REVIEW ARTICLE

Toll-like Receptors: Molecular Microbe Sensors in Periodontium

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Abstract

Aim: This review aims to highlight the emerging role of toll-like receptors (TLRs) in pathogenesis of periodontitis and negative regulation of TLR signaling.

Background: Periodontal disease is the common chronic bacterial infection of the supporting structures of the teeth characterized by the tissue destruction. Bacterial plaque stimulates the host inflammatory response. It is now known that the immune response utilizes a family of pattern-recognition receptors (PRRs) called TLR as a tool to trigger an inflammatory response to microbial invasion. The TLRs expressed by epithelial cells of gingiva are constantly stimulated which release cytokines and defensins required for maintenance of oral health. The chronic stimulation of TLRs may leas to the disruption of epithelial barrier and allows microorganisms to enter the underlying connective tissue. This further activates TLRs present on additional cells of the periodontium, i.e., resident and non-resident cells. These TLRs activation may cause host tissue destruction due to an overproduction of proinflammatory cytokines as well other biological mediators.

Review results: The electronic databases PubMed, MEDLINE, Cochrane, Scopus and Google Scholar were searched for available data in the present review. A database search yielded a total of 94 articles out of 56 included based on the core data. The results and subsequent conclusions were extracted and reviewed.

Conclusion: It may be concluded that TLR signaling is crucial for maintenance of periodontal health as well as initiation and progression of periodontal disease. In spite of this, there are still lacks of information regarding the functional polymorphisms of genes that are involved in the stimulation and regulation of lipopolysaccharide mediated inflammatory processes.

Clinical significance: Overactive TLRs might pivot into chronic inflammation, and so targeting TLRs might therefore lead to remission from this chronic inflammation. Therefore, further investigations are necessary to expand our knowledge to understand and develop therapies for major pathologic conditions.

Keywords: Cytokines, Inflammatory response, Innate immunity, Pattern-recognition receptors.

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Introduction

The interplay between the oral microflora and the host can result in innate and adaptive immune responses that either contribute to development of disease or host immunity. Innate immunity serves as a primary host defense and denotes inherited resistance against infection. Hallmarks of innate immune responses include the ability to (i) discriminate between pathogens and self (ii) activate effector mechanisms that will destroy pathogens within hours, (iii) activate and orient an adaptive immune response.[1–3]

Toll-like receptors (TLRs) have essential role in the recognition of microbial components and in the initiation of cellular innate immune responses.[4] Inflammation is the central hallmark of this TLR induced response to microbial invasion. Toll-like receptors are able to recognize molecules unique to microbes, which help in self vs nonself discrimination. The family of TLRs is now known to represent the major microbe-sensing system in mammals, detecting molecules derived from viruses, fungi, bacteria, and protozoa.

Periodontitis is a chronic inflammatory disease caused by microorganisms and affects the supporting structure of the teeth.[5] Mainly gram-negative bacteria associated with periodontitis interact with the host to initiate a cascade of immune responses. The gingival epithelial cells (GEC) are the first cells to encounter these bacteria. The host-bacteria interplay is facilitated by TLR expressed by GEC that interact with molecules unique to these bacteria.[6–8]

Toll-like receptors trigger the inflammatory response through intracellular signaling pathways.[9] However, chronic activation of TLRs is responsible for an increased production of proinflammatory cytokines which may lead to tissue destruction. Further, this TLR mediated innate immune response has prime role of activating the adaptive immune response. Like other chronic inflammatory diseases, TLRs have an imperative role in pathogenesis of periodontitis. This review highlights a vital role of TLRs in preserving the periodontal health, initiation of periodontitis and negative regulation of TLR signaling.

