

## Review

# Toll-like receptors and their dual role in periodontitis: a review

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**Abstract:** Innate immunity is the first line of host defense and represents inherited resistance to infection. Innate immunity works through toll-like receptors (TLRs), which recognize the conserved molecular patterns on pathogenic bacteria known as pathogen-associated molecular patterns. The periodontium is a unique environment in which oral microorganisms are in constant contact with the host immune system. The TLRs present on gingival epithelial cells are continuously stimulated, resulting in production of cytokines and defensins that help to maintain oral health. If the epithelial barrier is breached, allowing invasion of bacteria into the underlying connective tissue, the TLRs on other resident and non-resident cells of the periodontium become activated. This leads to an exaggerated release of pro-inflammatory cytokines and other biological mediators, which may cause host tissue destruction. The present review examines the role of TLRs and their signaling in periodontal health and disease. (J Oral Sci 53, 263-271, 2011)

Keywords: toll-like receptors; pathogenesis; periodontitis; innate immunity.

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## Introduction

Innate immunity is the first line of immunological defense. Previously it was thought to involve a non-specific immune response, but lately it has been recognized

to show remarkable specificity of discrimination between host and pathogens through a sophisticated system based on toll-like receptors (TLRs). These germline-encoded receptors, collectively known as pattern-recognition receptors (PRRs), can detect and respond to conserved and generally distinct microbial structures that are shared by related groups of microorganisms (1). These microbial structures are referred to as pathogen-associated molecular patterns (PAMPs), and include bacterial lipopolysaccharides, peptidoglycan, lipoproteins, bacterial DNA, and double-stranded RNA. Upon interaction with these PAMPs, TLRs activate the innate immune cells through intracellular signaling pathways. This TLR-mediated innate immune response is also critical for activation of adaptive immunity. Since periodontitis is a chronic inflammatory disease, TLRs play an important role in its pathogenesis. Recent research has indicated an essential role of these receptors in maintaining the periodontium in a healthy state, as well as in the causation of periodontitis.

## History

Toll-like receptors are so-called because their similarity to the protein encoded by the Toll gene, which was identified in *Drosophila* in 1985 by Christiane Nüsslein-Volhard (2). The product of the Toll gene was later found to be responsible for dorso-ventral polarity during embryonic development. In 1996, Jules A. Hoffmann et al. demonstrated that Toll plays an essential role in the immunity of *Drosophila* to fungal infection, which it achieved by activating the synthesis of antimicrobial peptides (3). The first reported human TLR was described by Nomura et al. in 1994 (4), and mapped to a chromosome by Taguchi et al. in 1996 (5). Because, at that time, the immune function of Toll in *Drosophila* was not known, it was assumed that this TLR (now known as

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TLR1) might participate in mammalian development. In 1997, it was observed that a TLR now known as TLR4 could, when artificially ligated using antibodies, induce the activation of certain genes necessary for initiating an adaptive immune response (6). The association of TLRs with innate immunity was recognized with the discovery of mouse TLR4, which acts as a receptor of bacterial lipopolysaccharide (7). To date, ten human TLRs have been identified, and these are believed to detect a discrete collection of molecules of microbial origin, and to signal the presence of infections.

### The toll-like receptor superfamily

Toll-like receptors, together with the interleukin-1 receptors, form a receptor superfamily known as the “Interleukin-1 Receptor/Toll-Like Receptor Superfamily”; all the members of this family have in common a so-called Toll-IL-1 receptor (TIR) domain. Three subgroups of TIR domains exist. Proteins with subgroup 1 TIR domains are receptors for interleukins that are produced by macrophages, monocytes and dendritic cells, and all have extracellular immunoglobulin (Ig) domains. Proteins with subgroup 2 TIR domains are classical TLRs, and bind directly or indirectly to molecules of microbial origin. A third subgroup of proteins containing TIR domains consists of adaptor proteins that are exclusively cytosolic and mediate signaling from proteins of subgroups 1 and 2 (8).

