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Review Article

Mechanism of alveolar bone destruction in periodontitis — Periodontal bacteria and inflammation

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ABSTRACT

Periodontal disease is an inflammatory disease caused by periodontopathogenic bacteria, which eventually leads to bone tissue (alveolar bone) destruction as inflammation persists. Periodontal tissues have an immune system against the invasion of these bacteria, however, due to the persistent infection by periodontopathogenic bacteria, the host innate and acquired immunity is impaired, and tissue destruction, including bone tissue destruction, occurs. Osteoclasts are essential for bone destruction. Osteoclast progenitor cells derived from hematopoietic stem cells differentiate into osteoclasts. In addition, bone loss occurs when bone resorption by osteoclasts exceeds bone formation by osteoblasts. In inflammatory bone disease, inflammatory cytokines act on osteoblasts and receptor activator of nuclear factor-κB ligand (RANKL)-producing cells, resulting in osteoclast differentiation and activation. In addition to this mechanism, pathogenic factors of periodontal bacteria and mechanical stress activate osteoclasts and destruct alveolar bone in periodontitis. In this review, we focused on the mechanism of osteoclast activation in periodontitis and provide an overview based on the latest findings.

1. Introduction

Periodontitis is a chronic inflammatory disease that is triggered by bacteria in the oral cavity. As inflammation persists, periodontal tissues such as gingiva, periodontal ligament, cementum, and alveolar bone are destroyed. In particular, severe destruction of the alveolar bone results in loss of the support for the teeth, which eventually fall out. Tooth loss leads to occlusal instability that reduces the quality of life [1]. In recent years, periodontal tissue regeneration therapy has been introduced to regenerate periodontal tissues lost by periodontitis. However, complete restoration to the original state has not been achieved so far [2]. Periodontitis has also been suggested to cause systemic diseases. Therefore, it is important to understand the pathogenesis of periodontitis to overcome this disease and establish new treatment methods.

In 1976, Page and Schroeder observed activation of the acquired immune system, consisting of T cells and B cells, in periodontitis-affected donors by histological analysis [3]. Since the 1990s, development and spread of molecular biology has revealed various molecular mechanisms underlying the pathogenesis of periodontitis. Page and Kornman explained the relationship between periodontal bacteria and the host response, production of various factors during inflammation, and destruction of the periodontal tissue [4]. The present review explains the mechanism underlying the destruction of alveolar bone caused by periodontitis, in particular, new findings and concepts introduced through experiments using periodontitis model with ligature ligation in the past few years.

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2. Osteoclasts and receptor activator of nuclear factor-κB ligand (RANKL)

2.1. Osteoclasts

Osteoclasts differentiate from the macrophage cell lineage in the blood, called as osteoclast precursor cells. Osteoclasts are multinucleated giant cells with a diameter of 20–100 μm, and are the only cells capable of destroying and resorbing bone tissue in vivo [5]. Histologically, they express tartrate-resistant acid phosphatase (TRAP), a bone matrix degrading enzyme, which is used as an osteoclast-specific marker [6]. Osteoclasts use αVβ3 integrin to attach to the bone surfaces, which activates the Src-dependent signaling pathway, promoting the formation of actin rings and wavy edges. Subsequently, proteolytic enzymes such as cathepsin K and H^+ ions are secreted into the resorption foci, and bone resorption begins. Pleckstrin homology domain-containing family M member 1 (PLEKHM1) and sorting nexin 10 (SNX10) play critical role in vesicular transport of proteolytic enzymes. Their mutations can cause autosomal recessive marble bone disease [7,8]. To maintain homeostasis, bone tissue undergoes continuous bone remodeling, including bone resorption by osteoclasts and bone formation by osteoblasts. Bone mass is controlled by the functions of osteoclasts and osteoblasts. However, in diseases that cause bone loss, such as osteoporosis and periodontal disease, osteoclasts are activated and bone resorption exceeds bone formation by osteoblasts [9]. In particular, when alveolar bone destruction occurs due to periodontitis, connective tissue and gingival epithelium invade the space created after destruction by osteoclasts, making it difficult for osteoblasts to form bone.