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History

Toll gene products were first discovered by Anderson et al. in 1985, in the fruit fly, *Drosophila* melanogaster. As there are the

human homologs, TLRs are entitled due to their resemblance to the protein moiety encoded by the toll gene. Lemaitre et al. in 1996 verified that toll gene has an essential role in regulating the antifungal defense in *Drosophila*, which is attained by initiating the production of antimicrobial peptides.[10] Charles Janeway and Ruslov Medzhitov identified the first human homolog of *Drosophila* toll in 1997, initially termed as human toll and subsequently as TLR 4.[11] The link between innate immunity and TLRs was cleared with the mouse TLR 4 discovery. This mouse TLR 4 act as a bacterial lipopolysaccharide receptor.[12] Till now at least 11 different human TLRs and 13 TLRs in mice have been discovered.[4]

TLRs and their Family

Pattern-recognition receptors (PRRs) are germline-encoded receptors which can detect and respond to pathogen-associated molecular patterns (PAMPs).[13] Pathogen-associated molecular patterns are conserved and large diverse microbial structures which are typically shared by enormous group of microorganisms, e.g., bacterial lipopolysaccharide.[3] The two principal classes of PRR which interact with PAMPs include (1) those that mediate phagocytosis and (2) those that lead to the activation of proinflammatory pathways.[14] The PRRs are expressed on the cell surface, in intracellular compartments, or secreted into the blood stream and tissue fluids.[15] Toll-like receptors are important PRRs which upon interaction with PAMPs, activate the transcription factor NF-κB and induce the synthesis of antimicrobial and antiviral cytokines, chemokines and peptides.[16]

Mammalian TLRs comprise a large family consisting of at least 11 members. Toll receptors are type I transmembrane glycoproteins present on host cell. Toll-like receptors structure comprises two different domains, extracellular domain and intracellular domain (Fig. 1).[17] Extracellular domain comprises multiple leucine-rich repeats (LRRs) and one or two cysteine-rich regions which represent pathogen binding domain. Intracellular domain known as toll/IL-1 receptor (TIR) domain shows high similarity to that of the IL-1 receptor family.[18] Both TLR and interleukin-1 receptor together, constitute a receptor superfamily that is recognized as the "Interleukin-1 Receptor/Toll-Like Receptor Superfamily." The TIR domain is responsible for protein–protein interaction and has three different subgroups. Proteins produced by monocytes, macrophages, and dendritic cells that contain subgroup I TIR domains are receptors for interleukins. These proteins also have extracellular immunoglobulin (Ig) domains. Proteins containing subgroup II TIR domains are typical TLRs which directly or indirectly binds to microbial origin molecules. A subgroup III TIR domain represents the adaptor proteins which are entirely cytosolic and facilitate subgroups I and II proteins signaling.[19]

