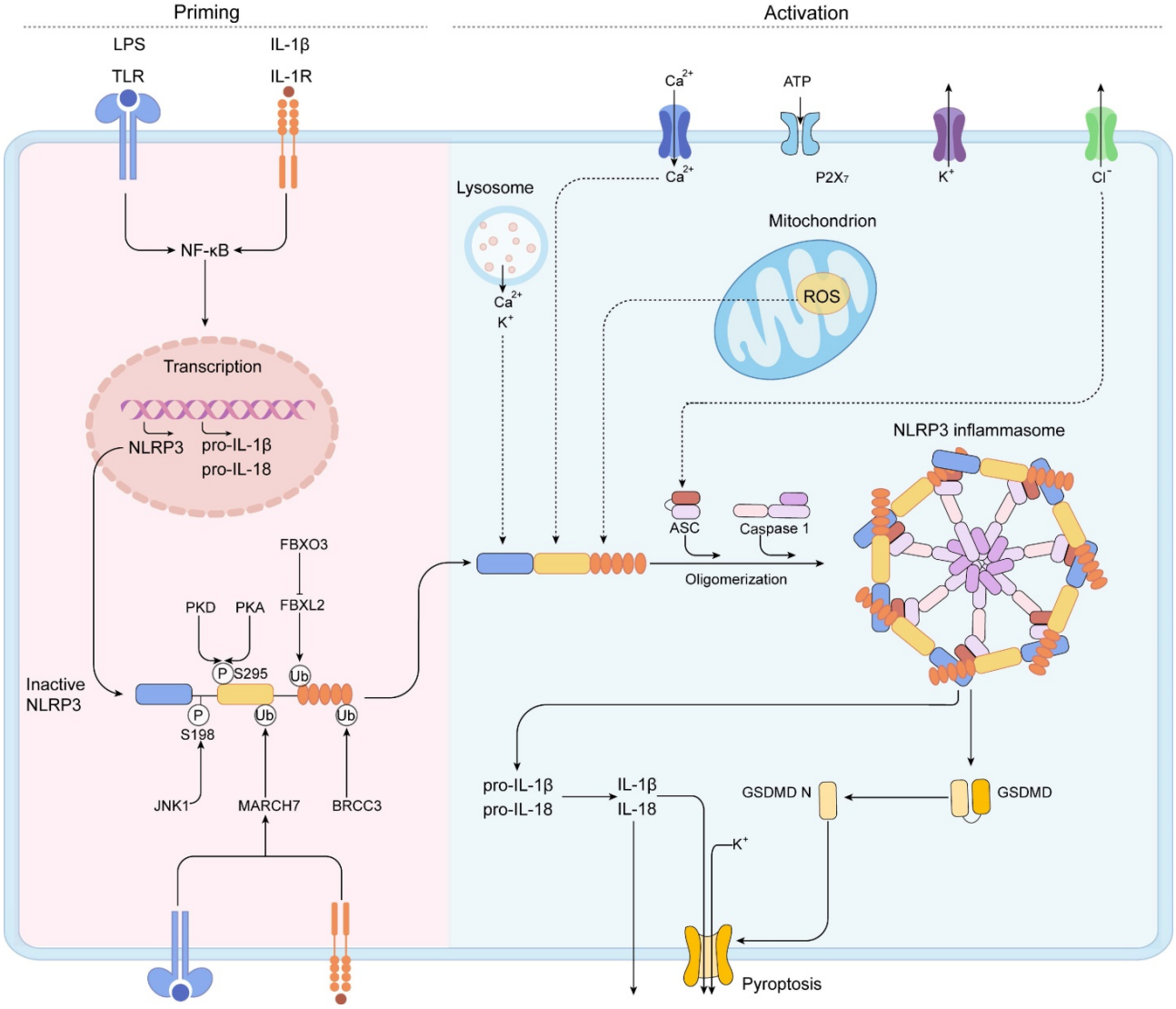

Figure 3. A two-step mechanism of NLRP3 inflammasome activation. The priming is triggered by the activation of cytokines or PAMPs, such as LPS and IL-1β, leading to the transcriptional upregulation of NLRP3 inflammasome components, including NLRP3, pro-IL-1β, pro-IL-18, caspase 1, and the activation of NF-κB signaling pathway. Post-translational modifications (PTMs) in the priming signal maintain NLRP3 in an auto-suppressed inactive conformation before stimulation. The self-oligomerization of NLRP3 occurs and the downstream recruitment of ASC is achieved by PYD–PYD interaction. Subsequently, aggregated ASC recruits pro-caspase-1, resulting in the activation of caspase-1. IL-1β, IL-18, and GSDMD can be activated by activated caspase-1. Then, caspase-1 cleaves GSDMD to liberate gasdermin D N-terminal form (GSDMD N). The GSDMD N can form membrane pores to mediate the nonconventional release of IL-1β and IL-18 and triggers pyroptosis.

Activation step

When a primed cell is subjected to an activating stimulus, complete activation and the creation of an NLRP3 inflammasome ensue. The activation of the NLRP3 inflammasome is essential for caspase-1 autocatalytic activation. When NLRP3 is activated, it self-oligomerizes, allowing the downstream of ASC to be recruited via the PYD–PYD interaction. As a result of the CARD–CARD interaction, aggregated ASC attracts the effector, pro-caspase-1, culminating in caspase-1 activation [64, 65]. The proteolytic activation of the proinflammatory cytokines IL-1β and IL-18, and of a pore forming protein, GSDMD, can be activated by activated caspase-1 heterotetramers through cleaving these substrates [66-68]. After proteolysis, caspase-1 cleaves GSDMD to liberate GSDMD N. The oligomerized GSDMD N can generate membrane holes, allowing the nonconventional release of IL-1 and IL-18 and the induction of pyroptosis, a type of proinflammatory cell death. Multiple upstream cellular signals, including K^+ or Cl^- efflux, Ca^{2+} flux, lysosomal disruption, mitochondrial dysfunction, metabolic alterations, and ROS generation, all contribute to NLRP3 inflammasome activation [65, 69]. Despite the abundance of evidences defining the upstream signaling processes, no precise molecular events of NLRP3 activation have been identified yet.

The role of NLRP3 inflammasome in periodontitis

