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DOES TOLL LIKE RECEPTORS HAVE DOUBLE EDGE? : A REVIEW

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ABSTRACT

Vertebral immunity can be broadly classified into adaptive and innate immunity .Innate immunity is the first line of defense. Adaptive immunity are regulated by clonally distributed B and T lymphocytes and are characterized by specificity and memory .Innate immunity works through toll-like receptors (TLRs), which recognize the molecular patterns on virulent bacteria known as pathogen-associated molecular patterns(PAMP). The periodontium is constantly exposed to microorganisms which inturn stimulates the innate immune sytem. The toll like receptors 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

KEYWORDS: Toll like receptors ;Pathogenesis ;Periodontitis,;Innate immunity

INTRODUCTION

Vertebral immunity can be broadly classified into adaptive and innate immunity. Adaptive immunity are regulated by clonally distributed B and T lymphocytes and are characterized by specificity and memory.Adaptive immune responses are delayed typically for 4 to 7 days and the infection during the first few days are controlled by innate immune system.[1,]The main function of innate immune system is opsonization, complement activation,activation of pro-inflammatory signaling cascade and apoptosis. The innate immune system also has an important function in activation and shaping of adaptive immune response through the induction of co stimulatory molecules and cytokines.In contrast to clonotypic receptors expressed by T and B lymphocytes,the innate immune system uses non clonal sets of recognition molecules called pattern recognition receptors.[2]

The innate immune system employs germline encoded pattern recognition receptors for the initial detection of microbes.These pattern recognition receptors recognize microbe specific signatures called as Pathogen associated molecular pattern (PAMP) and self -derived molecules from damaged cells referred as the damage associated molecular pattern (DAMP) .The pattern recognition receptors lead to induction of innate immune responses by producing inflammatory cytokines,interferon(INF) and other mediators.These responses are essential for the clearance of infecting microbes as well as crucial for the consequent instruction of antigen-specific adaptive immune responses.[3]

Once the pathogen associated molecular pattern (PAMPs) and the damage associated molecular pattern (DAMPs) is recognized the toll like receptors recruit the domain containing adaptor proteins such as MyD88 and TRIF which initiate the signal transduction pathways that culminate in the activation of NF-κ B,IRFs or MAP kinases to regulate the expression of cytokines ,chemokines ,and type -I IFNs that ultimately protect the host from microbial infection[4]

HISTORY OF TOLL LIKE RECEPTORS

Timeline of the history of toll like receptors

1988-IL-1R1 is cloned ,CD 14 and LPS binding protein was identified [5]
1989-Toll is described in the fly with a role in development IL-1 shown to activate NK-Kb[6]
1990-CD14 and LPS binding protein are identified as the components of LPS complex [7]
1991-IL-1RL toll homology and tol il-1RL pathway[8]
1993-Pathogen specific immune signalling is found to involve induction of antimicrobial peptides by the members of NF-kappa B in drosophillia family .[9]
1994-Plant protein N is shown to be involved increased resistance and TLR domain that is similar to toll and IL1R1[10]
1996-Toll pathway is shown to regulate the antifungal response in D.Melanogaster[11]
1997-Human toll described MyD88 identified as adaptor in IL-1R signalling [12]
1998-5 Toll like reeptorsdescribed .Toll like receptors 4 identified as lipopolysaccaharide receptor [13]
2000-Ligands for toll like receptor 2 hetrodimeric complexes was identified [14]
2003 -TRAM identified as TLR -4 adaptor and TLR inhibitors were described [15]
2004-Toll like receptors 7 and 8 are reported to recognize viral ssRNA1[16]
2006-The first function for mammalian SARM1(a regulatory TRIF) is reported [17]
2007-Structure of several TLR complexes (TLR-1,2,3,4) are solved.[18]
2009-TAG(Tram splice variant) as inhibitor of MyD88 pathway [19]