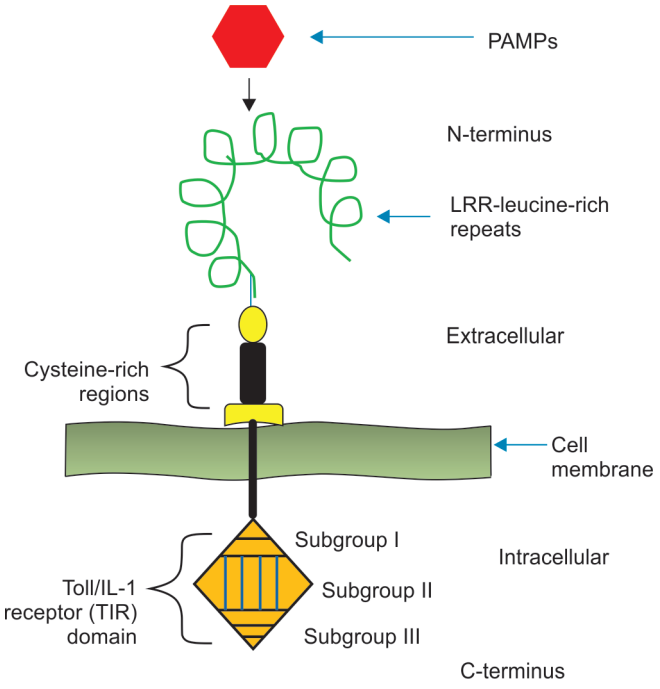

Fig. 1: Schematic structure of human toll-like receptor

TLRs and their Ligands

Toll-like receptor family receptors interact with variety of ligands, ranging from hydrophilic nucleic acids to hydrophobic LPS or lipoproteins. Toll-like receptors form homodimers or heterodimers induced by the simultaneous binding of ligands.[20] Toll-like receptors are primarily expressed by immune cells, i.e., neutrophils, macrophages, B cells, T cells, mast cells, NK cells and dendritic cells (Fig. 2) and even by nonimmune cells such as fibroblasts, parenchymal cells and epithelial cells.[21] The expression of the various TLRs is also modulated in response to a distinct stimuli. The Table 1 depicts the distinct ligands and their sources for human TLRs.[22]

TLRs in Periodontal Tissues

In addition to immune cells, TLRs are also expressed in cells of the periodontium viz. GECs, gingival fibroblast, endothelium, cementoblast, periodontal ligament fibroblast, osteoblast, osteoclast, etc., (Fig. 3). The oral cavity is home to a several bacterial species. More than 500 different bacterial species have been found in a cultivated human subgingival plaque samples.[23] As the gingiva is constantly exposed to plaque bacteria, GECs and fibroblasts may directly interact with bacteria and bacterial products. It is suggested that TLR signaling may activate the innate immune response to maintain the periodontium in a healthy state. Consistently, the expressions of the TLR in gingival and periodontal tissues have been reported both in healthy and diseased tissues.[24]

The expression of TLRs by human GECs was considered in gingival biopsies obtained during periodontal surgery. Toll-like receptor 2, 3, 4, 5, 6, 9 are expressed by the GECs. The expression of TLR 2 is denser in the spinous epithelial layer compared to the basal epithelial layer while expression of TLR 4 is low in epithelial layers.[25]

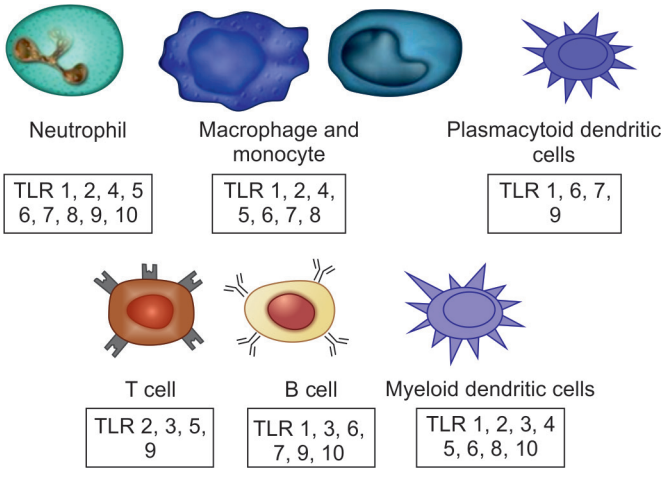

Fig. 2: Toll-like receptors expressed by immune cells

Table 1: Human toll-like receptor ligands and their source

Toll-like receptor	*Ligands*	*Source*
TLR 1	Triacyl lipopeptides	Gram-positive bacteria, fungi
TLR 2	Lipoproteins/lipopeptides, peptidoglycan/lipoteichoic acid, fimbriae, lipopolysaccharide and zymosan	Gram-positive bacteria, fungi
TLR 3	Double stranded RNA, polyinosine–polycytidylic acid	Viruses
TLR 4	Lipopolysaccharide	Gram-negative bacteria
TLR 5	Flagellin	Bacteria
TLR 6	Peptidoglycan, lipoteichoic acid, diacyl lipopeptides, zymosan	Gram-positive bacteria, fungi
TLR 7	Imidazquinoline	Viruses
TLR 8	Single stranded RNA, imidazquinoline	Viruses
TLR 9	Bacterial DNA, CpG oligodeoxynucleotide	Bacteria, viruses, fungi
TLR 10	Lipopeptides (prediction)	–
TLR 11	Flagellin	Bacteria