TLRs are present in vertebrates, as well as in invertebrates. It has been estimated that most mammalian

species have between ten and fifteen types of TLRs. Thirteen TLRs (named simply TLR1 to TLR13) have been identified in humans and mice together, although equivalents of certain TLRs found in humans are not present in all mammals. For example, a gene encoding a protein analogous to TLR10 in humans is present in mice, but appears to have been damaged at some point in the past by a retrovirus (9). On the other hand, mice express TLRs 11, 12, and 13, none of which are represented in humans. Other mammals may express TLRs that are not found in humans.

### Structure of the human toll-like receptor

Human TLRs respond to various PAMPs shared by numerous microorganisms. TLRs are transmembrane glycoproteins possessing varying numbers of extracellular N-terminal leucine-rich repeat (LRR) domain, followed by a cysteine-rich region, a transmembrane domain and a C-terminal cytoplasmic Toll/IL-1R (TIR) domain (10) (Fig. 1). The LRR domain is important for ligand binding and associated signaling, and is a common feature of PRRs. The TIR domain is important in protein-protein interaction and is typically associated with innate immunity.

### Toll-like receptors and their ligands

Toll-like receptors are primarily expressed by first-line professional phagocytes, i.e. neutrophils, macrophages and dendritic cells. These receptors sense and respond to distinct microbial structures. Toll-like receptors (TLRs 1, 2, 4, 5 and 6) that recognize extracellular microbial structures are expressed on the host cell surface; for example, TLR 5 responds to bacterial flagellin. TLR 2 is unique in that it heterodimerizes with the signaling partner TLR1 or TLR6 for detecting and responding to microbial cell wall components. Toll-like receptors (TLRs 3, 7, 8 and 9) specifically detecting viral or bacterial nucleic acids are expressed intracellularly on endocytic vesicles; for example, TLR 3 recognizes double-stranded viral DNA (11). The ligands for human TLRs are enumerated in Table 1.

### Expression of toll-like receptors in immune cells

Toll-like receptors are predominantly expressed on cells of the innate immune system, including neutrophils, monocytes/macrophages, and dendritic cells. These cells express different TLRs, allowing them to induce a wide variety of immune responses to specific pathogens. Neutrophils are the first innate immune cells to migrate to sites of infection and utilize TLRs to recognize and