TYPES OF TOLL LIKE RECEPTORSAND THEIR LIGANDS

Toll like receptors can be divided into five subfamilies Toll like receptor 3 ,Toll like receptor 4,Toll like receptor 5,Toll like receptor 2 and Toll like receptor 9 subfamilies .The toll like receptor 2 sub family is composed of Toll like receptor 1,Toll like receptor 2 ,Toll like receptor 6 and Toll like receptor 10.The Toll like receptor 9 subfamiles is composed of Toll like receptor 7,Toll like receptor 8,Toll like receptor 9.The Toll like receptor 2 subfamily is composed of Toll like receptor 1 and Toll like receptor 6 are found.[20]

Table 1 Toll like receptors and its ligands

TOLL LIKE RECEPTORS	LIGANDS
Toll like receptor 1	Triacyl Lipopeptides
Toll like receptor 2	Lipoproteins, Peptidoglycan, Lipoteichoic acid, Zymosan, *Porphyromonas gingivalis* lipopolysaccharide & fimbriae, *Capnocytophaga ochracea* lipopolysaccharide
Toll like receptor 3	Double stranded RNA, Polyinosine-polycytidylic acid
Toll like receptor 4	Escherichia coli, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans and Fusobacterium nucleatum lipopolysacchride
Toll like receptor 5	Flagellin
Toll like receptor 6	Peptidoglycan, Lipoteichoic acid, Diacyl lipopeptides, Zymosan
Toll like receptor 7	Imidazquinoline
Toll like receptor 8	Single stranded RNA, Imidazquinoline
Toll like receptor 9	Bacterial DNA, CpG oligodeoxynucleotide
Toll like receptor 10	Not determined

SIGNALLING OF TOLL LIKE RECEPTORS

MyD88 Dependent pathway

After TLR engagement, MyD88 forms a complex with IRAK kinase family members, referred to as the Myddosome .During Myddosome formation, IRAK4 activates IRAK1, which is then autophosphorylated at several sites and released from MyD88 .IRAK1 associates with the RING-domain E3 ubiquitin ligase TRAF6. TRAF6, along with ubiquitin-conjugating enzyme UBC13 and UEV1A, promotes K63-linked polyubiquitination of both TRAF6 itself and the TAK1 protein kinase complex. TAK1 is a member of the MAPKKK family and forms a complex with the regulatory subunits TAB1, TAB2, and TAB3, which interact with polyubiquitin chains generated by TRAF6 to drive TAK1 activation .Although the mechanisms of TAK1 activation within this complex remain unclear, K63-linked ubiquitination or close proximity-dependent transphosphorylation may be responsible for TAK1 activation. TAK1 then activates two different pathways that lead to activation of the IKK complex-NF-κB pathway and -MAPK pathway. The IKK complex is composed of the catalytic subunits IKKα and IKKβ and the regulatory subunit NEMO (also called IKKγ). TAK1 binds to the IKK complex through ubiquitin chains, which allows it to phosphorylate and activate IKKβ. The IKK complex phosphorylates the NF-κB inhibitory protein IκBα, which undergoes proteasome degradation, allowing NF-κB to translocate into the nucleus to induce proinflammatory gene expression. TAK1 activation also results in activation of MAPK family members such as ERK1/2, p38 and JNK, which mediates activation of AP-1 family transcription factors or stabilization of mRNA to regulate inflammatory responses

TRIF-Dependent pathway

TRIF interacts with TRAF6 and TRAF3. TRAF6 recruits the kinase RIP-1, which in turn interacts with and activates the TAK1 complex, leading to activation of NF-κB and MAPKs and induction of inflammatory cytokines . In contrast, TRAF3 recruits the IKK-related kinases TBK1 and IKKi along with NEMO for IRF3 phosphorylation. Subsequently, IRF3 forms a dimer and translocates into the nucleus from the cytoplasm, where it induces the expression of type I IFN genes .

