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Review Article

A Boolean Network Approach to Study the Mechanism Associated with Inflammatory Response Induced by *Porphyromonas gingivalis*

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Abstract

Anaerobic *Porphyromonas gingivalis* is a rod-shaped bacterium and is a primary agent of periodontal inflammation and thus periodontitis. This bacterium disturbs the normal flora of the oral cavity and causes dysbiosis. Databases including Google Scholar Scopus and PubMed were employed to find the evidence by using keywords like '*Porphyromonas gingivalis*,' 'Boolean network,' 'inflammatory response and *Porphyromonas gingivalis*,' 'inflammation and *Porphyromonas gingivalis*. Only articles that reviewed the role of *Porphyromonas gingivalis* in oral inflammation were selected. *Porphyromonas gingivalis* promotes and reorganizes host immune systems against normal host flora, which causes a dysbiotic state. A reorganized immune system induces dysbiosis and periodontitis. Specifically, the role of the C5a receptor in the complement system is vital in this mechanism. *P. gingivalis* can change the metabolic pathways of phagocytic cells without impeding inflammation. Toll-like receptor and complement signaling are inverted by *Porphyromonas gingivalis*, which aids them in overcoming immunological responses. However, they sustain the inflammation process, which promotes dysbiosis. Instead of a subjective approach, a systems perspective is required to comprehend this intricate process. A Boolean network is a system approach that seems to be a better approach to understanding this complicated interaction process of *Porphyromonas gingivalis* with the immune system and inflammation. In short, attempts to understand the complex process using the Boolean network will ultimately help in the early detection of periodontitis, and immediate treatment can prevent soft tissue destruction and dentition loss.

Keywords: Boolean network, Oral health, *Porphyromonas gingivalis*, Oral inflammation, the Complement system

1. Context

Normal flora inside the human body affects health to a great extent. Disruption of normal flora destabilizes not only human health but also causes various life-threatening diseases. There are different complex interactions between the normal flora and the human body. One of the significant physiological systems to study such interactions is undoubtedly oral health. Periodontal disease or gum disease is one such oral health issue that is affected by the disturbance of normal oral flora. Periodontal disease involves the inflammation of the gum surrounding a tooth. Bacterial invasion and colonization within gum tissue is the leading cause of the periodontal disease (1). The keystone organism associated with periodontal disease is *Porphyromonas gingivalis (P. gingivalis)*. *P.*

gingivalis is an anaerobic, non-motile, gram-negative bacterium identified by black colonies on blood agar. It is predominant in human oral flora, gastrointestinal (GI), and respiratory tract. A unique property of this species is the production of collagenase, which could break collagen (2). They could invade the epithelial cells of human gum and persist along with the epithelial cell for a long time (Katz et al. 2002). Infection with *P. gingivalis* also associated with some complex diseases like rheumatoid arthritis and Alzheimer's disease (3). Gingipain is the most important among various virulence factors of *P. gingivalis* (4). These are endopeptidases responsible for their survival. Other than that, capsular polysaccharides and fimbriae are also important. The interaction of *P. gingivalis* with the human immune system is exciting and complex. *P. gingivalis* (Pg) promotes and reorganizes host immune systems against normal host flora, which causes a dysbiotic state. It causes inflammation but evades the inflammatory response through some complex mechanism that is unclear until today. Rather than a reductionist approach, a systems perspective is required to comprehend this intricate process. This review attempts to understand the complex process using the Boolean network.