2.2. RANKL

Osteoclast differentiation factor (ODF), a factor that activates osteoclasts, was cloned by Dr. Suda's group in Japan in 1998, and found it to be identical to RANKL that has already been reported [10]. RANKL (encoded by the TNFSF11 gene) is a major regulator of osteoclast differentiation and function [9]. Osteoclast did not form in mice lacking the RANKL gene or patients with mutations in the RANKL gene, resulting in osteopetrosis with increased bone mass, indicating that RANKL is an essential factor for osteoclastogenesis [11,12]. In addition, bone erosion did not occur in RANKL-deficient mice with rheumatoid arthritis, indicating that osteoclastogenesis in rheumatoid arthritis is also dependent on RANKL [13]. Osteoclasts are stimulated by RANKL and macrophage colony-stimulating factor (M-CSF) from macrophages, which are osteoclast precursor cells. RANKL binds to RANK, a receptor on osteoclast progenitor cells, and M-CSF induces the expression of RANK. RANKL induces the expression of NF-κB, c-Fos, and nuclear factor of activated T cells c1 (NFATc1). Osteoclast-specific NFATc1-deficient mice exhibited severe osteopetrosis, therefore NFATc1 is considered as the most important transcription factor in osteoclast differentiation [14]. On the other hand, osteoprotegerin (OPG), an osteoclast suppressor produced by osteoblasts and stromal cells, is a secreted protein with a structure similar to RANK but without transmembrane domain. OPG is a "decoy receptor" for RANKL that blocks RANK signaling and inhibits bone resorption [9]. Elevated RANKL/OPG ratio serve as a potential biomarker for periodontitis progression [15].

3. Periodontal tissue cells and RANKL expression

RANKL is expressed on osteoblasts [16], T cells [17], chondrocytes [18], osteocytes [19], and synovial fibroblasts [20], and periodontal tissue also express RANKL. Periodontal tissue is composed of alveolar bone, periodontal ligament, gingiva, and cementum, all of which express RANKL. The periodontal ligament is a soft tissue that intervenes between the alveolar bone and the teeth. Periodontal ligament cells express RANKL, and formed osteoclasts when co-cultured with osteoclast progenitor cells [21]. In addition, periodontal ligament cells produce proinflammatory cytokines and modulate osteoclast formation upon stimulation by periodontal bacterial lipopolysaccharide (LPS) [22]. However, it has been reported that osteoclasts formed in co-culture of periodontal ligament cells had no bone resorption ability [23,24]. The gingiva is composed of gingival epithelial cells and fibroblasts. The gingiva is the outermost part of the periodontal tissue and the first site to be invaded by periodontal bacteria. It has been reported that gingival epithelial cells and fibroblasts express RANKL [25,26]. Gingival epithelial cells formed osteoclasts with bone resorption ability when co-cultured with osteoclast progenitor cells [27]. On the other hand, osteoclasts formed in co-culture of gingival fibroblasts were not capable of bone resorption [26]. Cementoblasts are the cells that form the cementum attached to the tooth root. Cementoblasts express RANKL, and formed osteoclasts when co-cultured with osteoclast progenitor cells. This osteoclastogenesis process has been shown to be enhanced by interleukin (IL)-1 and parathyroid hormone-related peptide (PTHrP) [28,29].