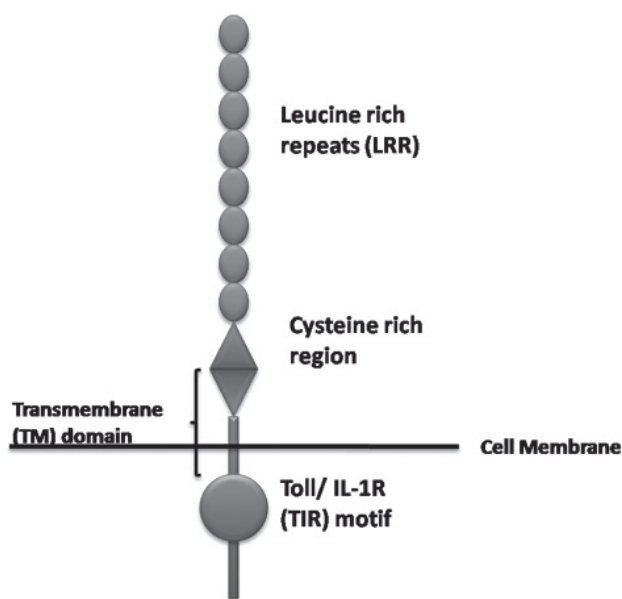


Fig. 1 Structure of a toll-like receptor

Table 1 Ligands for toll-like receptors

Toll-like receptor	Ligands
TLR 1	Triacyl Lipopeptides
TLR 2	Lipoproteins, Peptidoglycan, Lipoteichoic acid, Zymosan, <i>Porphyromonas gingivalis</i> lipopolysaccharide & fimbriae, <i>Capnocytophaga ochracea</i> lipopolysaccharide
TLR 3	Double stranded RNA, Polyinosine-polycytidylic acid
TLR 4	<i>Escherichia coli</i> lipopolysaccharide, <i>Porphyromonas gingivalis</i> lipopolysaccharide, <i>Actinobacillus actinomycetemcomitans</i> lipopolysaccharide, <i>Fusobacterium nucleatum</i> lipopolysaccharide
TLR 5	Flagellin
TLR 6	Peptidoglycan, Lipoteichoic acid, Diacyl lipopeptides, Zymosan
TLR 7	Imidazquinoline
TLR 8	Single stranded RNA, Imidazquinoline
TLR 9	Bacterial DNA, CpG oligodeoxynucleotide
TLR 10	Not determined

Table 2 Toll-like receptors expressed by immune cells

Cells	Toll like receptors
Neutrophils	TLR 1, 2, 4, 5, 6, 7, 8, 9, 10
Monocytes/macrophages	TLR 1, 2, 4, 5, 6, 7, 8
Myeloid dendritic cells	TLR 1, 2, 3, 4, 5, 6, 8, 10
Plasmacytoid dendritic cells	TLR 1, 6, 7, 9
B lymphocytes	TLR 1, 3, 6, 7, 9, 10
T lymphocytes (Th1/Th2)	TLR 2, 3, 5, 9
T lymphocytes (regulatory)	TLR 2, 5, 8

respond to microbial challenge. Neutrophils express TLR 1, TLR 2, and TLRs 4 to 10, but not TLR 3 (12). Macrophages/monocytes, also the first line of defense, bind to microbial pathogens through TLRs. They play a key role in host defense by recognizing, engulfing and killing microorganisms. The binding of PAMPs to monocyte TLRs can influence the type of adaptive immune response (13).

Recent research has clarified that adaptive immunity did not evolve to replace innate immunity, but rather evolved around it. Development of adaptive immunity is controlled through activation of innate immune cells, especially antigen-presenting dendritic cells. TLRs of resident immature dendritic cells detect the PAMPs and transmit the information through signaling pathways, resulting in activation of dendritic cells. This activation results in production of cytokines and chemokines critical for T-cell priming and differentiation (14). Two subpopulations of dendritic cell precursors have been

identified in human blood: myeloid dendritic cells and plasmacytoid dendritic cells expressing different TLRs.

It is evident that both B and T lymphocytes are present in diseased periodontal tissue. It has been hypothesized that T cells predominate in stable periodontal lesions, while the proportion of B cells and plasma cells is increased in progressive lesions. More recently, it has been shown that TLRs are also expressed on the cells responsible for adaptive immunity, i.e. B and T lymphocytes. The TLRs expressed on T lymphocytes and their respective ligands can directly modulate T cell function. These TLRs act as co-stimulatory receptors to enhance proliferation and/or cytokine production of T-cell receptor-stimulated T lymphocytes. Also, TLRs on CD25+CD4+ regulatory T cells are thought to modulate their suppressive activity (15). TLRs expressed by all of these immune cells are listed in Table 2.

### Toll-like receptors in periodontal tissues

Research has established that along with immune cells, TLRs are also expressed in periodontal tissues (16-21). Since the gingiva is constantly exposed to microbes present in plaque biofilm, TLR signaling plays an important role in the innate immune response and maintenance of periodontal health. However, over-production of pro-inflammatory cytokines due to chronic stimulation of TLRs may lead to tissue destruction. The TLRs present on different periodontal cells are listed in Table 3.