Dysbiosis could be described as an imbalance of normal flora in a specific organ. As previously described, this deviation from normal flora could cause various physiological problems and even be detrimental to human health. The modified immune systems of the host also favor this dysbiosis. Without the active immune system, Pg fails to evoke dysbiosis and periodontitis. Precisely, the C5a receptor of the complement system plays an important role here. C5a deficient mice mutant also fails to induce dysbiosis and periodontitis, as reported by Hajishengallis and colleagues (5). To cause dysbiotic disease, Pg must overcome the host immunity but at the same time provoke inflammation. This complex process could be possible only if Pg could suppress only the killing process of the immune system but not the process of inflammation. Pg uses products from tissue breakdown for their nutritional needs (6). So, they selectively inhibit immune reactions against them without compromising the process of inflammation. Thus, it could be hypothesized that Pg could somehow alter the metabolic pathways of phagocytic cells without hampering the inflammation process. Pg inverts Toll-like receptor (TLR) and complement signaling, which helps them to overcome immune threats against them. However, they preserve inflammation which helps in dysbiosis (7).

This study aims to understand how *P. gingivalis* promotes inflammatory response in phagocytic cells. As described above, *P. gingivalis* induces the inflammatory response in a highly complex way, enabling them to bypass the bactericidal activity. In phagocytic cells like neutrophils, *P. gingivalis* disarms the TLR2-MyD88 pathway from the TLR2-Mal-PI3K pathway. It happens through crosstalk between *P. gingivalis*, C5aR, and TLR2. Due to the crosstalk, *P. gingivalis* can raise the bacterial count in periodontal tissue. P. gingivalis may circumvent phagocytosis and phagosomal maturation with the aid of PI3K and RhoA (Ras homolog family member A). *P. gingivalis* also promotes C5aR/TLR2-dependent ubiquitination of MtD88, which blocks the bactericidal activity. On the other hand, the increased level of PI3K helps maintain an inflammatory response in the periodontal tissue (7). So, the over-expression of PI3K is responsible for maintaining inflammation and avoiding phagocytic activity for *P. gingivalis*. This review also presents a system model using a Boolean network to help understand this complicated process.

2. Evidence Acquisition

Different keywords were used to find relevant research studies using two widely used databases, i.e., Google Scholar and PubMed. Keywords used to search the relevant studies were 'Boolean network,' '*Porphyromonas gingivalis*,'

'*Porphyromonas gingivalis* AND inflammatory response,' and '*Porphyromonas gingivalis* AND inflammation.'

Only studies were included in this review that described the interaction of *P. gingivalis* with the immune system and its role in periodontitis-associated inflammation. All other studies that do not review the role of *P. gingivalis* in periodontitis-associated inflammation were excluded as they were not up to the inclusion criteria of this study. Cross-references of selected articles were also searched to find more relevant articles. Only published articles were included in this review, and all other articles and studies were excluded.

