Chapter 3

Cellular Response Mechanisms in *Porphyromonas gingivalis* Infection

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Additional information is available at the end of the chapter

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Abstract

The pathogenicity of the periodontal biofilm is highly dependent on a few key species, of which *Porphyromonas gingivalis* is considered to be one of the most important pathogens. *P. gingivalis* expresses a broad range of virulence factors, of these cysteine proteases (gingipains) are of special importance both for the bacterial survival/proliferation and for the pathological outcome. Several cell types, for example, epithelial cells, endothelial cells, dendritic cells, osteoblasts, and fibroblasts, reside in the periodontium and are part of the innate host response, as well as platelets, neutrophils, lymphocytes, and monocytes/macrophages. These cells recognize and respond to *P. gingivalis* and its components through pattern recognition receptors (PRRs), for example, Toll-like receptors and protease-activated receptors. Ligation of PRRs induces downstream-signaling pathways modifying the activity of transcription factors that regulates the expression of genes linked to inflammation. This is followed by the release of inflammatory mediators, for example, cytokines and reactive oxygen species. Periodontal disease is today considered to play a significant role in various systemic conditions such as cardiovascular disease (CVD). The mechanisms by which *P. gingivalis* and its virulence factors interact with host immune cells and contribute to the pathogenesis of periodontitis and CVD are far from completely understood.

Keywords: host-microbe interaction, immune cells, pathogen recognition receptors, intracellular signaling, inflammatory responses, *Porphyromonas gingivalis*, gingipains, LPS, cardiovascular disease, treatment

1. Introduction

Evidence suggests that it is the early host-inflammatory and immune responses to the oral microbiota that changes the subgingival environment and favors the emergence of periodontal

opportunistic pathogens during the development of periodontitis. Substances released from the dental biofilm, such as lipopolysaccharides, proteolytic enzymes, and other virulence factors, activate the innate immune system and initiate an inflammatory response, which disrupts the host-microbe homeostasis. The activation of immune cells leads to a release of an array of inflammatory mediators, for example, cytokines, chemokines, proteases, reactive oxygen species (ROS), and eicosanoids, which struggle against the bacterial burden. However, the complexity of the microbial biofilm of the subgingival dental plaque and the failure of the acute inflammation to resolve lead to an accumulation of mediators of the innate and adaptive immune systems that collectively promote chronic inflammation and tissue destruction. How host cells discriminate commensal from pathogenic microbial species and why this ability seems to differ between individuals is currently unknown. The variation in individual susceptibility to develop periodontal disease appears to be determined by the magnitude of the inflammatory response to a dysbiotic microbial community and whether only the innate or also the adaptive immune pathways are activated.

2. *Porphyromonas gingivalis* in periodontitis

There are a number of bacterial species that are associated to periodontitis, based on their detection in periodontal pockets, their pathogenicity, and the immunological responses they evoke [1]. The red complex is a consortium of three periodontal bacterial species, *Treponema denticola, Tannarella forsythia*, and *Porphyromonas gingivalis*, which are linked to each other and to diseased sites [2]. The development and progression of periodontitis is believed to be due to a synergistic and dysbiotic polymicrobial community, and the oral biofilm (dental plaque) [3]. A biofilm is a highly structured, three-dimensional matrix with a simple circulatory system. The biofilm provides physical protection and a gradient of oxygen, allowing anaerobic species to grow in the deeper pocket, and aerobic species near the surface. Furthermore, metabolic by-products from one species can be used as nutrients by other species in the biofilm, the so-called cross-feeding [4]. The keystone species hypothesis suggests that some species, like *P. gingivalis*, exerts a disproportionally large effect in the biofilm. *P. gingivalis* can turn from a natural low-abundance microorganism residing in the oral cavity to an opportunistic pathogen that interferes with the host immune system and from a normal, symbiotic microbiota, and enables the transition and emergence into a dysbiotic bacterial society that drives the progress of periodontitis [2, 5]. *P. gingivalis* is a late colonizer usually found in a rather low number in the dental plaque, and interestingly, *P. gingivalis* is not able to induce periodontitis in germ-free mice, suggesting that *P. gingivalis* is dependent on the complex microbial community. Through synergistic interactions, the biofilm promotes colonization, nutrition acquisition and subvert, and evades host immune responses [4, 6].

P. gingivalis is a non-motile, proteolytic, and Gram-negative rod that expresses several virulence factors that are related to colonization of oral tissues, periodontal tissue destruction, and evasion of the host responses [7]. *P. gingivalis* exhibits genotypic and phenotypic diversity, which results

in differences in virulence and in the capacity of individual strains to colonize and induce destruction of periodontal tissues. Certain strains may therefore exhibit a higher pathogenic potential than others and may be linked to a more severe form of periodontitis [8–12]. The asaccharolytic bacterium *P. gingivalis* grows under anaerobic conditions and acquires metabolic energy by fermenting amino acids. *P. gingivalis* also uses micronutrients, such as metal ions for anabolic and catabolic purposes, as well as vitamin K. *P. gingivalis* expresses a broad range of virulence factors, all of which add to enhanced growth and survival in a hostile environment [7]. However, the virulence of *P. gingivalis* is affected by its surroundings, including other bacterial species in the biofilm and host-derived factors. By altering the gene expression of virulence factors, *P. gingivalis* can adjust to a more or less virulent phenotype depending on the environment [13].