4. Cytokines and immune cells

Periodontal tissue is continuously exposed to the oral microbiota and other physical stimuli generated by mastication. A fragile balance exists between the local immune response and the microbiota under physiological conditions. Immune surveillance and tolerance to the local microbiota can be achieved without a severe inflammatory response. Nevertheless, after colonization by a "keystone" pathogen such as *P. gingivalis*, the constituents of the microbiota and their total counts are altered, which elevates the pathogenicity of the whole community and disrupts tissue homeostasis. Under these conditions, the immune response is overactivated, which leads to infiltration by immune cells, overproduction of proinflammatory cytokines, activation of osteoclastic activity, and eventually the destruction of the gingiva and alveolar bone. In this section, cytokines and immune cells directly involved in osteoclastogenesis in periodontitis are described. The concept of keystone pathogens is explained in Section 7.

4.1. Cytokines

Various inflammatory cytokines are involved in osteoclast formation and activation. IL-1, 6, 11, and 17, and tumor necrosis factor (TNF)-α, produced by infiltrating immune cells, stimulate bone resorption in periodontitis. These cytokines have been shown to promote osteoclastogenesis [30–33]. Currently, the role of local inflammatory mediators produced by macrophages and T-lymphocytes in bone resorption has been studied in various ways [34]. In contrast, cytokines such as IL-4, 12, 27, and 33 inhibit osteoclastogenesis [35–38].

4.2. Immune cells

In the 1970s, histological analysis showed that the acquired immune system consisting of T and B cells was activated in donors with periodontitis [3]. In a recent study, levels of plasma cells, naive B cells, neutrophils, and activated CD4 memory T cells were found elevated in periodontal tissue compared to healthy controls [39]. To investigate the involvement of T cells in periodontitis, human peripheral blood lymphocytes collected from patients with periodontitis were transplanted into NOD/SCID mice deficient in T cells.

Oral administration of *Aggregatibacter actinomycetemcomitans* activated $CD4^+$ T cells in periodontal tissues and induced alveolar bone destruction, indicating that *A. actinomycetemcomitans* induces RANKL expression in $CD4^+$ T cells, which in turn activates osteoclasts [40]. $CD4^+$ T cells are divided into four major types (Th1, Th2, Th17, and Treg) according to the type of cytokine they produce and their gene expression patterns. In particular, Th17 cells produce IL-17 that eliminates bacteria and fungi in the intestinal mucosa and barrier sites, while Tregs produce IL-10 and transforming growth factor (TGF)-β that suppress autoimmunity. A recent study has reported the role of Th17 cells in periodontitis; Th17 cells accumulated in periodontal tissues in periodontitis, moreover, periodontitis induced by silk thread ligation was suppressed in Th17 cell-deficient mice. Furthermore, among these Th17 cells, Foxp3-negative and IL-17-positive cells differentiated from Treg (Foxp3-positive and IL-17-negative) cells and were found to be mainly involved in the destruction of periodontal tissue [41]. In accordance with these data, cells expressing Foxp3, a marker of Treg, and IL-17, a marker of Th17, have been detected in the periapical tissues of teeth extracted due to severe periodontitis [42].

5. Bacterial factors

5.1. Periodontal bacteria

Periodontopathic bacteria detected in the subgingival pockets of patients with periodontitis and responsible for the development and progression of periodontitis include *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*. In particular, three species, *P. gingivalis, T. forsythensis, and T. denticola*, are known as the red complexes, and are particularly pathogenic [43]. Administration of *P. gingivalis* into the oral cavity of mice increased alveolar bone resorption [44]. However, there are reports that dead *P. gingivalis* or some species of *P. gingivalis* do not cause alveolar bone resorption [45]. Additionally, studies reported that during alveolar bone resorption caused by oral administration of *P. gingivalis*, the number of osteoclasts increased while the number of osteoblasts decreased [46,47]. Moreover, oral administration of *A. actinomycetemcomitans* also causes alveolar bone resorption similar to that observed by *P. gingivalis* [48]. Recent studies have reported that alveolar bone resorption is more common in multiple bacterial infections than in single bacterial infections of *P. gingivalis* and *A. actinomycetemcomitans* [49–51]. These in vivo results were further supported by in vitro assays that demonstrated that *P. gingivalis* upregulated RANKL expression in osteoblasts, while decreased the expression of OPG, an antagonist of RANKL [52,53]. These periodontal bacteria have various pathogenic factors; the relationship between each of these factors and osteoclastogenesis is described in the next section.