The Pellino family E3 ubiquitin ligases are implicated in TLR signaling .Pellino-1-deficient mice display impaired TRIF-dependent NF-κB activation and cytokine production . Pellino-1 is phosphorylated by TBK1/IKKi and thereby facilitates ubiquitination of RIP-1, suggesting that Pellino-1 mediates TRIF-dependent NF-κB activation by recruiting RIP-1. Furthermore, Pellino-1 regulates IRF3 activation by binding to DEAF-1, a transcription factor that facilitates binding of IRF3 to the IFNβ promoter ..Recently, IRF3 activation was demonstrated to be regulated by an inositol lipid, PtdIns5P. PtdIns5P binds to both IRF3 and TBK1, and thus facilitates complex formation between TBK1 and IRF3. The accessibility of TBK1 to IRF3 mediated by PtdIns5P likely causes IRF3 phosphorylation in a closely proximal manner. Furthermore, PIKfyve was identified as a kinase responsible for production of PtdIns5P during virus infection

Balanced Activation Between MyD88- and TRIF-Dependent Pathways

TLR4 activates both the MyD88-dependent and TRIF-dependent pathways. Activation of these pathways is controlled by several molecules to induce appropriate responses. Balanced production of inflammatory cytokines and type I IFN may be important for controlling tumor cell growth and autoimmune diseases.

TRAF3 was shown to be incorporated into the MyD88 complex as well as the TRIF complex in TLR4 signaling. TRAF3 within the MyD88 complex is then degraded, which causes TAK1 activation. Thus, in addition its role in promoting TRIF-dependent pathway activation, TRAF3 has a role in inhibiting the MyD88-dependent pathway. NRDP-1, an E3 ubiquitin ligase, binds and ubiquitinates MyD88 and TBK1, inducing the degradation of MyD88 and augmenting the activation of TBK1, which attenuates inflammatory cytokine production and induces preferential type I IFN production, respectively

MHC class II molecules that are localized in endosomes in antigen-presenting cells interact with the tyrosine kinase Btk via the costimulatory molecule CD40 and maintain Btk activation. Activated Btk interacts with MyD88 and TRIF to promote the activation of the MyD88-dependent and TRIF-dependent pathways and thus to enhance production of inflammatory cytokines and type I IFNs, respectively [21]

TOLL LIKE RECEPTORS IN PERIODONTAL TISSUES

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-α (TNF-α) and biologic mediators responsible for bone resorption. Increased expression of receptor activator of nuclear factor κ B ligand (RANKL).

Osteoclasts -TLR 2, 4 Enhanced survival of osteoblasts and increased osteoclastic activities.

Cementoblasts -TLR 2, 4 Down regulation of RANKL[22]

Periodontal ligament fibroblasts TLR 2, 4 Enhanced production of pro-inflammatory cytokines, release of proteases causing direct destruction of surrounding tissues.

ROLE OF TOLL LIKE RECEPTORS IN PERIODONTITIS

Numerous studies have shown that Toll like receptorss are involved in the initiation and progression of chronic periodontitis, and altered expression of Toll like receptorss can be detected in chronic periodontitis patients [23] .Toll like receptors4, which is capable of sensing lps, has been clearly implicated in chronic periodontitis .the gene expression levels of Toll like receptors2 and Toll like receptors4 were significantly higher in chronic periodontitis patients than in individuals with healthy periodontium. based on immunohistochemical analysis of the periodontal tissues, clinical studies have demonstrated the levels of Toll like receptors2 and Toll like receptors4 in chronic periodontitis patients were significantly higher than the levels in control groups . both human periodontal ligament fibroblasts (hpdlfs) and human gingival fibroblasts, purified from chronic periodontitis patients, expressed significantly higher levels of Toll like receptors1, Toll like receptors4, and Toll like receptors7 than those from periodontally healthy subjects. in addition to Toll like receptors2 and Toll like receptors4, Toll like receptors9 was also detected and its expression was significantly elevated in subjects with chronic periodontitis.[24] the nf-jb pathway was activated and the levels of cytokines were enhanced. additionally, smoking habits are known to exacerbate chronic periodontitis. revealed that upregulated expression of Toll like receptors2 and Toll like receptors4 was closely associated with chronic periodontitis and that smoking could promote their mrna levels. however, the expression of Toll like receptors4 was not always promoted in patients with chronic periodontitis. demonstrated that the Toll like receptors4 levels in patients with chronic periodontitis and in a control group were not significantly different. instead, Toll like receptors2 and Toll like receptors9 expression levels in subjects with chronic periodontitis were markedly higher than those in periodontally healthy subjects.[25]