NLRP3 and IL-1 are substantially expressed in human gingival tissues with severe chronic periodontitis [70, 71]. IL-1β is essential for the pathogenesis and development of periodontitis, and NLRP3 inflammasome is engaged in the maturation of IL-1β and IL-18 [72]. The activation of NLRP3 inflammasomes has both beneficial and harmful impacts on the host's defensive system [73, 74]. This section focuses on the role of NLRP3 inflammasomes in the pathogenesis and progression of periodontitis, provides an update on what is currently known about the effects of NLRP3 inflammasome activity on various cell types (including but not limited to osteoclasts, osteoblasts, gingival fibroblasts, periodontal ligament cells, and immune cells), and summarizes the current research on the potential role of NLRP3 inflammasomes in the treatment of periodontitis.

NLRP3 inflammasome and osteoclast

The result of periodontitis is the disorganization of ligament and alveolar bone [75]. Studies on the pathogenesis of periodontitis have always focused on alveolar bone loss, especially the role of osteoclasts and osteoblasts during this process [76]. A growing number of researches have revealed the critical role of osteoclasts and osteoblasts in the pathological changes of periodontitis [77-79].

Osteoclasts serve an important role in bone resorption during the process of periodontitis. The RANK/ receptor activator of NF-kB ligand (RANKL)/osteoprotegerin (OPG) axis is critical for osteoclastogenesis [80]. The ability of RANKL to bind to receptor RANK upregulates nuclear factor of activated T cells 1 (NFATC1), a key transcription factor in osteoclast differentiation via recruiting TNF receptor-associated factor-6 (TRAF6) [81]. Under physiological conditions, immature myeloid progenitors convert into multinucleated osteoclasts that correctly resorb bone tissue to ensure healthy bone turnover, whereas pathological states result in excessive bone loss [82]. OPG is a RANKL receptor with a higher affinity than RANK, which inhibits osteoclastogenesis by binding to RANKL [81, 83]. Some early inflammatory related proteins, such as transglutaminases, may regulate the alveolar bone loss by affecting the ratio of RANKL/OPG [28]. Cytokines, particularly IL-1β and IL-18, processed by the effector caspase-1, may modulate osteoclast differentiation and activity either directly by effects on osteoclasts or indirectly by regulating RANKL expression by other cell types [84, 85]. By upregulating the expression of cathepsin K and MMPs in periodontal tissues, IL-1β can boost the ability of osteoclasts to degrade extracellular matrix [86, 87].