3. Results

3.1. *Porphyromonas gingivalis* and Inflammation

P. gingivalis induces bone loss and damage to soft tissue by inducing the expression of potent inflammatory cytokines such as TNF-α, IL-1, and IL-6. These inflammatory cytokines can cause several pathological and physiological changes associated with periodontitis (8). In a rat periodontitis model, topical application of LPS derived from *P. gingivalis* (*P. gingivalis*-LPS) into the palatal gingival sulcus caused significant alveolar bone loss. It also induced memory and learning impairment in rats. Application of *P. gingivalis*-LPS led to the activation of the pro-inflammatory TLR4/NF-κB signaling pathway. Importantly, periodontitis was related to neuroinflammation, aberrant - and -secretase activity, microglia, and astrocyte activation (9). Recent experimental observations suggest that *P. gingivalis* induces the expression of inflammatory microRNA miR-132. The induction of miR-132 was dependent on TLR2/4 and NF-κB signaling. Suppression of miR-132 significantly reduced the expression of TNF-α in THP-1-derived macrophages. The study highlights micro RNAs' crucial role in inflammation and periodontitis's progression (10). Papadopoulos *et al.* demonstrated that the TLR2 receptor is needed for *P. gingivalis*-induced alveolar bone loss. Moreover, TNF-α deficient mice do not develop alveolar bone loss suggesting a central role of TNF-α in periodontal inflammation. The initial inflammatory response mounted by naïve macrophages against *P. gingivalis* is MyD88 dependent and requires cooperation between TLR2 and TLR4. The study identified a TLR2- and TNF-dependent mechanism that involves macrophages and is implicated in bone loss caused due to inflammation (11). Studies have shown that treatment with beneficial gut microbes such as *Akkermansia muciniphila* (*A. muciniphila)* can lower the inflammation induced by *P. gingivalis*. *A. muciniphila* treatment lowered the infiltration of immune cells and prevented alveolar bone loss. Additionally, *A. muciniphila* increased the expression of anti-inflammatory cytokine IL-10 and lowered the expression of IL-12. Finally, *A. muciniphila* co-cultured with *P. gingivalis* reduced the mRNA expression of gingipains in *P. gingivalis* (12). In experimental animal models, injection of *P. gingivalis*-LPS in systemic circulation can induce periodontal inflammation and alveolar bone destruction. Experimental periodontitis also increased the systemic levels of inflammatory mediators such as IL-6 and PTX3 24 hours post-*P. gingivalis*-LPS injection. *P. gingivalis*-LPS injection maintained an inflammatory state for 21-days, and the anti-inflammatory cytokine IL-10 remained low during the study period (13). Studies have shown that the NLRP3 Inflammasome is induced by *P. gingivalis* and NLRP3. Inflammasome-medicated secretion of pro-inflammatory cytokine IL-1β is instrumental in periodontitis. Further, Inflammasome like NLRP3, NLRP6, NLRP12, and AIM2 show abnormal upregulation in gingival tissues of periodontitis patients. Importantly, THP-1 cells with si-RNA-induced knockdown of NLRP-3, caspase-1, and

caspase-4 displayed lower secretion of IL-1β upon infection with *P. gingivalis*, suggesting that NLRP3/Caspase-4 and NLRP3/Caspase-1 dependent IL-1β synthesis in periodontitis is crucial for the abnormal inflammatory response in periodontitis (14). Interestingly, catechin prevents *P. gingivalis*-induce alveolar bone damage and inflammation by reducing the production of IL-1β. At the molecular level, catechin inhibits the expression of pro-IL-1β by downregulating NF-kB, p38-MAPK, and TLR signaling. Additionally, catechin prevented the activation of the Inflammasome, thus further reducing the inflammatory environment. Taken together, the anti-inflammatory properties of catechin can be exploited for the treatment of periodontitis (15). Uric acid acts as a danger-associated molecular pattern (DAMP) and elicits inflammation by activating pro-inflammatory cytokines. Studies have demonstrated that *P. gingivalis* gingipains facilitate the production of uric acid by upregulating the expression of xanthine oxidoreductase (XOR) in THP-1 macrophages. Once synthesized, uric acid modulates several physiological and immunological pathways and promotes the secretion of IL-1α, IL-6, and IL-8. Moreover, it induces apoptosis by activating the caspase-1 enzyme. The results suggest that gingipains-induced uric acid synthesis is an important strategy used by *P. gingivalis* to promote inflammation in the periodontal tissue cells (16). Long-term exposure to *P. gingivalis*-LPS induces tolerance in the host called endotoxin tolerance. This tolerance generates a hyporesponsive state in the host. It has been observed that endotoxin tolerance induced by *P. gingivalis*-LPS adversely affects the functioning of neutrophils which reflects in a distinct immunoprogramming. *P. gingivalis*-LPS-tolerized neutrophils displayed significantly lower phagocytosis, lower production of reactive oxygen species, and reduced apoptosis. Tolerized neutrophils also secrete lower inflammatory cytokine TNF-α but higher IL-10 levels. Moreover, ERK1/2 phosphorylation was suppressed in tolerized neutrophils (17). *P. gingivalis* modulates the functions of neutrophils and also helps in bacterial survival. It has been shown that *P. gingivalis* can dysregulate the TLR2-MyD88 pathway in neutrophils by promoting the proteasomal degradation of MyD88. Also, *P. gingivalis* modulates the complement pathway (C5aR receptor) and activates the TLR2-Mal-PI3K pathway, which blocks *P. gingivalis* phagocytosis by neutrophils. This activation provides a "bystander" protection to the pathogen and promotes dysbiotic inflammation (7). In certain cases, *P. gingivalis* reaches from periodontal pockets to the systemic circulation and binds with circulating red blood cells. This attachment protects the pathogen from immune cells/phagocytosis. Neutrophils isolated from localized aggressive periodontitis (LAgP) patients show increased production of IL-6, CCL2, and TNF-α in the presence of RBCs suggesting that RBC provides a selective growth advantage to *P. gingivalis* (18). Figure 1 shows the role of *P. gingivalis* in several metabolic and microbial disturbances in the oral cavity.