### Toll-like receptor signaling

Toll-like receptor signaling takes place by two separate pathways: the myeloid differentiation primary

Table 3 Toll-like receptors in periodontal tissues

Periodontal Cells	Toll-like receptors	Function
Gingival Epithelial cells	TLR 2, 3, 4, 5, 6, 9	Increased attachment and migration of leucocytes towards antigen on lumen of the pocket, also induces production of Interleukin-8 (IL-8) as well as Matrix metalloproteinases.
Gingival fibroblasts	TLR 2, 4, 9	Increased production of Interleukin-8 as well as other pro-inflammatory cytokines.
Endothelium	TLR 1, 3, 4, 5	Production of pro-inflammatory cytokines and chemokines, migration of immune cells towards gingival sulcus.
Osteoblasts	TLR 1, 4, 5, 6, 9	Upregulation of pro-inflammatory cytokines like IL-8, Tumour necrosis factor- $\alpha$ (TNF- $\alpha$ ) and biologic mediators responsible for bone resorption. Increased expression of receptor activator of nuclear factor $\kappa$ B ligand (RANKL).
Osteoclasts	TLR 2, 4	Enhanced survival of osteoblasts and increased osteoclastic activities.
Cementoblasts	TLR 2, 4	Down regulation of RANKL
Periodontal ligament fibroblasts	TLR 2, 4	Enhanced production of pro-inflammatory cytokines, release of proteases causing direct destruction of surrounding tissues.

response protein 88 (MyD88)-dependent pathway and the MyD88-independent pathway (22). The MyD88-dependent pathway is essential for most TLR-mediated cell activation, whereas stimulation of TLR 3 and 4 takes place through the MyD88-independent pathway (Fig. 2).

In the MyD88-dependent pathway, TLR signaling is mediated by adaptor molecule MyD88, which either alone or in combination with another adaptor protein, Toll-IL-1 receptor domain-containing adaptor protein (TIRAP), activates interleukin-1 receptor-associated kinase-4 (IRAK-4). This IRAK-4 then unites with tumor necrosis factor receptor-associated factor 6 (TRAF6), leading to activation of two distinct signaling pathways. One pathway leads to activation of activator protein 1 (AP-1) through activation of mitogen-activated protein kinase (MAK). The other pathway activates transforming growth factor- $\beta$ -activated kinase 1 (TAK1), which enhances the activity of inhibitor of the nuclear factor- $\kappa$ B kinase complex. This leads to degradation of the inhibitor of nuclear factor- $\kappa$ B and release of nuclear factor- $\kappa$ B (NF- $\kappa$ B), which translocates to the nucleus. Both the end products of these signaling pathways, AP-1 and NF- $\kappa$ B, induce the expression of pro-inflammatory cytokines and chemokines.

TLR 7, 8 and 9 recognize viral nucleic acid through another pathway, which is also MyD88-dependent. MyD88 associates with interleukin-1 receptor associated kinase-1 (IRAK1), which in turn phosphorylates interferon-regulatory factor-7 (IRF7). The phosphorylated IRF7 translocates to the nucleus and regulates the expression of type I interferon. TLR signaling through the MyD88-independent pathway results in induction of type 1 interferon through interferon-regulatory factor-3

(IRF-3). The adaptor molecules for this pathway are Toll-IL-1 receptor domain-containing adaptor inducing interferon- $\beta$  (TRIF) and TRIF-related adaptor molecules (TRAM).

### Specificity of innate immunity

Until recently, the innate immune response was viewed as non-specific and a temporary expedient to 'buy time' until the activation of adaptive immunity, which comprises the B and T lymphocyte systems, each of which expresses antigen receptors of strict specificity (23). Although innate immunity is unable to make fine structural distinctions, it is endowed with considerable specificity. This specificity occurs due to a collection of receptors and their specific ligands that have evolved to allow rapid identification and response to non-self. These receptors, collectively known as PRRs, can detect and respond to conserved and generally distinct microbial structures that are shared by related groups of microorganisms (24). Toll-like pattern recognition receptors recognize different molecular components, thereby activating different innate pathways. This activation involves expression of co-stimulatory molecules and production of cytokines and chemokines which are critical for T-cell priming and differentiation. It is now recognized that immune cells release quantitatively and qualitatively different cytokines on stimulation with gram-positive and gram-negative bacteria, indicating the specificity of the response (25).