Periodontal pathogens, such as *P. gingivalis*, can induce inflammatory responses associated with NLRP3 inflammasome signal transduction [88]. Yohei et al. reported that the involvement of NLRP3 inflammasome was evaluated in *P. gingivalis*-induced periodontitis using NLRP3-KO mice [89]. Infection-induced alveolar bone loss was significantly inhibited in NLRP3-KO mice, suggesting that NLRP3 inflammasome has mediated the production of inflammatory cytokines and has an important impact on *P.* gingivalis-induced bone loss [89]. They also found significantly lower levels of RANKL and increased levels of OPG in NLRP3-KO mice, suggesting that NLRP3 inflammasomes may have functioned in promoting osteoclastogenesis in periodontitis mice [89]. Kelk P et al. found that A. actinomycetemcomitans leukotoxicity mediates activation of the NLRP3 inflammasome in THP-1-differentiated macrophages which further facilitates osteoclasts differentiation [90]. Another aging-related model of periodontitis also suggested that NLRP3 played an inevitable role in osteoclastogenesis during aging [91]. An overactive

NLRP3 inflammasome can boost osteoclast ability to resorb bone by rebuilding the actin cytoskeleton [92], autophagy, or ubiquitination [32]. Therefore, by modulating osteoclast activity and differentiation, the NLRP3 inflammasome may be exploited as a target to treat periodontitis through governing bone resorption.

NLRP3 inflammasome and osteoblast

Collagen fibers, osteocalcin (OCN), and osteonectin are released by osteoblasts and function in bone deposition and mineralization [93]. During osteoblast differentiation, other osteogenic markers such as alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2), and osterix are also expressed. After the newly formed osteoid has calcified, osteoblasts evolve into osteocytes [93, 94]. Then, osteocytes, osteoblasts, and osteoclasts form a network that demonstrates bone turnover [95]. Osteoblasts express the core protein of inflammasome NLRP3 [96]. Infection of osteoblastic cells with pathogens results in the generation of IL-1β and IL-18, as well as apoptosis, which is mediated by the activation of the NLRP3 inflammasome [97, 98]. These may affect the inflammatory bone resorption and bone turnover. Osteoblasts enhance osteoclastogenesis by increasing RANKL synthesis or decreasing OPG levels when the NLRP3 inflammasome is activated [99]. In osteoblasts, chemokines induced by IL-1 govern osteoclast precursor migration and differentiation [100].

Study has indicated that multiple pathogens associated with periodontitis can cause NLRP3 inflammasome activation and apoptosis of osteoblast. A study has indicated that osteoblastic MG63 cells infected with periodontal bacteria *Aggregatibacter actinomycetemcomitans* promote apoptosis by activating the NLRP3 inflammasome [98]. McCall et al. found that the apoptosis of osteoblasts after salmonella challenge requires functional expression of NLRP3 inflammasome [101].

Besides pathogenic bacteria, other factors have an impact on the NLRP3 inflammasome activation and osteogenic dysfunction. LPS treatment leads to the activation of NLRP3 inflammasome to mediate cell death, reduce cell migration and boost osteogenic dysfunction [102]. ROS is a key factor in the NLRP3 inflammasome activation. Some studies have shown that the pathogenesis of periodontitis is related to ROS-induced oxidative stress [103, 104], which could accumulate in periodontal tissue and aggravate the damage to periodontal tissues. A study has shown that LPS-mediated increase of ROS elevates the NLRP3 inflammasome components IL-1β and IL-18, activates pyroptosis, and causes functional impairment in osteoblasts [105]. These results suggest that ROS may promote alveolar bone loss in periodontitis by affecting the ROS-NLRP3-IL-1β pyroptosis axis in osteoblasts. It is possible to conclude that NLRP3 inflammasome activation lowers osteoblast activity by lowering its bone forming ability, differentiation, and proliferation, as well as triggering pyroptosis in osteoblasts and promoting bone resorption in periodontitis.