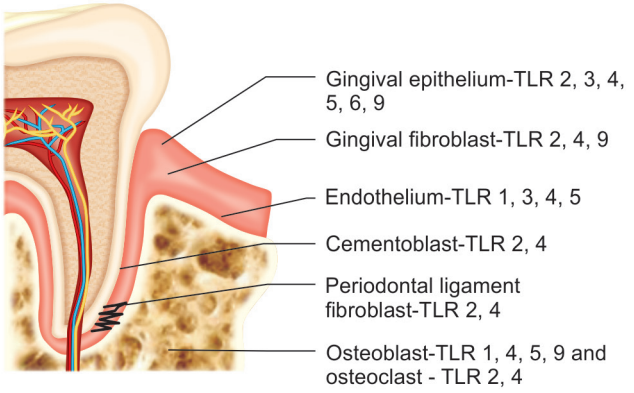

Fig. 3: Toll-like receptors expressed by periodontal tissues

These TLR's increases movement of leucocytes towards antigen and attachment on the pocket lumen.[26] Asai et al.[27] demonstrated that TLR 2 signaling appears to produce matrix metalloproteinases (MMPs) and interleukin-8 (IL-8) by GECs in response to *P. gingivalis* fimbriae. Gingival epithelial cells also express TLR 3 and TLR 9 which provide the ability for epithelial cells to respond to both viral and bacterial nucleic acids.[27]

Gingival fibroblasts constitutively express TLR 2, 4, 9 which increase production of interleukin-1, interleukin-6, and interleukin-8 upon stimulation by bacteria and their components.[28–30] Human microvascular endothelial cells play an important active role in regulation of immune and inflammatory responses. Toll-like receptor 1, 3, 4, 5 are expressed by endothelium which encourages synthesis of proinflammatory cytokines, chemokines as well as immune cell movement to gingival sulcus.[26]

Osteoblasts, the bone-forming cells, regulate directly the bone matrix synthesis and mineralization by their synthetic activity, and indirectly regulate the bone resorption by osteoclasts, the bone-resorbing cells. Human osteoblastic cells, constitutively express TLR 1, 4, 5, 6, 9. Toll like receptor signaling, stimulate tumor necrosis factor-α (TNF-α), IL-8 upregulation and increase receptor activator of nuclear factor κB ligand (RANKL) expression. The increased expression TNF-α and RANKL produced by osteoblasts are involved in osteoclast formation. Toll-like receptor 2 and 4 expressed by osteoclasts, boosts survival of osteoblasts and osteoclastic activities.

Cementoblasts present in cementum on root surface, plays important roles in mineralization. These cells express TLR 2 and 4 responsible for down regulation of RANKL.[26] TLR 2 and 4 are also expressed by periodontal ligament fibroblasts which enhance formation of proinflammatory cytokines as well as protease release resulting in surrounding tissues destruction.[26]

Activation of TLRs

The expression of the various TLRs is modulated in response to distinct stimuli. The extracellular microbial structures that are expressed by host cell surface are distinguished by TLR 1, 2, 4, 5 and 6. Toll-like receptors 3, 7, 8 and 9 are expressed intracellularly on endocytic vesicles for detecting and responding to viral or bacterial nucleic acids. Identification of such stimuli by TLRs induces diverse of immune responses by cells to specific pathogens. The first immune cells to migrate to sites of infection are neutrophils which utilizes TLRs to identify and respond to microbial challenge.[31]