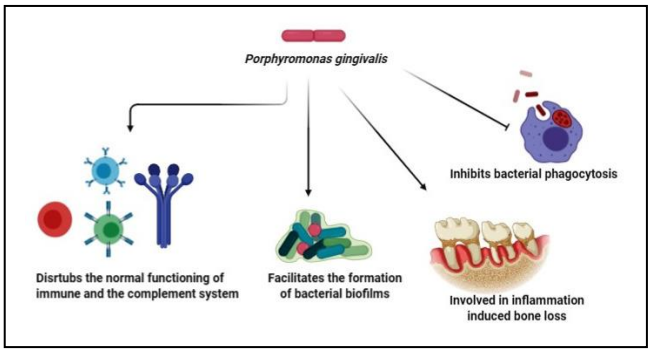
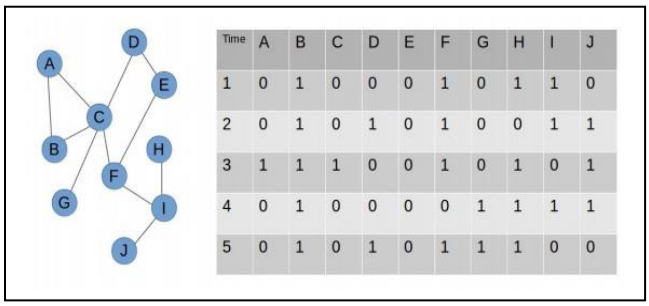


Figure 1. Implications of *P. gingivalis* on the oral cavity as a cause of several metabolic and microbial disturbances

3.2. Boolean Network

A Boolean network is a set of nodes connected via edges where each node is associated with a Boolean function. A Boolean network is used in biology to

model regulatory networks. It helps to model dynamic systems. In a Boolean network, each node in a particular state is either 'ON' (represented by 1) or 'OFF' (represented by 0). The state of a node at a particular time step (t + 1) is determined by its Boolean function, which is associated with a set of control nodes and their states in the previous time step (t). In the case of a gene regulatory network, the expression of a gene (either ON or OFF) is determined by its control genes and their expression states. The Boolean function is associated with the complex interactions of the control genes. Various models represent a Boolean network, among which the Kauffman model is extensively studied (19). Figure 2 shows a Boolean network.



Time	A	B	C	D	E	F	G	H	I	J
1	0	1	0	0	0	1	0	1	1	0
2	0	1	0	1	0	1	0	0	1	1
3	1	1	1	0	0	1	0	1	0	1
4	0	1	0	0	0	0	1	1	1	1
5	0	1	0	1	0	1	1	1	0	0

Figure 2. Representation of a Boolean network (20)

A Boolean network can be employed to study the complex interaction of various immune components with *P. gingivalis*. The overexpression of PI3K could be responsible for maintaining inflammation and avoiding phagocytic activity for *P. gingivalis*. Figures 3 and 4 shows the interaction network of various immune components with *P. gingivalis.*

Using the truth table, this Boolean representation helps simulate the system at various time steps. *P. gingivalis* infection may induce inflammation and dysbiosis but at the same time bypasses phagocytosis. So, by using the Boolean network to simulate the network starting from *P. gingivalis*, at t=0, all the components show an OFF (blue) state, while at t=1, components show ON (Yellow-green) *P. gingivalis*. At the end of the simulation, t= 6, *P. gingivalis* induce inflammation and dysbiosis, as shown in figure 5. In addition, phagocytosis has been bypassed using the complex interaction with the help of PI3K.