Fimbriae are hair-like protrusions emanating from the outer cell surface that facilitate the adherence and colonization of the bacterium. Indeed, fimbriae are critical for mediating the initial bacterial interaction with the host tissue. *P. gingivalis* expresses major and minor fimbriae, encoded by the *fimA* and *mfa1* genes, respectively. Today, six *fimA* allele types are known (fimA I, Ib, II, III, IV, and V). These variants are more or less associated to periodontitis [14]. *P. gingivalis* isolated from periodontally healthy persons more often expresses type I, II, or V. Types Ib, II, and IV, on the other hand, are more associated to diseased periodontal pockets [9, 15]. Major fimbriae can attach and bind to host cells, extracellular matrix (ECM), as well as salivary proteins. Major fimbriae can also facilitate binding to other bacteria, both *P. gingivalis* itself and other species. Minor fimbriae have a role in biofilm formation [14, 16].

As a Gram-negative species, *P. gingivalis* possesses lipopolysaccharides (LPS). Intriguingly, the lipid A part of *P. gingivalis* LPS has a structure that is heterogeneous. The number of associated fatty acids coupled to the disaccharide core varies, resulting in penta- or tetra-acylated lipid A moieties that allows interaction with both Toll-like receptors (TLR) 2 and TLR4 [17]. It is the availability of hemin in the microenvironment that defines which lipid A form that *P. gingivalis* expresses, enabling the bacteria to determine how it interacts with the host to elicit various inflammatory responses [8, 18].

Gingipains are cysteine proteases which probably are the most vital virulence factor expressed by *P. gingivalis*. Gingipains are membrane-bound, as well as secreted from the bacterium, thus, *P. gingivalis* can exert all the various gingipain activities at distant sites. *P. gingivalis* possesses arginine-specific gingipains, Rgp (RgpA and RgpB), encoded by *rgpA* and *rgpB*, respectively, and the lysine-specific gingipain, Kgp, encoded by *kgp*. *P. gingivalis* expresses numerous proteolytic enzymes, but the gingipains are by far the most important ones, accounting for at least 85% of the total proteolytic activity. Furthermore, they are implicated and play key roles in adherence and colonization of the host, in nutrition acquisition by cleaving host proteins, in neutralization of host defense mechanisms, and in manipulation of the host inflammatory response. In summary, gingipains are vital for bacterial survival and proliferation *in vivo* [7]. In the process of adherence and colonization, *P. gingivalis* utilizes fimbrial adhesions, but nevertheless,

gingipains are also necessary in these steps. RgpA and Kgp contain hemagglutinin-adhesin domains, which are directly involved in conjugation with other bacterial species, thereby promoting the construction of the bacterial biofilm. These domains also enable binding to ECM, as well as interaction with host cells [19–21]. Rgp is also important for processing various *P. gingivalis*-derived proteins. For instance, Rgp is necessary for the modification of major fimbriae to the mature form [22]. Gingipains are also key mediators in dysregulation of the host immune response [23, 24].

Some *P. gingivalis* strains possess a capsule. Encapsulated strains are more virulent since they have been shown to be more invasive and more resistant to phagocytosis [25–27]. *P. gingivalis* also releases outer membrane vesicles, small cargos that are shed from the outer bacterial membrane that are loaded with LPS, gingipains and other proteases, fimbriae, and capsule (encapsulated strains). The shedding of outer membrane vesicles occurs at a higher rate during colonization and biofilm formation, enabling immune modulation at sites distant from the actual site of infection [28].

3. Mechanisms of *P. gingivalis* interaction with host cells

P. gingivalis, as a keystone pathogen, has the ability to interfere with the host in such ways that the growth and survival of the entire biofilm is promoted and enhanced. It is vital for *P. gingivalis* in a hostile environment to be able to counteract, modify, and manipulate the host immune response in order to survive and evade the various host defense mechanisms. Although it is important to evade the host defense mechanisms, it is also of essential importance to induce inflammation to secure a constant delivery of nutrients to the biofilm through the formation of the nutrient-rich-inflammatory exudate that constitutes the gingival crevicular fluid. *P. gingivalis* has indeed evolved elaborated strategies to diminish as well as promote inflammation [5]. The complement system, which targets microbes, is itself a target for proteolysis by gingipains. In fact, *P. gingivalis* can both inhibit and stimulate the complement system [29]. Also, depending on the type of lipid A expressed, *P. gingivalis* can act as both a TLR4 agonist and an antagonist and regulate the TLR4-dependent immune responses [10, 18]. Realizing all the clever ways of escaping, it may not come as a surprise that *P. gingivalis*, as an additional function on the repertoire, also is resistant to oxidative killing by phagocytes and can survive phagocytosis by macrophages [26, 30]. Furthermore, *P. gingivalis* is able to activate the coagulation cascade and the kallikrein/kinin cascade, thereby enhancing inflammation [31–33]. *P. gingivalis* can invade host cells and replicate within the cell [34]. *P. gingivalis* is also able to protect itself from neutrophil-released reactive oxygen species, leaving the oxidative burst effortless and instead contributing to the destruction of the periodontium [13, 30].