Innate immunity also faces the challenge of discriminating the host from among a large number of periodontal pathogens using a limited number of cell surface receptors. One complicating factor is that microorganisms have

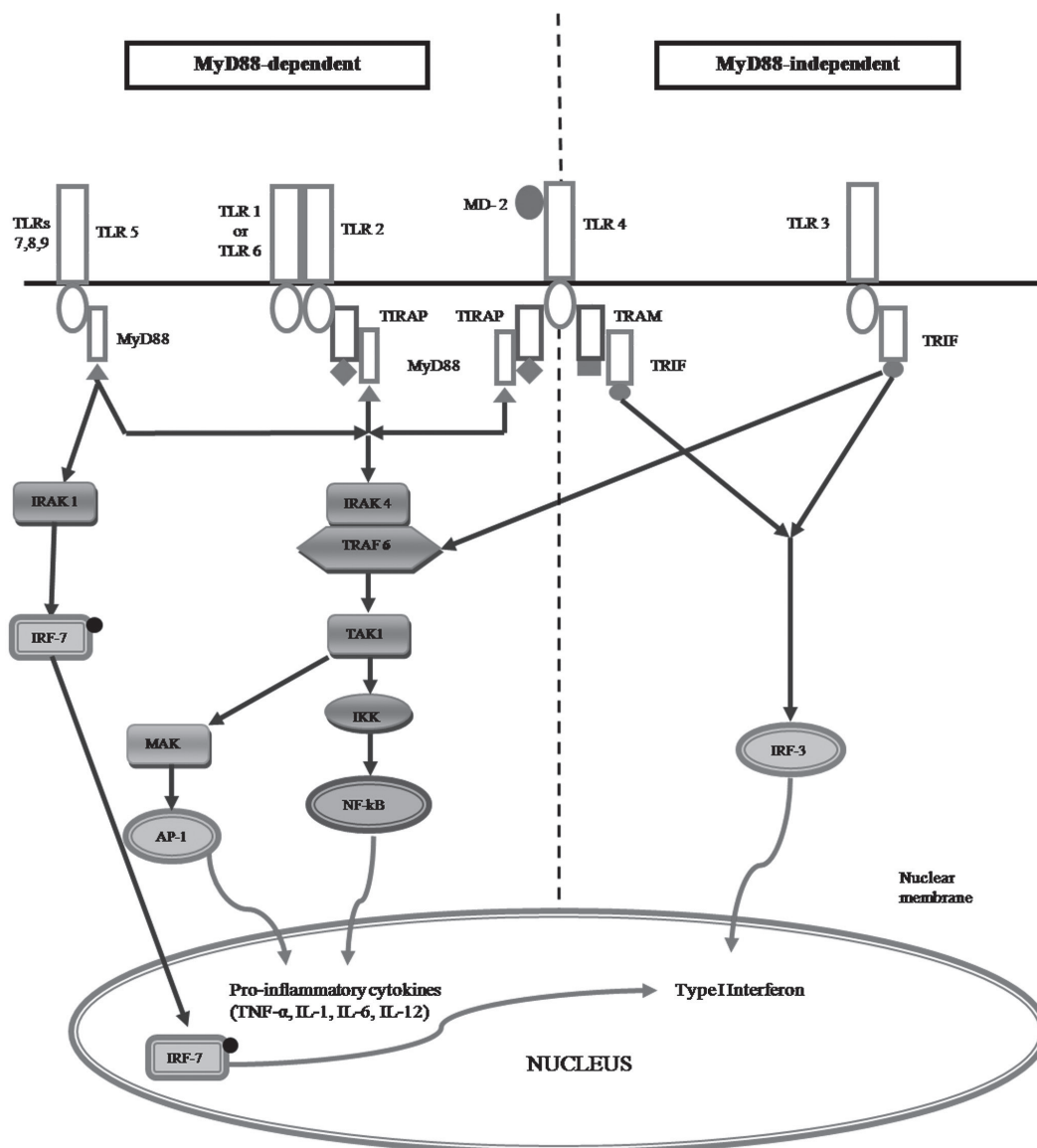


Fig. 2 Toll-like receptor signaling. TLR: toll-like receptor; MyD88: myeloid differentiation primary response protein 88; TIRAP: toll-IL-1 receptor domain-containing adaptor protein; IRAK-1: interleukin 1 receptor-associated kinase-1; IRAK-4: interleukin 1 receptor-associated kinase-4; TRAF6: tumor necrosis factor receptor-associated factor 6; MAK: mitogen-activated protein kinase; AP-1: activator protein 1; TAK1: transforming growth factor- $\beta$ -activated kinase 1; NF- $\kappa$ B: nuclear factor- $\kappa$ B; IRF-3: interferon-regulatory factor-3; IRF-7: interferon-regulatory factor-7; TRIF: Toll-IL-1 receptor domain-containing adaptor inducing interferon- $\beta$ ; TRAM: TRIF-related adaptor molecules, ● phosphorylation

the ability to mutate in order to escape host recognition. Innate immunity meets this challenge through recognition of evolutionarily conserved structures – known as PAMPs – on pathogens that are not present in higher eukaryotes. These play a role in the ability of pathogens to evade host defense, and are therefore not subject to high mutation rates. These PAMPs are shared among pathogens but are not expressed by the host (26). Thus, a limited number of receptors are able to recognize a large

number of pathogens.

Also, recent information suggests that a specific TLR-mediated signaling pathway differentially select adaptors to initiate the MyD88-dependent or independent pathway. As discussed above, only four adaptor molecules have been identified for both of these pathways: Myeloid differentiation primary response protein 88 (MyD88), Toll-IL-1 receptor domain-containing adaptor protein (TIRAP), Toll-IL-1 receptor domain-containing adaptor