The time series and the network status are shown in figure 5.

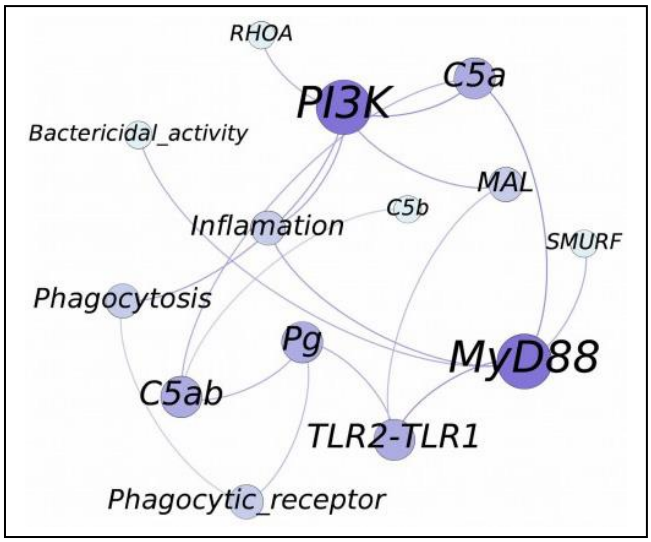


Figure 3. Network representation of crosstalk between *P. gingivalis* (Pg) and other immune components

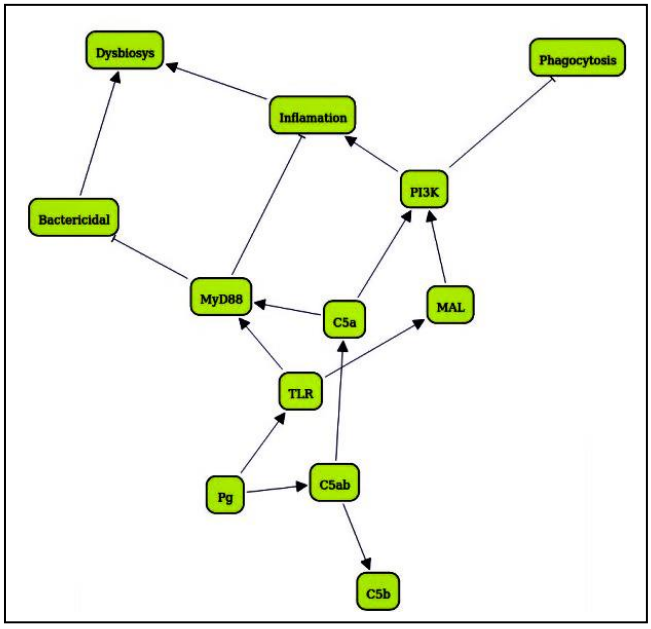


Figure 4. Boolean representation of *P. gingivalis* (Pg) and other immune components