As chronic periodontitis is defined as an infectious disease, the long-term inflammatory state of periodontal tissues may lead to endotoxin tolerance, which can weaken the host innate immune response to subsequent stimulation with lps. previous reports have described endotoxin tolerance, in which Toll like receptors2 and Toll like receptors4 levels in human monocytes are elevated following the first stimulation with lps and then downregulated after the second stimulation[26] .Recently, sun et al (2012) demonstrated that when lps-pretreated peritoneal macrophages from mice were rechallenged with lps, the gene and protein levels of Toll like receptors2 and Toll like receptors4 were markedly downregulated. epigenetic regulations might also be involved in endotoxin tolerance. benakanakere et al (2015) discovered that chronic porphyromonas gingivalis stimulation of human gingival epithelial cells (hgecs) could increase dna methylation in Toll like receptors2, leading to reduced expression of Toll like receptors2 and inhibiting the inflammatory reaction. this alteration promoted host susceptibility to chronic periodontitis. endotoxin tolerance can be observed in thp-1 cells as well (sun et al, 2014).[27] after lps-pretreated thp-1 cells were re-exposed to lps, the levels of tnf-a, il-1b, Toll like receptors2, and Toll like receptors4 decreased. endotoxin tolerance has also been observed in hpdlfs. the second stimulation with lps downregulated the expression of Toll like receptors2, Toll like receptors4, il-6, and il-8 in hpdlfs . these findings indicated that repeated stimulation with periodontopathic bacteria might induce endotoxin tolerance, which is modulated by Toll like receptors2 and Toll like receptors4. therefore, either hyper-responsiveness or hyporesponsiveness to lps stimulation in the periodontium can initiate chronic periodontitis binding of monocytes to periodontal

fibroblasts is regulated by Toll like receptorss. when u937 cells were exposed to a Toll like receptors4 agonist, the gene expression levels of lfa-1 and vla-4 were enhanced, which facilitated the adherence of u937 cells to hgfs or human periodontal ligament cells (hpdlcs) .this partly confirmed the involvement of Toll like receptorss in host innate immunity[27] .Gaddis et al (2013) revealed that il-10 regulated by Toll like receptors2 was significantly elevated and that ifn-c secretion from t cells was inhibited in mice with periodontitis. these findings further confirmed that Toll like receptorss are a potential bridge between innate immunity with adaptive immunity[28]. Hutcherson et al (2015) also found that after primary human monocytes were exposed to porphyromonas gingivalis ragb, il-8 production increased, which was linked to Toll like receptors2 and Toll like receptors4 expression. however, suppressing the expression levels of Toll like receptors2 or Toll like receptors4 by pretreating dendritic cells (dcs) with anti-Toll like receptors2 or anti-Toll like receptors4 induced the opposite results (diaz-zuniga et al, 2015). in addition, after thp-1 cells were stimulated with porphyromonas gingivalis, the il-1b level was reduced by suppressing the expressions of myd88 and/or traf6.pretreating hpdlcs with anti-Toll like receptors2 and antiToll like receptors4 could also reduce il-17 and il-23 production in lps-stimulated cells[29] . Taken together, these results suggest that the production of pro-inflammatory cytokines and chemokine receptors is positively associated with Toll like receptors2 and/or Toll like receptors4 expression and that antibody or sirna may be effective tools to inhibit Toll like receptors2 and Toll like receptors4. recent studies further support the association between host immunity and periodontal bacteria that contributes to the initiation and progression of periodontitis. data obtained from these studies indicated that Toll like receptorss play a pivotal role in chronic periodontitis. besides,[30] .Toll like receptorss are associated with the production of pro-inflammatory and inflammatory cytokines, activation of adaptive immunity, resorption of alveolar bone, and endotoxin tolerance in chronic periodontitis[31]