inducing interferon- $\beta$  (TRIF), and TRIF-related adaptor molecules (TRAM). The response of a limited number of TLRs to an extensive number of PAMPs through only four adaptor molecules indicates differential signaling to different PAMPs. Thus, clusters of many genes must be selectively regulated to control needed immune processes (27).

### Role of toll-like receptors in periodontal health and disease

The periodontium is continually exposed to dental plaque, which harbors many commensal and pathogenic oral microorganisms. Periodontal tissues express different types of TLRs, allowing them to actively participate in the innate immune response against these oral microorganisms. Thus, these TLRs provide first line of defense in maintaining periodontal health.

It has been suggested recently that the oral mucosa develops tolerance after repeated exposure to bacterial products (28). Down-regulation of TLR expression and inhibition of intracellular signaling may be the underlying mechanisms of tolerance. However, recent

research has indicated that under steady-state conditions, activation of TLRs by commensal bacteria is critical for the maintenance of oral health. Gingival epithelial cells express TLR 2, 3, 4, 5, 6 and 9 and recognize various microorganisms with the help of these receptors (16). These TLRs expressed on gingival epithelium continually interact with oral microorganisms that form biofilms on tooth surfaces. This TLR signaling results in innate immune responses involving the release of the antibacterial  $\beta$ -defensins cathelicidin and calprotectin, as well as neutrophil chemoattractant (interleukin-8) (29). Therefore, TLR signaling limits microbial invasion and prevents commensal organisms from breaching the epithelial barrier, thereby maintaining gingival health. Further investigations are required to better understand the interaction between oral microorganisms and gingival epithelial cells, and how the periodontium prevents disease initiation and progression through TLR signaling.