The interactions between the host immune system and the oral microbial flora involve complex cellular and molecular mechanisms. Several cell types, for example, epithelial cells, dendritic cells, osteoblasts, and fibroblasts that reside in the periodontium, are part of the innate host response, as well as platelets, neutrophils, and monocytes/macrophages. Cells of the innate immune system recognize and respond to pathogens (e.g., LPS, fimbriae, DNA, and proteases) through pathogen recognition receptors (PRRs). Important PRRs are TLRs and protease-activated receptors

(PARs). Ligation of PRRs induces downstream signaling pathways that modify the activity of transcription factors that regulates the expression of genes linked to inflammation. Early cellular events leading to a phosphorylation cascade of mitogen-activated protein kinase (MAPK) signaling include the activation of Protein kinase C (PKC) by diacylglycerol and calcium. Signals transduced via MAPK pathways lead to the assembly and activation of the transcription factor AP-1. TLR activation results in the recruitment of an adaptor protein, which in many cases involves MyD88, followed by a signaling cascade that phosphorylates, polyubiquitylates, and degrades IκB. This allows the transcription factor NFκB to translocate to the nucleus and induces gene expression (**Figure 1**). AP-1, NFκB, and other transcription factors cooperatively regulate genes, such as inflammatory mediators and growth factors that are important in many biological processes [35, 36]. This is followed by the release of inflammatory mediators such as CXCL8 and interleukin (IL)-6. The chemokine CXCL8 attracts and recruits neutrophils to the site of infection and promotes monocyte adhesion to the vessel wall. The infiltrating neutrophils, as well as resident cells and macrophages, release cytokines, such as tumor necrosis factor-α (TNF-α), IL-1,

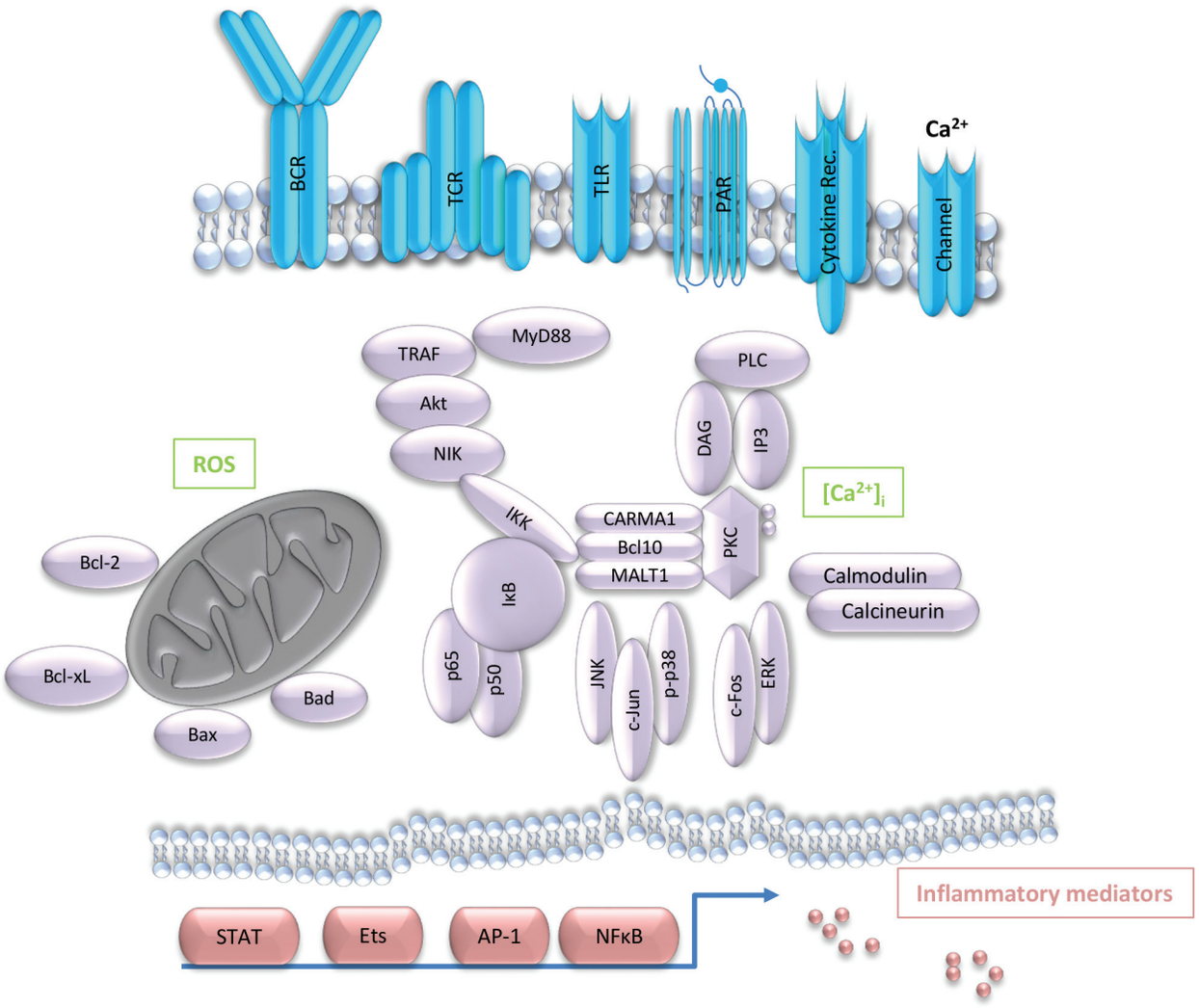

Figure 1. Overview of receptors and intracellular signaling pathways in response to virulence factors of *P. gingivalis*. See text for details.

and IL-6. These inflammatory mediators will eventually contribute to tissue destruction with alveolar bone loss and a sustained chronic inflammation. In addition, the innate immune system will in turn also activate the adaptive immune system with the involvement of lymphocytes [1, 2, 5].