5.2. LPS

Periodontal bacteria are gram-negative bacteria with LPS in their outer membrane. Structurally, the lipid A is embedded in the outer membrane to form a membrane structure, and polysaccharide chains extend from lipid A through an oligosaccharide region called the core. LPS is known as a virulence factor and an endotoxin because the released lipid A portion produces marked activation of the immune response and causes constant dysfunction of vital organs [54]. In addition to this, LPS is known to be directly involved in osteoclastogenesis. When Raw264.7 cells, an osteoclast progenitor cell line, were treated with *E. coli*-derived LPS alone, osteoclasts were formed [55]. Further, *E. coli*-derived LPS and *P. gingivalis*-derived LPS induced the formation of osteoclasts from mouse leukocytes [56]. However, it has been reported that LPS alone does not form osteoclasts [57,58]. When pretreated with RANKL, osteoclasts were formed by LPS stimulation [59,60]. It has been reported that the multinucleated giant cells formed from Raw264.7 cells by LPS alone did not express osteoclast-specific enzymes such as cathepsin K, unlike the osteoclasts formed by RANKL [61]. Further research is needed to assess the significance of the cell type used in these studies. LPS used in in vitro osteoclastogenesis assays was mainly derived from three bacterial species: *E. coli* and *P. gingivalis*, which are commercially available, and *A. actinomycetemcomitans*, which is extracted from a bacterial strain. Lipid A of LPS has nearly similar structure except for the difference in the number of carbon atoms in the constituent fatty acids, and shows considerably similar biological activity across species and genera. There was no significant difference in the ability of LPS derived from the three species to promote osteoclastogenesis. However, different responses corresponding to the production of inflammatory cytokines have been reported in mast cells, gingival fibroblasts, and periodontal ligament cells, depending on the species from which LPS has been derived [62–64], which might be due to the difference in the downstream signals activated during osteoclastogenesis; therefore, further studies are needed.

As mentioned above, osteoclastogenesis occurs when osteoblasts and other cells produce RANKL, which in turn causes osteoclast progenitor cells to differentiate into osteoclasts. Toll-like receptor 4 (TLR4) is known as a receptor for LPS, and osteoblasts involved in osteoclastogenesis also have TLR4, similar to osteoclast progenitor cells [65,66]. *E. coli* LPS induced the production of RANKL and prostaglandin E2 (PGE2) via TLR4 present in osteoblasts, and promoted osteoclast formation in a co-culture system of osteoblasts and bone marrow cells, including osteoclast progenitor cells [67,68]. Periodontal ligament cells also express TLR4, and *P. gingivalis* LPS increased both TLR4 and TLR2 expression [69,70], along with RANKL expression [71]. Similarly, *E. coli* LPS and *A. actinomycetemcomitans* LPS increased RANKL expression in periodontal ligament cells [72,73]. In a co-culture system of periodontal ligament cells and bone marrow-derived macrophages, which are osteoclast precursor cells, the addition of LPS increased the number of osteoclasts formed [74,75]. Cementoblasts also have TLR4 receptors, but RANKL expression is decreased and OPG expression is increased by *P. gingivalis* LPS treatment [76]. Interestingly, the cells that constitute the same periodontal tissue respond differently to LPS with respect to RANKL expression.

LPS induced bone resorption in vitro as well as in vivo in mice and rats. In many studies, direct administration of LPS to periodontal tissues has been successful in inducing alveolar bone resorption by osteoclasts, similar to periodontitis. Previous study has used LPS derived from *E. coli* to destroy the alveolar bone [77]. Recently, models presenting alveolar bone destruction by osteoclasts induced by LPS derived from periodontal bacteria, *P. gingivalis* and *A. actinomycetemcomitans*, have been increasingly used to reflect the reality of periodontitis [78–82].