TOLL LIKE RECEPTORS A DOUBLE EDGE SWORD IN PERIODONTAL DISEASE

Positive edge of toll-like receptors

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 a 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.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. These TLRs expressed on the 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 β-defensinscathelicidin and calprotectin, as well as neutrophil chemoattractant (IL-8)[32] .Therefore, TLR signaling limits microbial invasion and prevents commensal organisms from breaching the epithelial barrier, thereby maintaining gingival health. 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 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[33]

NEGATIVE EDGE OF TOLL-LIKE RECEPTORS

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 leukocytes toward the gingival sulcus. IL-8, a known neutrophil chemoattractant, is also released by epithelial cells, further enhancing the migration of neutrophils. Epithelial cells are also known to produce matrix metalloproteinases (MMPs) in response to PAMPs, causing direct damage to periodontal tissues.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 IL-1, IL-6, TNF-α)[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).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 κB ligand (RANKL). 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 . 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. 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 a type of co-stimulatory molecule.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. 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 RANK present on osteoclasts and osteoclast precursors and activates them.A bone-protecting factor, osteoprotegrin (OPG), produced by osteoblasts and bone marrow stomal cells, inhibits the RANK/RANKL interaction and prevents bone resorption. 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.[35\]

CONCLUSION

TLR 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 the 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

REFERENCE

1.Shizuo akira ,satoshiuematsu and osamutakeuchi Pathogen recognition and innate immunity Cell 124,783-801 ,February 24,2006

2.Kiyoshi takeda .tsuneyasukaisho and shizuoakira Toll like receptors ,Annu.Rev.Immunology 2003.21:335-76

3.Takumi Kawasaki and taro kawai Toll like receptor signalingpathways .Laboratory of molecular immunology,September 2014 volume 5 article 461

4.Mallenahally kushka ,V.Girishkumar ,Veerasamysejian ,Madiajaganbagath,Govindan Krishnan Toll like receptors :Significance ,ligands ,signaling pathways ,and function in mammals International review of immunology 13 October 2017