How host-derived factors such as cytokines, hormones, and reactive oxygen species affect periodontal biofilm formation and bacterial virulence is poorly studied and thus not well understood. A recent study suggests that the host-inflammatory responses affect the physiology of bacteria, for example, by utilizing inflammatory mediators as transcription factors [37]. It thus seems quite reasonable that bacteria have evolved mechanisms to sense their environment and to respond to their surrounding by using inflammatory mediators as regulators to be able to adjust and adapt to a changing environment. Consequently, it is possible that early host-inflammatory and immune responses affect and modulate the composition and function of the oral biofilm and the progression of periodontitis.

TLRs are a family of receptors which are of high importance in the innate immune response in sensing pathogens and other danger-associated signals. LPS and fimbriae originate from *P. gingivalis* signals mainly through TLR2, which mediates the release of inflammatory mediators like CXCL8 [38–40]. *P. gingivalis*-mediated activation of TLR2 has been demonstrated to stimulate differentiation and formation of osteoclasts [40]. A study showed that TLR2−/− mice more rapidly cleared *P. gingivalis* infection, had a more efficient phagocytosis of *P. gingivalis*, and also resisted alveolar bone loss despite being repeatedly infected with *P. gingivalis* [41]. TLR2 expression has also been found to be upregulated by *P. gingivalis* [42]. During inflammation, the hemin concentration in the gingival crevicular fluid is high and the tetra-acylated lipid A form is expressed. The tetra-acylated lipid A is acting as a TLR4 antagonist, suppressing TLR4-mediated inflammatory events. The TLR4 antagonist also competitively blocks the binding of TLR4; hence, TLR4 is unable to respond to other bacterial species as well. In addition, since the outer membrane vesicles contain LPS, and can penetrate through the gingival tissue, *P. gingivalis* can dampen the TLR4 effects for the entire oral microbial community. When the hemin concentration is low, inflammation is promoted by expressing penta-acylated lipid A, which works as a TLR4 agonist [10, 18, 43].

PARs have been found to be activated by proteolytic cleaving by gingipains, leading to increased inflammatory response with the release of inflammatory chemokines [39, 44]. PAR2 activation has been demonstrated to induce alveolar bone loss in rats. Since PAR2 is expressed by the cells in the periodontium, *P. gingivalis* and its gingipains are able through PAR2 activation to significantly contribute to the release of several pro-inflammatory mediators that cause degradation of the periodontal tissue [45]. Furthermore, *P. gingivalis* per se has been demonstrated to upregulate the PAR2 expression in gingival fibroblasts [39].

A gradient of CXCL8 is normally established in the healthy periodontal tissue with the highest concentration at the border of the symbiotic dental plaque. This gradient establishes a "wall" of neutrophils, a continuous flow of migrating neutrophils that transit from the vasculature into the periodontium and the gingival crevice. *P. gingivalis* can interact with CXCL8 and this gradient in several ways [2]. In contact with gingival epithelial cells,

P. gingivalis expresses phosphoserine phosphatase SerB, which contributes to CXCL8 inhibition [46]. Gingipains are well known to cleave CXCL8, as well as other cytokines and chemokines, such as IL-6, IL-6 receptor, CXCL10, TNF-α, CD14, IL-4, and IL-12 [23, 24, 44, 47–52]. By targeting inflammatory mediators such as CXCL8, the resulting chemokine paralysis leads to inhibited neutrophil recruitment, thereby promoting the growth of the biofilm. Consequently, *P. gingivalis* undermines innate immunity [2]. Furthermore, CXCL8 is secreted in two different isoforms, as a 72 amino acid (CXCL8-72aa) variant from immune cells and as a 77 amino acid variant (CXCL8-77aa) from non-immune cells such as fibroblasts. CXCL8-72aa is a stronger chemoattractant than CXCL8-77aa, but after cleavage of CXCL8-77aa by gingipains, this is shifted so that the CXCL8-77a has a higher chemotactic potential. This could be a mechanism whereby *P. gingivalis*, by creating a gradient of gingipains across the periodontal tissue can suppress neutrophilic response in the periodontal pocket where the concentration of gingipains is the highest. At a more distant site, with lower concentrations of gingipains, the chemotactic function of CXCL8-77aa is increased, enhancing the inflammatory response and thereby promoting leaky vessels and a constant delivery of nutrients to the biofilm [47, 53].