5.3. Peptidoglycan (PGN)

PGN is a polymer consisting of peptides and sugars. The sugar component consists of alternating residues of β-(1,4)-linked *N*-acetylglucosamine and *N*-acetylmuramic acid. Unlike LPS, both gram-positive and gram-negative bacteria possess PGN. While LPS activates NF-κB signaling via TLR4, PGN is known to activate NF-κB via intracellular signaling activated by TLR2 [83]. *Staphylococcus aureus* PGN, but not *E. coli* PGN, induced alveolar bone resorption, similar to LPS in mice [84]. PGN derived from *Actinomyces naeslundii,* a biofilm-forming bacterium, increased the number of osteoclasts formed in a co-culture system of osteoblasts and bone marrow cells. Furthermore, the bone mass of *Actinomyces*

naeslundii-infected rats was comparable to that of *P. gingivalis*-infected rats, however significantly greater than that of the sham group [85]. Additionally, PGN promoted osteoclast cell fusion in a system that used Raw264.7 cells [86]. Alveolar bone resorption and RANKL expression were suppressed in *P. gingivalis*-induced periodontitis in mice lacking Nod2, the receptor for PGN, strongly suggesting the involvement of PGN in the pathogenesis of periodontitis [87].

5.4. Gingipain

Gingipains are major proteases secreted by *P. gingivalis*, and are classified into Lys-specific gingipains (Kgp) and Arg-specific gingipains (Rgp) based on the cleavage site of the peptide. Kgp and Rgp work in cooperation to cause degradation of biological proteins and damage host cells, resulting in various pathologies related to periodontal disease [88]. To determine which gingipain is important in the pathogenesis of periodontal disease, a mouse periodontitis model was established using *P. gingivalis* containing mutations of each gingipain. The results showed that *P. gingivalis* with mutations in RgpA did not cause significant alveolar bone resorption, suggesting that RgpA is not involved in alveolar bone resorption [89]. Contrarily, it has been reported that RgpA is important for alveolar bone resorption in a mouse periodontitis model using mutant strains of gingipains [90]. The difference in the results of the two studies might be due to the difference between *P. gingivalis* wild-type strains (W50 versus W83), the oral infection protocols used, and techniques used to measure bone loss (two dimensional versus three dimensional). In a study by Wilensky [90], mice were infected three times every other day with 0.2 mL of 5×10^{10} CFU/mL of *P. gingivalis* (W83) or mutants in PBS and 2% carboxymethylcellulose, whereas Pathirana et al. infected the mice four times, once every 2 days, with 1×10^{10} of *P. gingivalis* W50 or mutants in PG buffer, and repeated this protocol in the same mice at 2 weeks following the first infection [89]. Both gingipains were added to a co-culture system of osteoblasts and osteoclast progenitor cells for the osteoclastogenesis assay. Rgp treatment did not change the number of osteoclasts, whereas Kgp treatment promoted osteoclastogenesis in the co-culture system [91]. The mechanism underlying the Kgp action involves degradation of OPG, an osteoclast inhibitory factor produced by osteoblasts, resulting in the promotion of osteoclastogenesis [92].

6. Mechanical stress

Our bodies are constantly exposed to mechanical stress from outside world, and we recognize and respond to this stress to maintain a state of homeostasis. The fact that the bone mass of astronauts decreases during their stay in the microgravity environment of space indicates the importance of mechanical stress in maintaining bone mass. Nowadays, the combination of bisphosphonates and exercise helps astronauts to maintain their bone mass [93,94]. Dentists are well aware of alveolar bone resorption at the tooth extraction site in the oral cavity. In recent years, the detailed mechanism of this process has been elucidated, according to which osteocytes in bone are sensitive to mechanical stress, and express and produce RANKL that forms osteoclasts resulting in bone remodeling [95].