Periodontal health represents a dynamic state in which pro-inflammatory and anti-microbial activities for control of infection are optimally balanced by anti-inflammatory mechanisms to prevent unwarranted inflammation. This

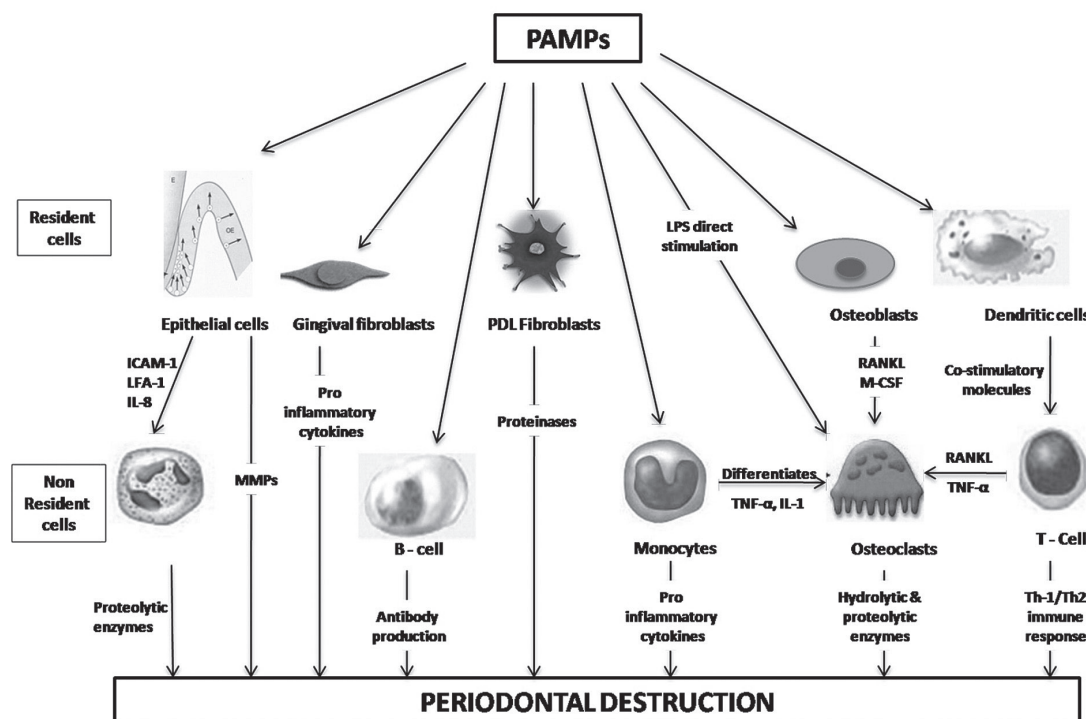


Fig. 3 TLR-mediated effects of PAMPs on cells of the periodontium and their interactions. ICAM-1: intercellular adhesion molecule-1; LFA-1: ligand for lymphocyte function-associated antigen-1; IL-8: interleukin-8; MMPs: matrix metalloproteinases; PDL: periodontal ligament; LPS: bacterial lipopolysaccharides; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; IL-1: interleukin-1; RANKL: receptor activator of nuclear factor kappa B ligand; M-CSF: macrophage colony-stimulating factor

homeostasis is disrupted when pathogens present in dental plaque undermine the host defense mechanism. Chronic stimulation of TLRs in periodontal tissues by bacterial PAMPs can lead to excessive production of pro-inflammatory mediators, resulting in tissue destruction. Also, periodontitis induced by bacterial plaque may start with disruption and penetration of the gingival epithelial barrier by invasive bacteria or their cytotoxic products. Through this invasion into deeper tissues, TLRs in other cells such as macrophages, fibroblasts, osteoblasts, osteoclasts and antigen-presenting cells become activated. These cells, when stimulated, produce various pro-inflammatory cytokines that lead to inflammation and immune cell infiltration. The infiltrated cells, such as memory T-cells, further produce cytokines and amplify the inflammatory reaction, leading to destruction of connective tissue and bone (30). Thus, TLRs act as a double-edged sword, not only maintaining periodontal health but also contributing to periodontal tissue destruction.