Microbial recognition of TLRs facilitates dimerization of TLR ectodomains and undergo the conformational changes that are propagated to the intracellular TIR domain. Dimerization of TLRs triggers activation of signaling pathways, which originate from an intracellular TIR domain. Toll-like receptor signaling depends critically on a total of four adaptor proteins—(1) myeloid differentiation primary-response protein 88 (MyD88), (2) TIR domain-containing adaptor protein (TIRAP) also called MyD88-adaptor-like (Mal), (3) TIR domain-containing adaptor molecule 1 (TICAM-1) also called toll-IL-1 receptor domain comprising adaptor inducing interferon-β (TRIF) and (4) TIR domain-containing adaptor molecule 2 (TICAM-2) also called TRIF-related adaptor molecules (TRAM).[13,20]

Toll-like receptor signaling occurs through the two pathways first one, the myeloid differentiation primary response protein 88-dependent pathway (MyD88-dependent) and second, the myeloid differentiation primary-response protein 88-independent pathway (MyD88-independent) (Fig. 4).[32] The myeloid differentiation primary-response protein 88 is a key adaptor molecule essential for most TLR mediated cell stimulation. This stimulation results in activation of transcription factors, such as nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) and modulation of cytokine production.[33] The induction of type I interferon is mediated by MyD88-independent pathway through interferon-regulatory factor-3 and also leads late phase of NF-κB activation.[34]

Toll-like Receptors in the Periodontal Health

In numerous body organs, inflammation is a protective mechanism necessary for survival of the individual. The exacerbations of the inflammatory response in the gingiva, due to pathogenic biofilms, may represent protective responses of an individual to both local