Mechanical stress also plays an important role in the pathogenesis of periodontal disease. Occlusal forces exert constant stress on the periodontal tissues. Ericsson et al. showed that strong forces do not destroy healthy (non-periodontitis) alveolar bone. However, strong forces, such as premature contact and bruxism, are applied to the periodontal tissue with inflammation caused by plaque, and bacterial factors rapidly induce alveolar bone resorption [96,97]. When rat periodontal tissues inflamed by LPS were subjected to occlusal trauma, the number of periodontal ligament cells producing RANKL increased, though there was no significant difference in the production of RANKL by osteoblasts [98]. In addition, occlusal trauma damaged collagen fibers of periodontal tissues and increased the tissue permeability of antigens, resulting in the expansion of the area of immune complex formation and promotion of inflammatory reactions; eventually the number of osteoclasts increased [99]. Recently, a study suggested the mechanism of periodontal tissue destruction due to occlusal trauma. Dutzan et al. found that Th17 cells accumulated in the gingiva of mice raised on hard food, resulting in alveolar bone resorption. Furthermore, Th17 cells increased in a similar manner in germ-free (GF) mice, and alveolar bone resorption occurred. In addition, when the gingival epithelium of mice was rubbed with a cotton swab, the inflammatory cytokine IL-6 was produced by gingival epithelial cells, and Th17 cells accumulated in the gingiva [100], indicating that Th17 cells may accumulate in the gingival epithelium and activate osteoclasts when mechanical stress is applied, even in the complete absence of bacteria.

7. Ligature ligation–induced periodontitis model and new concept

Several mouse models of periodontitis have been developed to study the mechanisms of periodontitis and to test the potential of novel therapeutic agents. Mice have different tooth arrangements and occlusion conditions than humans. However, mouse periodontitis models have been used because of the rich background information regarding their immune system, wide range of genetically engineered strains (e.g., gene knockouts of key immune receptors and signaling molecules), and availability of high-quality immunochemical and cellular reagents. In particular, the ligature ligation–induced periodontitis model used in recent years has been shown to cause alveolar bone loss within a few days due to the deposition of anaerobic bacteria. Abe et al. reported that 5-0 silk threads were ligated to the maxillary second molars of C57BL/6 mice to induce periodontitis. In this ligature ligation model, the number of anaerobic bacteria causing periodontitis increased from 3 days after ligation, and alveolar bone resorption occurred 5 days after ligation [101]. The destruction of periodontal tissues over this short period of time was accompanied by an increase in anaerobic bacteria, and this reproducible and well-established phenotype will be beneficial for periodontal disease research. The ligature ligation–induced periodontitis model has also been used in a recent study that clarified the role of Th17 cells in periodontitis; Tsukasaki et al. used this model to transplant exFOXp3 Th17 cells into T cell–deficient mice, resulting in substantial alveolar bone destruction [41]. Dutzan et al. also used ligature ligation and found that Th17 cells and associated neutrophil accumulation were required for the destruction of inflammatory tissue [100]. In addition, this murine periodontitis model has been used in several studies that led to the keystone pathogen hypothesis by Prof. Hajishengallis. The details of this are described below.

As mentioned above, many studies have shown that the relationship between the components of periodontal bacteria and the host immune response affect the pathogenesis of periodontal disease. However, new concepts called the keystone pathogen hypothesis have emerged in recent years. Keystone pathogens are defined as bacteria that cause disease by engulfing other microorganisms, even when present in very small quantities; in contrast, majority of bacteria affect the human body by multiplying and increasing their populations to very large numbers. Although *P. gingivalis* caused periodontitis in specific pathogen free (SPF) mice with commensal bacteria, however, did not induce periodontitis