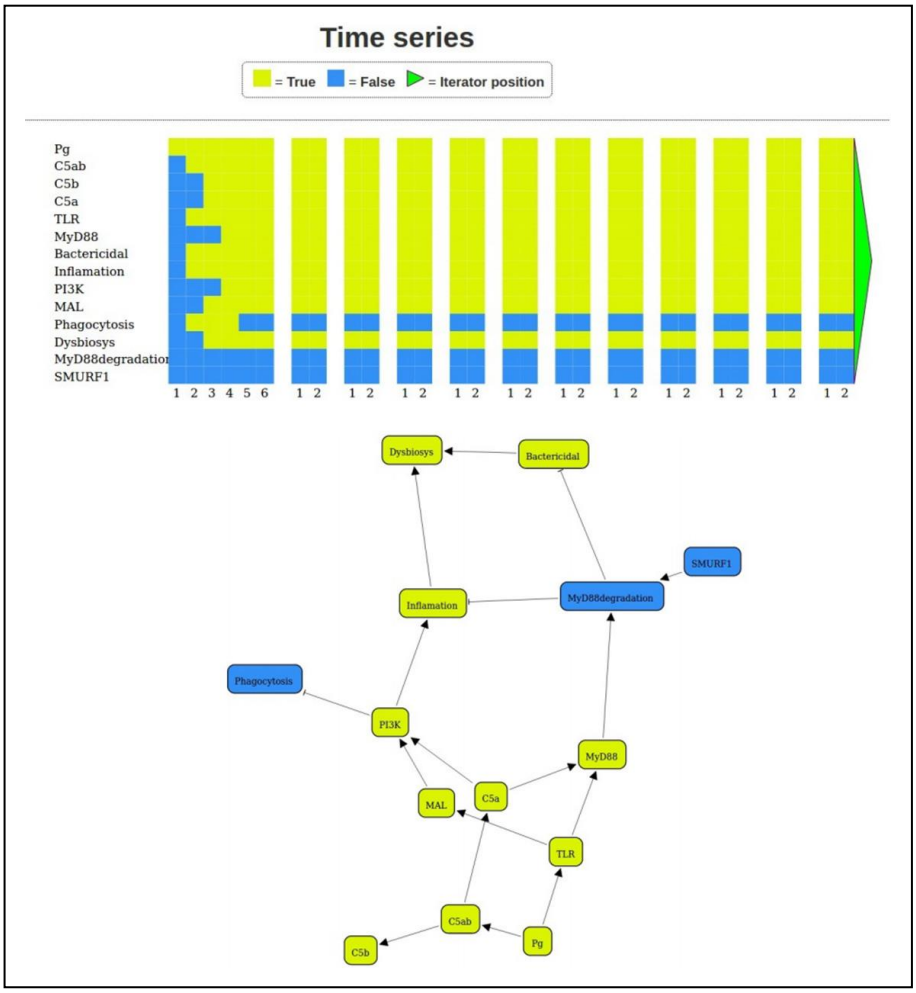


Figure 5. The time series of the simulation and the end state of the network

4. Conclusions

P. gingivalis is an anaerobic gram-negative bacterium that is considered a most common pathogen in altering the normal oral microbiota, which can lead to a dysbiotic microbial population, consequently causing inflammatory responses and periodontitis. It has been found that *P. gingivalis* could escape phagocytosis but induce inflammation in dysbiosis. Studies have described that the production of neutrophils due to modulation of the complement pathway (C5aR receptor) and activation of the TLR2-Mal-PI3K pathway by *P. gingivalis* help block phagocytosis of *P. gingivalis*. This activation provides a "bystander" protection to the pathogen and promotes dysbiotic inflammation. A Boolean network can be employed to find this complex interaction. A Boolean network showed that *P. gingivalis* escaped from phagocytosis by using the complex interaction with PI3K. The results of the Boolean system further required proper wet lab validation.

Authors' Contribution

Study concept and design: A. G.
Acquisition of data: A. G. B.
Analysis and interpretation of data: S. N.
Drafting of the manuscript: A. M.
Critical revision of the manuscript for important