4. Host cell responses in the oral cavity

4.1. Gingival epithelial cells

The first line of host defense in the gingiva consists of the epithelial cells forming a physical barrier against mechanical stress, exogenous substances, and pathogenic bacteria. This is achieved through different cell-cell junctions, including tight junction and gap junction. *P. gingivalis* uses different strategies to survive and persist in the oral cavity, and invasion of epithelium is one tactical approach in its lifestyle. The advantages of intracellular translocation of *P. gingivalis* into the cells include evasion from immune responses and antibiotics, and accessibility to disseminate to other sites, which collectively leads to persistence and proliferation [4]. The mechanism by which *P. gingivalis* enters epithelial cells is initiated by fimbriae that bind to α5β1-integrin, followed by the formation of cellular pseudopodia and entry through early endosomes. Intracellular bacteria are then either sorted to late endosomes followed by lysosomes for degradation, or fused with autophagosomes and subsequently degraded in autolysosomes. However, a large number of bacteria are able to escape through recycling pathways for exocytosis and are able to infect new cells, which facilitate deeper penetration into the host tissue [54]. While in other cell types, such as endothelial and smooth muscle cells, *P. gingivalis* has been reported to reside and persist within autophagosomes, followed by the prevention of lysosomal fusion and formation of autolysosomes [55, 56]. Interestingly, α5β1-integrin on epithelial cells has recently been shown to positively correlate with cells in S phase of the cell cycle, and *P. gingivalis* persistence may be associated with the ability to preferentially target dividing cells [57]. The virulence of intracellular *P. gingivalis* is associated with its ability to degrade paxillin and focal adhesion kinase, and may explain the significant periodontal tissue degradation and lack of wound healing and tissue regeneration processes in periodontitis [58, 59].

Epithelial cells also participate in innate immune responses by secreting a variety of cytokines and chemokines, such as TNF, IL-6, and CXCL8 [60]. *P. gingivalis* suppresses cytokine and chemokine accumulation below basal levels *in vitro*. These effects are most probably due to the potent enzymatic action of proteinases. Indeed, leukocytes are manipulated by *P. gingivalis* to express a limited repertoire of inflammatory mediators, while suppressing CXCL8 release, which is termed "local chemokine paralysis" [61]. Interestingly, *P. gingivalis* significantly increased TGF-β1 expression from gingival epithelial cells. TGF-β1 functions as a growth factor with anti-inflammatory characteristics. Besides TGF-β1, *P. gingivalis* was observed to induce the expression of a wide array of different growth factors, including Insulin-like growth factor (IGF), Platelet-derived growth factor (PDGF), endothelial growth factor (EGF), and Hepatocyte growth factor (HGF). We have previously shown that *P. gingivalis* induces high levels of HGF in clinical samples from patients with periodontitis. However, the activity of HGF was significantly reduced in patients compared to healthy controls [62].

4.2. Gingival fibroblasts

Gingival and periodontal ligament fibroblasts are the main cell types found in the connective tissue of the periodontium, and they are exposed to pathogens once the epithelial barrier is breached [2, 63]. Fibroblasts provide a structural tissue framework (stroma) and define the microanatomy of the tissue with the key function to regulate and maintain integrity of the connective tissue. Homeostasis of connective tissues is maintained through the production of ECM and by modifying existing ECM by secreting matrix metalloproteinases (MMPs) that cleave and degrade ECM components [64]. The ability of fibroblasts to secrete as well as respond to growth factors and cytokines/chemokines allows reciprocal communication with adjacent cells that facilitates homeostasis of the tissue. Considering the functions of fibroblasts makes it easy to realize that fibroblasts play a vital role in tissue development, differentiation, and repair. Fibroblasts are also of importance in tissue destruction by the release of MMPs and pro-inflammatory cytokines and chemokines [63–65]. PAR1 and TLR2 have been shown to be important in the interaction between gingival fibroblasts and *P. gingivalis*. Gingival fibroblasts can sense *P. gingivalis* through PAR1 and TLR2, and the activation of these receptors leads to the secretion of CXCL8 and IL-6, suggesting that fibroblasts could make a substantial contribution to the inflammatory process seen in periodontitis [38, 39, 66]. Furthermore, *P. gingivalis* is able to modify this response by cleaving fibroblast-derived cytokines through the proteolytic activity of the gingipains and thereby hampering the antimicrobial capacity of the fibroblasts [23, 24, 66].

4.3. Leukocytes

Periodontitis is characterized by interaction between a number of oral pathogens, such as *P. gingivalis*, and blood leukocytes. Neutrophils and monocytes are well equipped with PRRs, such as TLRs, nuclear-oligomerizing domains ½, and PARs. This arsenal of receptors enables the detection of invading pathogens and production of reactive oxygen species, cytokines, and chemokines. We have shown that *P. gingivalis* is capable of inducing ROS in isolated neutrophils and in whole blood, and stimulating the release of inflammatory mediators, such as IL-1β and CXCL8 [67]. Both these cytokines are capable of priming neutrophils, endothelial

cells, and other vascular cells in an autocrine and paracrine manner. Studies have demonstrated that gingipains hydrolyze pro-inflammatory cytokines, but not growth factor/anti-inflammatory cytokines, which result in aberrant immune cell recruitment to the site of infection, ensuring a continued low-grade infection.

The critical balance of different T-cell subsets has previously been described to play an important role in the inflammatory process underlying periodontitis. The presence of specific antibodies for oral bacteria in patients with periodontitis indicates an involvement of adaptive immune responses [68], of which different T-cell subsets play a detrimental role in the pathogenesis of this inflammatory disease. The T-cell-associated cytokine profile in gingival tissue suggests an engagement of T-helper (Th) 1, Th2, and Th17 cells [69–71]. These T-cell subsets are associated with host-derived tissue destruction and bone loss, through, for example, Receptor activator of nuclear factor kappa-B ligand (RANKL) expression. Exaggerated pro-inflammatory responses from T-cells can be controlled by regulatory T-cells (Tregs) that display protective effects through the secretion of anti-inflammatory IL-10 and TGF-β1. Tregs have a central role in maintaining homeostasis by regulating other leukocyte functions and thereby avoiding extensive immune cell activation and its pathological consequences, for example, in periodontitis. Interestingly, we have previously shown that T-cell interaction with *P. gingivalis* leads to a gingipain-mediated inactivation of IL-2 [72], which may thus downregulate Tregs and support the process of periodontitis. Thus, the inhibition of gingipains and maintenance of a Treg-mediated beneficial homeostasis may be a successful strategy for the prevention and treatment of periodontitis.