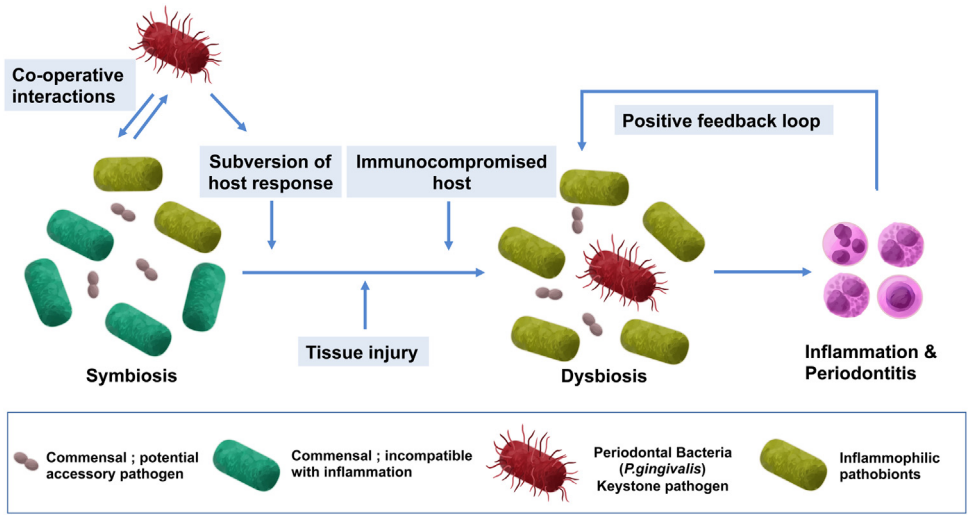

Fig. 1. Dysbiosis of the subgingival microbiota.
Inflammatory mucosal diseases such as periodontal disease are induced under certain conditions by a polymicrobial community in which different members have distinct and synergistic roles that promote destructive inflammation. A keystone pathogen (e.g., *P. gingivalis*), with the help of accessory pathogens in terms of nutrition and colony formation, initially subverts host immunity leading to the emergence of dysbiotic microbiota, in which commensal bacteria–turned pathobionts overactivate the inflammatory response and cause tissue destruction. Pathobionts are organisms that are generally benign within an indigenous community but become pathogenic when host-microbe homeostasis breaks down under certain conditions, such as with antibiotic treatment and tissue injury, and especially in immunocompromised hosts. These conditions can potentially promote the outgrowth of pathobionts and disrupt the symbiotic microbiota, resulting in dysbiosis and inflammation. Dysbiosis can be promoted by these factors, either individually or in combination. A poorly controlled host immune response, in turn, can generate a self-perpetuating pathogenic cycle where dysbiosis and inflammation reinforce each other by forming a positive feedback loop. This figure is modified from Ref. [108].