NLRP3 inflammasome and periodontal ligament fibroblasts

By producing cytokines and chemokines, human periodontal ligament fibroblasts (hPDLFs) contribute to periodontal inflammation, such as apical periodontitis and periodontitis [106]. hPDLFs connect the teeth root to the alveolar bone and play an important role in repairing periodontal tissues and producing bone cells. Therefore, the therapeutic effect of periodontitis can be evaluated by cell proliferation, inflammation, and osteogenic induction ability of hPDLFs [107]. Previous study suggested that NLRP3 and ASC were expressed stably in hPDLFs and mouse PDLFs [108]. The activity of NLRP3 inflammasome induces periodontal inflammation and increasing proinflammatory cytokines such as IL-1β and IL-6, damaging the periodontal ligament [109]. At present, there are relatively few studies on NLRP3 inflammasome in periodontal ligament fibroblasts, this is an issue warranting further research.

NLRP3 inflammasome and leukocytes

Previous study showed that fibroblasts and other stromal cells can specifically recruit leukocytes by expressing chemokines during the development of periodontitis [110]. PAMP can be recognized by the host's PRRs on immune cells, leading to cell activation and production of cytokines and adhesion molecules [111]. Neutrophils, also known as polymorphonuclear leukocytes, are the most abundant leukocytes in inflamed periodontal tissues and show a hyperactive state. The presence of severe periodontitis in patients with defective neutrophils displays the key role of neutrophils in periodontitis. The neutrophils infiltration in the area of periodontal lesion is dependent on NLRP3 expression [112]. *Cheat et al.* reported that stimulation of neutrophils by *P. gingivalis* in WT mice increased NLRP3 and RANKL expression, activated osteoclasts and improved alveolar bone resorption. NLRP3 KO mice had almost no neutrophils in the gingival connective tissue, which may be responsible for the withdrawal of protective resorption of alveolar bone [33]. These studies suggest that NLRP3 may be a switch that maintains/drives neutrophils in inflammatory tissues

[112].

Macrophages play a critical role in the host defense system, and are involved in innate immune defense, activation of acquired immune response mediated by lymphocytes, initiation and resolution of inflammation, and alveolar bone resorption in periodontitis [113]. Macrophages have two phenotypes, pro-inflammatory M1 and selective anti-inflammatory M2, which are determined by the microenvironment of surrounding tissues [114]. Clinical studies have shown that the numbers of M1 and M2 macrophages in inflamed periodontal tissue were more than healthy tissue, of which M1 was dominant[115, 116]. The conversion from M2 to M1 macrophages is an important cause of periodontal tissue damage, the induction of M2 macrophage polarization may become a new alternative for treating periodontitis [117]. These indicate the participation of NLRP3 inflammasome in periodontitis by regulating diverse types of leukocytes.

Potential inhibitors of NLRP3 inflammasome in the therapy of periodontitis

The activation of NLRP3 inflammasome has been found in periodontal tissues of periodontitis patients. Negative regulation of the NLRP3 inflammasome is a potential therapeutic target for NLRP3-associated diseases. A number of NLRP3 inhibitors harbor the ability to inhibit NLRP3 inflammasome. And some of them have displayed their therapeutic potentials for treating periodontitis. However, the underlying mechanism or precise target is not fully understood. In the following part, we will discuss them in detail (Table 1).

MCC950

MCC950 (also referred to as CP-456,773) is a diarylsulfonylurea-containing compound that originally acts as an IL-1β inhibitor. Further study confirmed that MCC950 could directly interacts with the Walker B motif within the NLRP3 NACHT domain, and then blocking ATP hydrolysis and inhibiting NLRP3 activation and inflammasome formation. MCC950 has shown its therapeutic effects on periodontitis. MCC950 can significantly decrease the number and inhibit osteoclast differentiation, which ultimately results in the reduction of alveolar bone loss in mice with periodontitis [71, 91, 118]. It can also present a beneficial therapeutic effect on periodontitis in a ligature-induced periodontitis mouse model, and acts directly on osteoclast precursors, reducing osteoclast development and alveolar bone loss in periodontitis [89]. MCC950 could rescue the inhibition of osteogenesis in hPDLCs from inflammatory root resorption [39]. Besides, MCC950 is able to ameliorate osteoblast migration and restore the expression of osteogenic differentiation-related proteins, such as RUNX2 and ALP, through inhibiting the activity of NLRP3 inflammasome [105]. In summary, MCC950 may serve as a promising new treatment alternative for periodontitis by blocking NLRP3 inflammation and rescuing alveolar bone loss.