intellectual content: A. G.
Administrative, technical, and material support: A. G. B.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Samaranayake L, Matsubara VH. Normal oral flora and the oral ecosystem. Dent Clin North Am. 2017;61(2):199-215.
2. Holt SC, Kesavalu L, Walker S, Genco CA. Virulence factors of Porphyromonas gingivalis. Periodontol 2000. 1999;20(1):168-238.
3. Mikuls TR, Payne JB, Yu F, Thiele GM, Reynolds RJ, Cannon GW, et al. Periodontitis and Porphyromonas gingivalis in patients with rheumatoid arthritis. Arthritis Rheumatol. 2014;66(5):1090-100.
4. Travis J, Pike R, Imamura T, Potempa J. Porphyromonas gingivalis proteinases as virulence factors in the development of periodontitis. J Periodontal Res. 1997;32(1):120-5.
5. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. 2011;10(5):497-506.
6. Hajishengallis G. The inflammophilic character of the periodontitis-associated microbiota. Mol Oral Microbiol. 2014;29(6):248-57.
7. Maekawa T, Krauss JL, Abe T, Jotwani R, Triantafilou M, Triantafilou K, et al. Porphyromonas gingivalis manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. Cell Host Microbe. 2014;15(6):768-78.
8. Chen Y, Zhou R, Yi Z, Li Y, Fu Y, Zhang Y, et al. Porphyromonas gingivalis induced inflammatory responses and promoted apoptosis in lung epithelial cells infected with H1N1 via the Bcl‐2/Bax/Caspase‐3 signaling pathway. Mol Med Rep. 2018;18(1):97-104.
9. Hu Y, Li H, Zhang J, Zhang X, Xia X, Qiu C, et al. Periodontitis induced by P. gingivalis-LPS is associated with neuroinflammation and learning and memory impairment in Sprague-Dawley rats. Front Neurol. 2020;14:658.
10. Park MH, Park E, Kim H-J, Na HS, Chung J. Porphyromonas gingivalis-induced miR-132 regulates TNFα expression in THP-1 derived macrophages. SpringerPlus. 2016;5(1):1-9.
11. Papadopoulos G, Weinberg EO, Massari P, Gibson FC, Wetzler LM, Morgan EF, et al. Macrophage-specific TLR2 signaling mediates pathogen-induced TNF-dependent inflammatory oral bone loss. J Immun. 2013;190(3):1148-57.
12. Huck O, Mulhall H, Rubin G, Kizelnik Z, Iyer R, Perpich JD, et al. Akkermansia muciniphila reduces Porphyromonas gingivalis-induced inflammation and periodontal bone destruction. J Clin Periodontol. 2020;47(2):202-12.
13. Leira Y, Iglesias-Rey R, Gómez-Lado N, Aguiar P, Sobrino T, D'Aiuto F, et al. Periodontitis and vascular inflammatory biomarkers: an experimental in vivo study in rats. Odontology. 2020;108(2):202-12.
14. Ding P-H, Yang M-X, Wang N-N, Jin L-J, Dong Y, Cai X, et al. Porphyromonas gingivalis-induced NLRP3 inflammasome activation and its downstream interleukin-1β release depend on caspase-4. Front Microbiol. 2020;11:1881.
15. Lee HA, Song YR, Park MH, Chung HY, Na HS, Chung J. Catechin ameliorates Porphyromonas gingivalis-induced inflammation via the regulation of TLR2/4 and inflammasome signaling. J Periodontol. 2020;91(5):661-70.
16. Jun HK, An SJ, Kim HY, Choi BK. Inflammatory response of uric acid produced by Porphyromonas gingivalis gingipains. Mol Oral Microbiol. 2020;35(5):222-30.
17. Gu J-y, Liu Y-j, Zhu X-q, Qiu J-y, Sun Y. Effects of endotoxin tolerance induced by Porphyromonas gingivalis lipopolysaccharide on inflammatory responses in neutrophils. Inflammation. 2020;43:1692-706.
18. Damgaard C, Kantarci A, Holmstrup P, Hasturk H, Nielsen CH, Van Dyke TE. Porphyromonas gingivalis-induced production of reactive oxygen species, tumor necrosis factor-α, interleukin-6, CXCL 8 and CCL 2 by neutrophils from localized aggressive periodontitis and healthy donors: modulating actions of red blood cells and resolvin E1. J Periodontal Res. 2017;52(2):246-54.
19. Kauffman S. Homeostasis and differentiation in random genetic control networks. Nature. 1969;224(5215):177-8.
20. Bock M, Scharp T, Talnikar C, Klipp E. BooleSim: an interactive Boolean network simulator. Bioinformatics. 2014;30(1):131-2.