5. Periodontitis, systemic inflammation, and cardiovascular disease

Periodontal disease is today considered to play a significant role in various systemic conditions and, in the past decade, the enhanced prevalence of cardiovascular disease (CVD) among patients with periodontitis has received increased attention [73, 74]. Several periodontal bacteria and their agents have been identified in atherosclerotic plaques, for example, *P. gingivalis, Fusobacterium nucleatum, T. forsythia, Prevotella intermedia, Aggregatibacter actinomycetecomitans*, and *T. denticola* [75–78]. The occurrence of periodontal bacteria in coronary artery plaques was found to be 5-fold greater in patients with severe periodontitis compared to those with medium periodontitis [79], and DNA from periodontal bacteria, including *P. gingivalis*, was identified in more than 70% of carotid plaque samples [80]. Furthermore, *P. gingivalis* has been shown to influence the development of abdominal aorta aneurysm, involving the activation of TLRs and MMPs [81]. Several animal experiments have demonstrated that oral and systemic infection with periodontal bacteria induces atherosclerosis [74]. Hokamura and Umemura [82] showed that the administration of *P. gingivalis* in a mouse model induces arterial intimal hyperplasia associated with upregulation of the calcium-binding protein S100A9.

When the periodontal disease develops, the gingival epithelium becomes ulcerated by proteolytic activity, for example, by *P. gingivalis*, leading to exposure of the underlying connective tissues and blood capillaries to the bacterial plaque biofilm. At medium periodontitis, the

ulcerative area in the oral cavity ranges between 8 and 20 cm^2, which means that large amounts of periodontal bacteria and their toxins and metabolic products have a chance, during chewing and oral hygiene activities, to disseminate into the bloodstream and cause transient bacteremias and systemic inflammation [74]. By entering the circulation, the bacteria and/or their components (e.g., proteases, fimbrillin, and LPS) activate platelets and neutrophils, induce ROS production, and trigger inflammatory processes in coronary vessels.

Studies using knockout mice orally infected by *P. gingivalis*, demonstrate that atherosclerosis, involving the accumulation of macrophages and inflammatory mediators (CD40, IL-1ß, IL-6, and TNF-α) in atherosclerotic lesions, is highly dependent on TLR2 [41, 83]. In correlation, interaction between *P. gingivalis* and human blood cells, for example, platelets, neutrophils, monocytes, and T-cells, is mainly mediated by TLR2 and has dramatic inflammatory and immunomodulatory effects, including cellular aggregation, oxygen radical production, low-density lipoprotein (LDL) oxidation, and release and degradation of cytokines. Furthermore, *P. gingivalis* changes the expression of more than thousand genes in vascular smooth muscle cells [84]. For example, *P. gingivalis* upregulates genes involved in proliferation, for example, the TGFβ1 pathway and production of matrix proteins, but downregulates pro-inflammatory genes, such as those involved in IL-1β, IL-6, and CXCL8 production. *P. gingivalis* also caused a dramatic increase in the expression of angiopoietin2 (ANGPT2), which is highly correlated with inflammation and atherosclerosis, whereas ANGPT1, inhibitor of inflammation, was downregulated [85, 86]. These effects are mediated via gingipain R, possibly through PAR signaling. Furthermore, the level of another angiogenic factor, vascular endothelial