Table 1. NLRP3 inflammasome inhibitors in the therapy of periodontitis

Agents	Alias	Inhibition mechanism	Benefits
MCC950	CP-456,773	Directly interact with the Walker B motif within the NLRP3 NACHT domain, target the NLRP3 ATP-hydrolysis motif for inflammasome inhibition	Reduce the alveolar bone loss in periodontitis, decrease the differentiation of osteoclasts [71, 91, 118], and restore the osteogenic differentiation-related proteins expression [105].
Glyburide	Glibenclamide	Active ATP-sensitive K+ channel (K_{ATP}) inhibitor, block NLRP3 inflammasome activation	Prevent NLRP3 inflammasome activation and decrease IL-1β release in periodontal pathogen-induced inflammation (116), lessen the alveolar bone resorption and osteoclastogenesis [120], reverse inflammation [121]
Tranilast	N-(3',4'-dimethoxycinnamonyl) anthranilic acid	Bind to NLRP3 NACHT domain to block NLRP3-NLRP3 and NLRP3-ASC interaction	Alleviate apical periodontitis [122] and inhibit osteoclastogenesis [123, 124].
Irisin	FNDC5	Resist oxidative stress, formation and activation of NLRP3 inflammasome caused by lipopolysaccharides	Increase primary hPDLCs proliferation, promote osteogenic [126] and facilitate the osteogenic/ cementogenic differentiation of hPDLCs[127]
Melatonin	N-acetyl-5-methoxy tryptamine	Inhibitory function on NLRP3 inflammasome activation through inhibiting or activating several proteins and pathways	Improve key periodontal parameters including pocket depth and clinical attachment loss [129], promote new bone regeneration and increase the number of osteoblast-like cells [130].
Dioscin	CCRIS 4123 Collettiside III	Inhibit NF-κB, MAPK signaling and NLRP3 inflammasome	Inhibit the activation of NLRP3 inflammasome in macrophages and promote the osteogenesis, reduce excessive inflammation and promote macrophage polarization to M2 phenotype[133]
Parthenolide		Inhibit NLRP3 ATPase activity	Anti-inflammatory and anti-osteoclastogenic [134, 135]

Glyburide

Glyburide (also known as glibenclamide) is an orally active ATP-sensitive K+ channel (K_{ATP}) inhibitor which can be used for the study of diabetes and obesity. Previous study showed that glyburide could block NLRP3 inflammasome activation, decrease the production of proinflammatory mediators (TNF-α, IL-1β, and reactive oxygen species), and suppress the accumulation of inflammatory cells [119]. While in the study of periodontitis, glyburide can prevent NLRP3 inflammasome activation and decrease IL-1β release in periodontal pathogen-induced inflammation [118]. And oral administration of glyburide can lessen the alveolar bone resorption and osteoclastogenesis caused by traumatic occlusion in a rat model [120]. Likewise, Jiang M et al. demonstrated that glyburide could reverse inflammation and bone resorption in occlusal trauma with periodontitis [121]. These results suggest that glyburide application may achieve good treatment outcomes in periodontal therapy.

Tranilast

Tranilast (N-(3',4'-dimethoxycinnamonyl) anthranilic acid) was developed as an anti-allergic medication. Then it was utilized as an anti-inflammatory agent to treat inflammation-related diseases for its ability to bind to NLRP3 NACHT domain to block NLRP3-NLRP3 and NLRP3-ASC interactions. It has been shown to be therapeutically effective, exerting anti-inflammatory and anti-oxidative effects. Tranilast usage in periodontitis can partially alleviate apical periodontitis [122]. During regulating bone homeostasis, tranilast inhibited activation of nuclear factor-κB and reduced induction and nuclear translocation of nuclear factor of activated T cells, ultimately leading to the inhibition of osteoclastogenesis by RANKL signaling [123]. The same effect of tranilast inhibiting osteoclastogenesis has also been confirmed in the arthritis study [124]. At present, application research on tranilast in periodontitis treatment is rare, but it deserves further investigations.