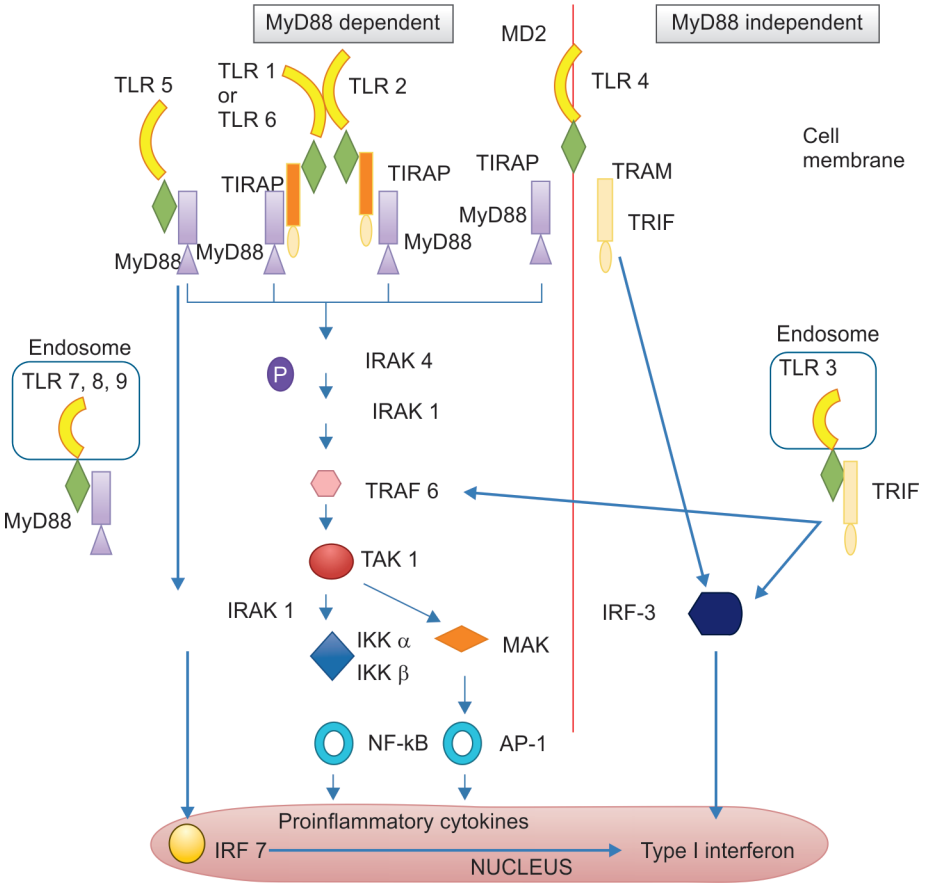

Fig. 4: Pathways of toll-like receptor signaling

and systemic environments by destroying and "walling off" the invading organisms.[35] Healthy periodontium signifies a dynamic state where anti-inflammatory and proinflammatory responses are optimally balanced to prevent unwarranted inflammation. Periodontium provides a habitat for many commensal and pathogenic oral microorganisms as it is persistently in-contact with dental plaque. Microorganisms present in this dental plaque damages the host defense mechanism that leads to the disruption of homeostasis. Therefore, to maintain periodontal health TLRs signaling actively contribute in providing primary defense through an innate immune response against these oral microorganisms.

Gingival epithelial cells are the first cells to express TLR 2, 3, 4, 5, 6 and 9 in response to PAMPs.[25] Toll-like receptors bind to their respective PAMPs, leading to the signaling of these receptors. These signaling results in the release of neutrophil chemoattractant (IL-8), antibacterial β-defensins, calprotectin and cathelicidin through induction of innate immune responses.[36] The ligand for lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) are expressed by GECs. This LFA-1 and ICAM-1 interact with leucocytes and guide their attachment and migration towards the gingival sulcus. Consequently, break in the epithelial barrier by commensal organisms is prevented with the help of TLR signaling. This signaling limits the microbial invasion and maintains the gingiva in a healthy state.

Toll-like Receptors in the Pathogenesis of Periodontal Disease

In the periodontal tissues, chronic stimulation of TLRs by bacterial PAMPs can result in unwarranted production of proinflammatory cytokines, leading to tissue destruction. Periodontal inflammation may arise with dissemination by invasive microorganisms or their cytotoxic product and disruption of the gingival epithelial barrier. With the epithelial invasion by PAMPs, epithelial cells have a role in production of MMPs causing direct damage to periodontal tissues.[37,38]

Once the epithelial cells are activated, TLRs present on the non-immune cells such as the fibroblasts, osteoblasts and osteoclasts also get activated. The epithelial cells secrete IL-8 which stimulates the blood vessel lining cells, i.e., endothelial cells through TLR 4. There is an increased production of adhesion molecules such as ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), E-selectin result in increased adhesion of monocytes.[39] These monocytes when stimulated via TLRs, produce proinflammatory cytokines and with the help of RANKL monocytes may differentiate into osteoclasts.[40] When neutrophils get exposed to PAMPs, the signaling results in improved chemotaxis and increased production of proinflammatory cytokines like IL-1, IL-6 and TNF-α.[41] The increased cytokine production can relate to tissue destruction, induction of tissue degrading proteinases and stimulation of bone resorption.

The most widely studied TLRs in periodontal destruction include TLR 2, 4, 7 and 9.[42] A study by El-Dessouky and Ahmed[43] showed the key role of TLR 2 and 4 in the pathogenesis of periodontal diseases. *A. actinomycetemcomitans* induced signaling pathways are mediated through both TLR 2 and TLR 4. This is in contrast to *P. gingivalis* and most other periodontal pathogens that appear to signal preferentially via TLR 2.[44] TLR 2 and 4 induce receptor

activator of RANKL expression and osteoclast differentiation.[44] A finding by Kajita et al.[45] suggest that TLR 9 inhibits RANKL-induced osteoclast differentiation. However, Kim et al.[46] demonstrated that TLR 9 signaling can mediate the induction of inflammatory and osteoclastogenic cytokines (IL-6, TNF, and RANKL), as well as bone loss.

Thus, chronic stimulation and upregulation of TLRs by periodontal pathogens plays a key role in pathogenesis of periodontitis.