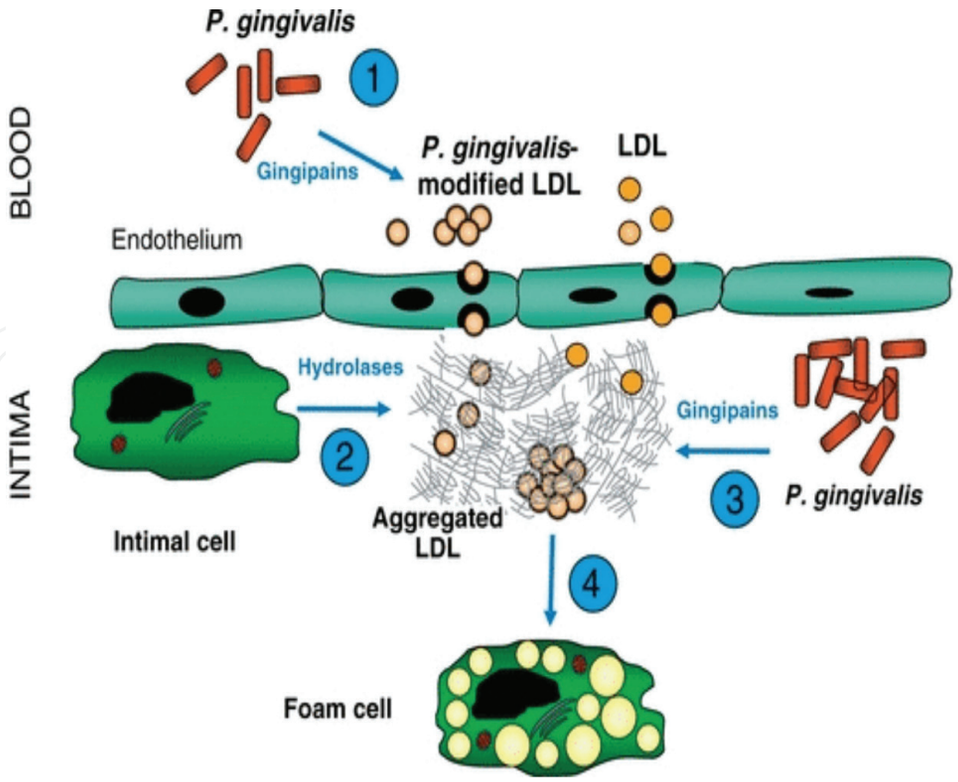

Figure 2. A novel biochemical link between periodontitis and cardiovascular disease.

growth factor (VEGF), increases in patients with periodontitis, and periodontal treatment reduces its concentration [87]. These data indicate that *P. gingivalis* causes a shift from contractile smooth muscle cells to proliferating and matrix-producing smooth muscle cells, which contributes to the growth of the fibrous atherosclerotic plaque, and promotes vascular inflammation and angiogenesis.

P. gingivalis has also been shown to modify LDL and promote phenotypic shift of monocytes to foam cells [75, 77, 88]. Our group has previously found fragmentation of the dominating apoprotein of LDL, apo B-100, by *P. gingivalis* and its Rgps [88]. Consequently, our findings together with others suggest that *P. gingivalis* during translocation in circulating blood modifies LDL to an atherogenic form which may represent a link between periodontal disease and atherosclerosis (**Figure 2**).

6. Host cell responses in the circulation and vascular wall

Endothelial cells possess secretory and immunological properties and play therefore important roles in the cardiovascular system. The association of periodontitis with cardiovascular complications includes the induction of endothelial dysfunction, oxidative stress, and systemic inflammation [89]. Furthermore, patients with periodontitis have increased levels of pro-inflammatory mediators, including C-reactive protein (CRP), IL-6, and TNF that may induce endothelial dysfunction [90]. Endothelial dysfunction, which is the initial step in the development and progression of atherosclerosis, is mediated by endotoxins and gingipains of periodontal bacteria. These toxins lead to an impairment of normal endothelial function, including vessel permeability and immune cell adhesion and function [91, 92]. Furthermore, *P. gingivalis* and other periodontal pathogens induce the expression of endothelin-1, a potent vasoconstrictor released by endothelial cells [93, 94]. Endothelin-1 expression has shown a positive correlation to pro-inflammatory cytokines TNF, IL-6, and IL-1β [95], and a negative correlation to anti-inflammatory mediators, for example, angiopoietin-1 [96, 97].

Platelets are key players in hemostasis and acute thrombosis and are initial actors in the development of atherosclerotic lesions often triggered by endothelial dysfunction [98]. However, they are also involved in the immune system and express a broad repertoire of immune cell features such as TLRs, the immunoglobulin γ-receptor FcγRIIA, complement receptors, inflammatory mediators, as well as microbicidal activities, for example, thrombocidins [99, 100]. Furthermore, platelets bind to and encapsulate bacteria, release ROS and recruit and activate leukocytes and regulate inflammatory processes of the vessel wall [101]. These characteristics make it possible for platelets to recognize and respond to pathogens, such as *P. gingivalis*, and engage other immune cells for enhanced bacterial clearance and inflammatory response.

Several studies suggest that platelet-leukocyte interaction is an essential underlying inflammatory process in atherosclerosis, and patients with cardiovascular disease have an increased number of neutrophil-platelet aggregates in the blood circulation [102, 103]. In correlation, we have shown that *P. gingivalis* markedly induces the formation of large aggregates of

neutrophils and platelets, associated with ROS production and lipid peroxidation, in whole blood and that this effect is dependent on CD11b/CD18-fibrinogen-GpIIb/IIIa interaction, and Rac2 and Cdc42 activation [104, 105] (**Figure 3**). In addition, mice challenged with *P. gingivalis* were found to form platelet-neutrophil aggregates, whereas knockout TLR2$^{-/-}$ mice did not. Human platelets express TLRs (TLR 1, 2, 4, 6, and 9), which could be key molecules linking periodontal infection and CVD. For example, TLR2-mediated platelet activation involving the activation of GpIIb/IIIa and P-selectin contributes to the formation of platelet-leukocyte complexes and ROS production [99].