Irisin

Irisin is a peptide hormone originated from the cleaved fibronectin type III domain containing protein 5 (FNDC5). It can serve as an NLRP3 inhibitor through inhibiting NLRP3 inflammasome formation and activation caused by lipopolysaccharides [125] . Previous study showed that irisin facilitated primary hPDLCs proliferation and promoted osteogenic via increasing extracellular matrix formation [126]. Under P. gingivalis-triggered inflammation, irisin facilitates the osteogenic/cementogenic differentiation of hPDLCs partially through the p38 signaling pathway [127]. Interestingly, compared with other inhibitors, irisin promoted osteogenesis without osteoclast differentiation suppression. This result suggests that irisin would likely play a crucial role in tiny alveolar bone defects in periodontitis.

Melatonin

Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone produced from L-tryptophan present mostly in the pineal gland, which is stored and released by salivary glands. Melatonin exerts inhibitory function on NLRP3 inflammasome activation through inhibiting or activating several proteins and pathways. Melatonin levels are associated with the severity of periodontitis. A systematic review by Balaji TM et al. has shown an initial reduction in melatonin levels followed by elevation with worsening of periodontitis [128]. The application of melatonin as a topical/systemic formulation to treat periodontitis was also reported by Balaji TM et al. Apart from the level and application of melatonin in periodontitis, melatonin is demonstrated to regulate immune response and prevent periodontal tissue from damage. A meta-analysis showed that melatonin supplementation (topical and systemic) in periodontitis patients improved key periodontal parameters including pocket depth and clinical attachment loss [129]. Researches of melatonin on bone repair and regeneration showed that melatonin could promote new bone regeneration and increase the number of osteoblast-like cells [130]. A systematic review and meta-analysis of melatonin adjuvant therapy for periodontitis illustrated that melatonin can significantly improve the periodontal status after non-surgical treatment for periodontitis, and the efficacy is correlated with drug dose [131]. On the contrary, it is noteworthy that different conclusions were also drawn by other researchers. Konečná B et al. reported that melatonin treatment had no significant impact on periodontitis [132]. The authors also analyzed the negative outcome and deduced that this may relate to the different study duration and different melatonin doses. Although numerous clinical studies on melatonin have been performed, the impact of melatonin on periodontitis and its precise molecular mechanisms remain to be elusive. Further studies are still needed.

Dioscin

Dioscin (also referred to as CCRIS 4123 or Collettiside III) is a natural steroid saponin isolated from several plants. Previously it was used an anti-cancer reagent against various kinds of tumor cell lines. Recent study showed its potential to be a

NLRP3 inflammasome inhibitor. Yin W et al. investigated the therapeutic effect of dioscin on periapical periodontitis in mouse, the results demonstrated that dioscin inhibited NLRP3 inflammasome activation in mouse macrophages and promoted the osteogenesis of mouse pre-osteoblasts [38]. In addition, other studies confirmed that dioscin could reduce excessive inflammation and promote macrophage polarization to M2 phenotype [133]. As a new candidate drug, the perspectives of dioscin are promising and deserve further investigations.

Parthenolide

Parthenolide is a sesquiterpene lactone found in the plant Tanacetum parthenium. Parthenolide exhibits anti-inflammatory activity by inhibiting NF-κB and HDAC1. It can also work as an NLRP3 inflammasome inhibitor through inhibiting NLRP3 ATPase activity. Parthenolide has been used to treat inflammation and inflammation related diseases. A few researches focused on the effects of parthenolide on treating periodontitis. Zhang X et al. revealed the anti-inflammatory and anti-osteoclastogenic effects of parthenolide and demonstrated its great potential for application in bone regeneration in periodontitis patients [134, 135]. Research also showed that parthenolide could inhibit osteoclast differentiation by down-regulation of RANKL-mediated osteoclastogenesis. Furthermore, a novel targeted nano-parthenolide molecule has recently been developed for the treatment of acute myeloid leukemia [136]. Based on the above research, whether parthenolide can be harnessed to develop target-directed drugs to periodontitis remains to be determined.