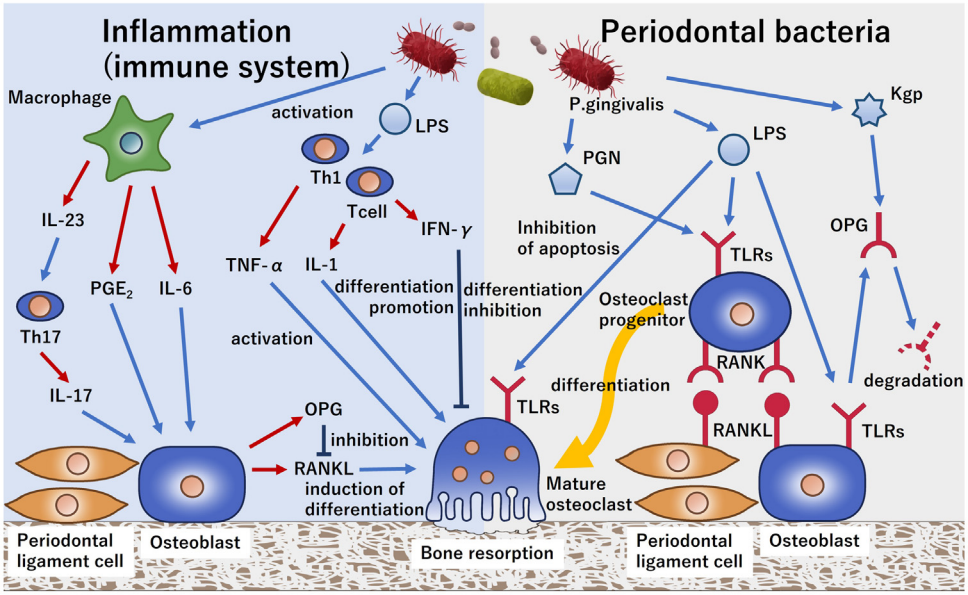

Fig. 2. Molecular mechanism of osteoclast formation and activation by periodontal bacteria.
In periodontitis, osteoclasts are formed by the direct action of periodontal bacteria and the immune system.

in GF mice, which were completely sterile [102]. The results of this experiment indicate that *P. gingivalis* alone does not cause periodontitis in mice; changes in the quality and quantity of oral commensal bacteria are important in the development of periodontitis. Although *P. gingivalis* occurs in low numbers during homeostasis, it is pathogenic in periodontitis owing to its ability to induce dysbiotic microbial communities; it, therefore, acts as a keystone pathogen [103,104]. Based on these studies, Prof. Hajishengallis developed the concept that *P. gingivalis* is a keystone bacterium that causes periodontitis by disturbing the commensal flora [105].

Neutrophils are the most common leukocytes collected in subgingival crevices and periodontal pockets. However, in neutrophils that recognize *P. gingivalis* via TLR2, complement component 5a receptor and TLR2 are co-activated, leading to ubiquitination and proteasomal degradation of the TLR2 signaling adaptor MYD88 and subsequent inhibition of the antimicrobial activity of neutrophils [106]. In other words, *P. gingivalis* manipulates neutrophils to sustain the survival of the microbial community and perpetuate inflammation. These findings explains that it is not the bacterium that causes periodontal tissue destruction due to periodontitis, but rather changes in the indigenous bacterial flora owing to the

increased *P. gingivalis* and disruption of host-microbe homeostasis in the oral barrier [107,108] (Fig. 1).

8. Conclusions

In the past, research on the pathogenesis of periodontal disease focused only on a small number of bacterial species, mainly members of the red complex group, and the effects of existence and components of other indigenous bacteria have not been investigated extensively. The currently conceivable pathways of alveolar bone desorption and destruction by periodontitis are shown in Fig. 2. Periodontal bacteria promote osteoclastogenesis both directly and indirectly via induction of the immune system. With the advent of the keystone hypothesis, attention has been focused on other commensal bacteria and their flora, but this does not eliminate the pathogenicity of the red complex, including *P. gingivalis*. Most studies based on the keystone hypothesis have been conducted in mice, so future studies in humans will be of great interest. Periodontitis is an inflammatory bone disease that has many similarities with rheumatoid arthritis, however, there are only few reports showing an association between periodontitis and rheumatoid arthritis [109]. Based on the findings that periodontal disease-associated bacteria, including *P. gingivalis*, are detected in the synovial fluid of patients with rheumatoid arthritis [110], various pathways have been proposed, including the possibility that oral bacteria reach the synovium directly via the bloodstream and exacerbate inflammation, and that *P. gingivalis* produces citrullinated protein in the oral mucosa and contributes to the production of anti-citrullinated protein antibodies. The involvement of Th17 cells, which are involved in bone destruction in rheumatoid arthritis, was also revealed in periodontitis. These new findings can elucidate the mechanisms of other inflammatory bone diseases, such as rheumatoid arthritis and bone metastasis of cancer.

Conflict of interest

The authors have no conflict of interest to disclose.

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