### **Toll-like receptor-mediated progression of periodontal disease**

The first cells to respond to PAMPs are the epithelial cells lining the sulcus. These cells express intercellular adhesion molecule-1 (ICAM-1) and the ligand for lymphocyte function-associated antigen-1 (LFA-1), which interact with and direct the attachment and migration of leucocytes towards the gingival sulcus. Interleukin-8 (IL-8), a known neutrophil chemoattractant, is also released by epithelial cells, further enhancing the migration of neutrophils (31,32). Epithelial cells are also known to produce matrix metalloproteinases (MMPs) in response to PAMPs, causing direct damage to periodontal tissues (33). MMPs are a structurally related group of endopeptidases collectively capable of degrading practically all extracellular matrix and basement membrane components. Once the epithelial cells are activated, they mediate the activation of other resident as well as non-resident cells. When stimulated via TLRs, neutrophils exhibit increased chemotaxis as well as production of pro-inflammatory cytokines [interleukin-1 (IL-1); interleukin-6 (IL-6); tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )] (34). These cytokines play a central role in periodontal tissue destruction. The properties of these cytokines that relate to tissue destruction involve stimulation of bone resorption and induction of tissue-degrading proteinases. The IL-8 secreted by epithelial cells stimulates the endothelial cells lining the blood vessels through TLR-4, leading to increased adhesion of monocytes by increased expression of the adhesion

molecules E-selectin, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) (35). When exposed to PAMPs, monocytes produce pro-inflammatory cytokines and may differentiate into osteoclasts upon direct stimulation with bacterial lipopolysaccharides with the help of receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) (36,37). Dendritic cells are resident immune cells present in both epithelium and connective tissue. TLRs present on these cells induce their maturation when stimulated with PAMPs. When activated, these cells not only act as antigen-presenting cells but also produce cytokines and co-stimulatory molecules that activate T-lymphocytes to produce a Th1 or Th2 immune response (38) (Fig. 3).

As the epithelial barrier is breached, microorganisms and their products access gain to the underlying connective tissue and directly activate the cells present there. Once stimulated by PAMPs, gingival fibroblasts produce pro-inflammatory cytokines leading to tissue destruction and bone resorption. Periodontal ligament fibroblasts, on the other hand, produce proteinases on TLR stimulation, resulting in direct degradation of periodontal tissues (39). With the PAMPs entering the circulation via blood vessels in connective tissue, lymphocytes move toward the site of infection. In the presence of biological mediators, naïve T-cells differentiate and initiate a Th1 or Th2 immune response, TLRs on T cells acting as type of co-stimulatory molecule (15). B lymphocytes are transformed into plasma cells, which produce antibodies against bacterial antigens. Osteoblasts also react to PAMPs through TLRs and produce biological mediators (MMPs, prostaglandin E2) responsible for bone resorption (40). Osteoblasts, marrow stromal cells, and T and B cells express RANKL, which is essential for activation of osteoclasts. RANKL in the presence of macrophage colony-stimulating factor (M-CSF) attaches to receptor activator of nuclear factor  $\kappa$ B (RANK) present on osteoclasts and osteoclast precursors, and activates them (41). A bone-protecting factor, osteoprotegerin (OPG), produced by osteoblasts and bone marrow stromal cells, inhibits the RANK/RANKL interaction and prevents bone resorption (42). If the destructive process continues, more subgingival plaque tends to accumulate, connective tissue attachment to the tooth is destroyed, epithelial cells proliferate apically along the root surface, and the periodontal pocket deepens. If not controlled, the bone and attachment loss extends to the apex, and the tooth is ultimately lost.

Toll-like receptor signaling at the dento-epithelial junction is critical in maintaining periodontal health as well as in the progression of periodontitis. However, there are still gaps in our knowledge of the mechanisms

by which TLRs maintain periodontal health and what leads to bacterial immune evasion and disease progression. At present it is uncertain which specific signaling pathways need to be blocked to attenuate the pathology or enhanced to promote host defense. Therefore, further investigations are required in this field to understand the initiation and progression of periodontal disease and develop therapeutic interventions to control it.

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