Other inflammasome inhibitors

Except for the inflammasome inhibitors described above, more inflammasome inhibitors have been developed, such as NLRP3-IN-2[137], JC124 [138], Arglabin [139], Isoandrographolide [140], Carvedilol [141], and JC-171 [142]. Unfortunately, their roles in periodontitis have not been clarified. But these indeed provide us enough alternative drugs for periodontitis treatment.

Conclusions and Perspectives

The overexpression and activation of the NLRP3 inflammasome has been linked to the development of periodontitis in recent years. NLRP3 inflammasome exerts different regulatory functions in cells of different types in PDL. NLRP3 inflammasome enhances osteoclastogenesis by increasing RANKL synthesis or decreasing OPG levels. At the same time, NLRP3 inflammasome promotes apoptosis of osteoblasts, elevates proinflammatory cytokines in periodontal ligament fibroblast, and controls the functions of immune cells. These indicate the great potential of NLRP3 inflammasome as a target for treating periodontitis. Numerous NLRP3 inflammasome inhibitors have been developed and shown broad potentials for the therapeutic treatment of periodontitis. Nevertheless, the therapeutic effects and side effects of NLRP3 inflammasome inhibitors on periodontitis are largely unknown, and the precise mechanisms of how NLRP3 inflammasome inhibitors influence periodontitis are far from full elucidation. Despite all this, revealing the role of NLRP3 inflammasome in periodontitis pathogenesis and developing safe and effective NLRP3 inflammasome inhibitors will greatly contribute to periodontitis treatment.

Abbreviations

AgP: aggressive periodontitis; AIM2: absent in melanoma 2; ALP: alkaline phosphatase; AP-1: activator protein 1; ASC: apoptosis-related speck-like protein containing a caspase activation and recruitment domain (CARD); CARD: caspase activation and recruitment domain; CASP1: caspase-1; CLRs: C-type lectin receptors; CP: chronic periodontitis; CVD: cardiovascular disease; DAMPs: damage-associated molecular patterns; FBXL2: Fbox/LRR-repeat protein 2; FBXO3: F-box only protein 3; FIIND: function-to-find domain; GSDMD: gasdermin D; GSDMD N: gasdermin D N-terminal form; GWAS: genome-wide association study; IL-17: interleukin-17; IL-1β: interleukin-1β; JNK1: JUN N-terminal kinase 1; LPS: lipopolysaccharide; LRR: leucine-rich-repeat domain; MARCH7: membrane-associated RING finger protein 7; MMPs: matrix metalloproteinases; NACHT: nucleotide-binding oligomerization domain; NFATc1: Recombinant Nuclear Factor Of Activated T-Cells Cytoplasmic 1; NF-κB: nuclear factor kappa B; NLRs: nucleotide-binding and oligomerization domain (NOD)-like receptors; NLRP1: Nod-like receptor pyrin domain-containing protein 1; OCN: osteocalcin; PAMP: pathogen-associated molecular pattern; PKD: protein kinase D; PRR: pattern recognition receptor; PTMs: post-translational modifications; PYD: pyrin domain; RANKL: receptor activator of NF-kB ligand; RIG: retinoic acid-inducible gene; ROS: reactive oxygen species; RUNX2: runt-related transcription factor 2; SENP6: sentrin-specific protease 6; SUMO: sumoylation; TLRs: Toll-like receptors; TNF: tumor necrosis factor; TRIM31: tripartite motif containing protein 31.

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Author contributions

Ying Zhao and Yue Quan drafted and proofread the manuscript. Sheng Hu and Xin Ge edited the manuscript. Ting Lei and Liumeizi Fan revised the manuscript. All authors have agreed upon the submission and publication of this work. Ying Zhao and Yue Quan contribute equally to this review and should be considered co-first authors.

Competing Interests

The authors have declared that no competing interest exists.

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