Negative Regulation of TLR Signaling

Even though the TLR pathway is integral to inflammatory defense mechanisms, it's aberrant activation by microbial components triggers the generation of inflammatory cytokines such as TNF-α, IL-6 and IL-12. The excessive production of these cytokines, induce serious systemic inflammatory diseases. The cells have evolved several mechanisms to maintain a balance between activation and inhibition of TLR signaling at almost each step of the pathway. Although several negative regulators have been identified which include; (1) soluble decoy receptors which blocks the signaling pathways of TLR, (2) dissociation of adaptor complexes, which act as antagonists and downregulate signaling pathways, (3) degradation of signal proteins like TIRAP/MAL or TRAF protein, (4) transcriptional regulation which controls a subset of TLR target genes without termination of signaling, (5) evasion by pathogen, i.e., pathogen use the host ubiquitin system.[47]

Nowadays, recent research has directed towards the development of TLR antagonists. Antagonists are immune system regulators developed for downregulating overactive TLR signaling which include small molecules, oligonucleotides, nucleotides, proteins, peptides, antibodies and polyphenols.[48] In recent times, some important TLR antagonists have been reported in the literature are summarized in Table 2. TLR targeting antagonists must be able to antagonize the harmful effects of TLR activation, without affecting host defense functions. However, some natural products targeting TLR 4 have been discovered to exert an anti-inflammatory action. The sources of natural products include mainly gram-negative bacteria and cyanobacteria or plants. The mechanism for natural TLR 4 antagonists was explained by their action in the extracellular compartment, by blocking the formation of the TLR 4/MD-2 complex and acting either on CD14 or on MD-2. Following are the currently reported molecules of natural origin with well-demonstrated TLR 4 antagonist activity, LPS and lipid A from *Rhodobacter sphaeroides*, LOS from *Bartonella quintana*, LPS from *Oscillatoria Planktothrix* FP1, curcumin from *Curcuma longa*, sulforaphane and iberin from cruciferous vegetables, xanthohumol from hops and beer, celastrol from *Tripterygium wilfordii*.[49]

Table 2: Toll-like receptors antagonists

Antagonist category	*Antagonist name*	*Target TLR*
Small molecules	CU-CPT22, C29 and ortho-vanillin	TLR 2
	TH1020	TLR 5
	AT791	TLR 7, 9
Oligonucleotides	Anti-microRNA oligonucleotides	TLR 7, 8
Nucleotides	INH-ODN, CpG ODN 2088, COV08-0064	TLR 9
Proteins	SSL3	TLR 2
Peptides	VB3323	TLR 4
	2R9	TLR 2, 4, 7, 9
Antibodies	OPN-305	TLR 2
	HT52, HTB2	TLR 4
	CNTO2424	TLR 3
	(+)-*N*-Phenethylnoroxymorphone, (+)-naltrexone and (+)-naloxone	TLR 4
Polyphenol	SsnB	TLR 2, 4

Conclusion

Our understanding about the functions of TLRs as a molecular microbe detection via intracellular signaling pathways has greatly advanced. The inflammatory response triggered by TLR stimulation is a double-edged sword, which defend host against a pathogenic infection but, also have potential harmful effects on host. The aberrant stimulation of TLRs may lead to autoimmune and inflammatory disease. Toll-like receptor signaling is crucial in maintaining periodontal health and development of periodontal disease. Yet, our knowledge regarding functional polymorphisms of genes involved in the stimulation and regulation of lipopolysaccharide mediated inflammatory processes is still limited. The known role of TLRs in the periodontal pathogenesis, bacterial immune evasion and disease progression is still expanding. As we know, TLRs are overactive (or their inhibitors are underactive), this might pivot into chronicity, and so targeting TLRs might therefore lead to remission from this chronic inflammation. But, in regard with the present situation it is unclear about which particular signaling pathways are essential to be blocked to enhance the host defenses by decreasing chronicity. For that reason, additional research is necessary to expand our knowledge regarding the TLR mediated periodontal disease initiation and progression.

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