Platelets activation by TLR1/2 receptor ligands results in aggregation as well as secretion of inflammatory mediators such as RANTES, macrophage migration inhibitory factor (MIF), and plasminogen activator inhibitor-1 (PAI-1) [105]. Interestingly, these platelet-derived factors are degraded by gingipains from *P. gingivalis* [105]. Regulated on activation, normal T-cell expressed and secreted (RANTES) is induced by *P. gingivalis* and its lipopolysaccharides and is thus implicated in periodontitis, where elevated levels have been detected in the gingival crevicular fluid of patients with periodontitis [106]. It has been demonstrated that *P. gingivalis*, in addition to TLR2, also can trigger platelet activation via PAR receptors. Through the action of Rgp on PARs, *P. gingivalis* activates platelets by increasing intracellular-free calcium and induces aggregation [105]. In correlation, Lourbakos et al. and McNiol and Israels [107, 108] have demonstrated that gingipains activate PAR1 and PAR4 on platelets leading to aggregation and secretion. We have shown that *P. gingivalis* triggers platelet aggregation through

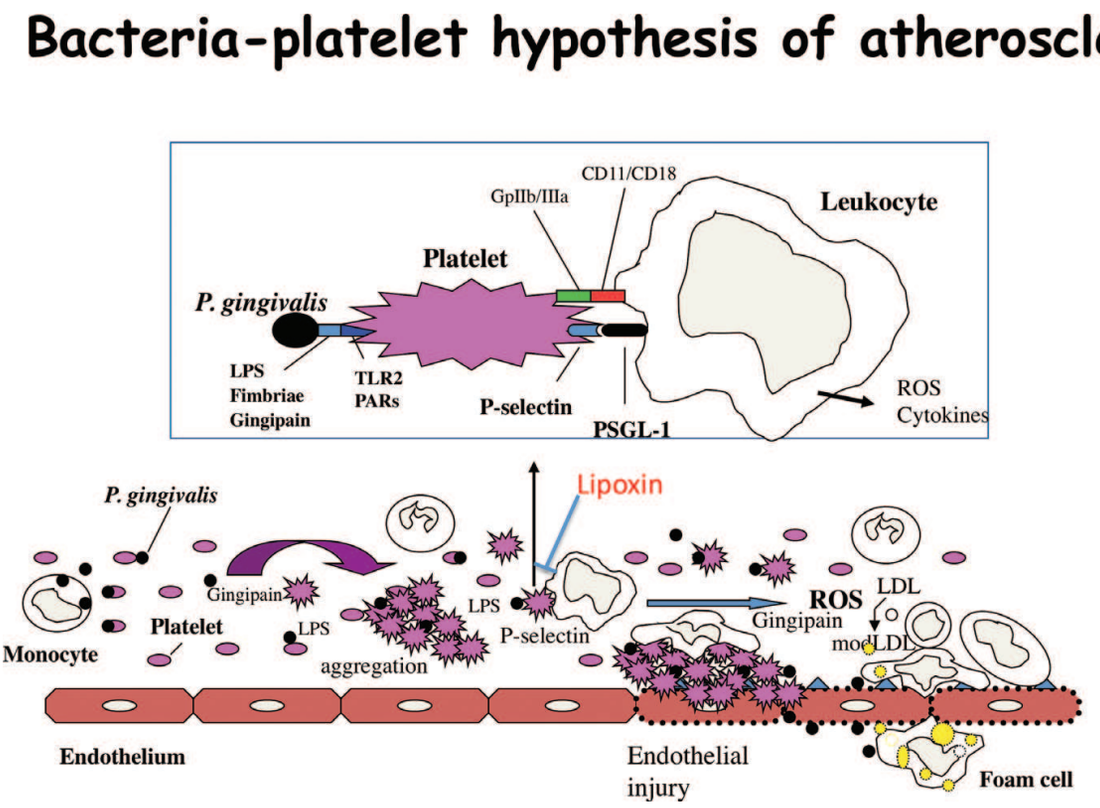

Figure 3. Model showing platelets as a linker between periodontal infection and innate immune response at the vessel wall.

gingipain interaction with PARs and sensitizes platelets for activation by epinephrine, which may explain the association between periodontitis, stress and CVD [109].

7. Preventive and treatment strategies

Periodontal pathogens reside in biofilms of subgingival dental plaque and form complex polymicrobial communities. The failure of the immune system to resolve bacterial biofilms results in an accumulation of inflammatory mediators that accelerates the disease state toward a chronic inflammatory condition. Bacterial biofilms are difficult to treat, and conventional methods, including mechanical removal and scaling and root planning (SRP), are still being used. These methods are less efficient and new preventive/treatment strategies are needed. A new approach includes the administration of adjunctive antibiotics systemically in combination with SRP. Different antibiotics have been applied, and a combination of metronidazole and amoxicillin was found to be effective at reducing pocket depth and clinical attachment gain compared to SRP alone, reviewed in [110]. Although antibiotic therapy is effective in modern medicine, microorganisms that are resistant to single or multiple antibiotics have emerged. The development of new families of antibiotics has significantly declined, which is associated with high costs and concerns for possible effects on the commensal microbiota and host health [111]. It is evident that new alternative strategies to traditional antibiotic therapy are needed. New approaches to combat bacterial infections include antibodies, vaccines, bacteriophages, probiotics, and antimicrobial peptides (host- and bacteria-derived) [111–114]. These strategies of promising candidates to traditional antibiotics deserve more consideration.

8. Concluding remarks

In summary, it is possible that *P. gingivalis* has a role in pathogenic oral biofilms to undermine important factors of innate immunity, by altering the functions of receptors and their intracellular signaling pathways and the levels of effector molecules, and thereby antagonizing an effective host response. These activities of key periodontal pathogens could contribute to an adaptation and maturation of dysbiotic biofilm communities and promote chronic inflammation and tissue destruction of periodontitis. Increased understanding of the interbacterial interactions that occur in the oral polymicrobial biofilm and its interplay with the host immune system is of uttermost importance for identifying novel targets for the prevention, diagnosis, and treatment of periodontitis and associated systemic disorders.

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