5.Sims, J. E. et al. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin

superfamily. Science 241, 585–589 (1988)
6.Janeway, C. A. Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. 54, 1–13 (1989)
7.Dinarello, C. A. Interleukin-1 and interleukin-1 antagonism. Blood 77, 1627–1652 (1990).
8.Hampton, R. Y., Golenbock, D. T., Penman, M., Krieger, M. &Raetz, C. R. Recognition and plasma clearance of endotoxin by scavenger receptors. Nature 352, 342–344 (1991)
9.Ip, Y. T. et al. Dif, a dorsal-related gene that mediates an immune response in Drosophila. Cell 75, 753–763 (1993)
10.Sims, J. E. & Dower, S. K. Interleukin-1 receptors. Eur. Cytokine Netw. 5, 539–546 (1994
11.Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. & Hoffmann, J. A. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86, 973–983 (1996)
12.Medzhitov, R., Preston-Hurlburt, P. & Janeway, C. A. Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388, 394–397 (1997).
13.Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A. & Bazan, J. F. A family of human receptors structurally related to Drosophila Toll. Proc. Natl Acad. Sci. USA 95, 588–593 (1998)
14.Rutschmann, S. et al. The Rel protein DIF mediates the antifungal but not the antibacterial host defense in Drosophila. Immunity 12, 569–580 (2000)
15. Lund, J., Sato, A., Akira, S., Medzhitov, R. & Iwasaki, A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2by plasmacytoid dendritic cells. J. Exp. Med. 198, 513–520 (2003)
16.Lemaitre, B. The road to Toll. Nature Rev. Immunol. 4, 521–527 (2004)
17.Kagan, J. C. &Medzhitov, R. Phosphoinositidemediated adaptor recruitment controls Toll-like receptor signaling. Cell 125, 943–955 (2006)
18. O'Neill, L. A. & Bowie, A. G. The family of five: TIRdomain-containing adaptors in Toll-like receptor signalling. Nature Rev. Immunol. 7, 353–364 (2007)
19.Kagan, J. C. &Medzhitov, R. Phosphoinositidemediated adaptor recruitment controls Toll-like receptor signaling. Cell 125, 943–955 (2006). for TLR2 signaling and inhibits signaling by TLR3. J. Immunol. 183, 3642–3651 (20091
20.Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu. Rev. Immunol. 2003; 21:335–376
21. Han KJ, Su X, Xu LG, Bin LH, Zhang J, Shu HB (2004) Mechanisms of the TRIF-induced interferon-stimulated response element and NF-kappaB activation and apoptosis pathways. J Biol Chem 279: 15652–15661
22. Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, Genco RJ (2000) Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. Infect Immun 68, 3140-3146.
23.Banu S, Jabir NR, Mohan R et al (2015). Correlation of Tolllike receptor 4, interleukin-18, transaminases, and uric acid in patients with chronic periodontitis and healthy adults. J Periodont 86: 431–439.
24.Becerik S, Ozsan N, Gurkan A, Ozturk VO, Atilla G, Emingil G (2011). Toll like receptor 4 and membrane-bound CD14 expressions in gingivitis, periodontitis and CsA-induced gingival overgrowth. Arch Oral Biol 56: 456–465. Beklen A, Sarp AS, Uckan D, Memet GT (2014).
25.The function of TLR4 in interferon gamma or interleukin-13 exposed and lipopolysaccharide stimulated gingival epithelial cell cultures. Biotech Histochem 89: 505–512.
26.Belstrom D, Damgaard C, Nielsen CH, Holmstrup P (2012). Does a causal relation between cardiovascular disease and periodontitis exist? Microbes Infect 14: 411–418.
27.Slots J (2010). Human viruses in periodontitis. Periodontology 2000: 89–110.
28.Soboku K, Kikuchi T, Fujita S et al (2014). Altered gene expression in gingival tissues and enhanced bone loss in rats with diabetes with experimental periodontitis. J Periodont 85: 455– 464.
29.Sorsa T, Tervahartiala T, Leppilahti J et al (2011). Collagenase2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. Pharmacol Res 63: 108– 113.
30.Sun Y, Shu R, Li CL, Zhang MZ (2010). Gram-negative periodontal bacteria induce the activation of Toll-like receptors 2 and 4, and cytokine production in human periodontal ligament cells. J Periodont 81: 1488–1496.
31. Kusumoto Y, Hirano H, Saitoh K, Yamada S, Takedachi M, Nozaki T, et al. Human gingival epithelial cells produce chemotactic factors interleukin-8 and monocyte chemoattractant protein-1

after stimulation with Porphyromonasgingivalis via toll-like receptor 2. J Periodontol 2004;75:370-9

32. Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, et al. Interactions between periodontal bacteria and human oral epithelial cells: Fusobacteriumnucleatum adheres to and invades epithelial cells. Infect Immun 2000;68:3140-6.

33. Warner RL, Bhagavathula N, Nerusu KC, Lateef H, Younkin E, Johnson KJ, et al. Matrix metalloproteinases in acute inflammation: Induction of MMP-3 and MMP-9 in fibroblasts and epithelial cells following exposure to proinflammatory mediators in vitro. ExpMolPathol 2004;76:189-95.

34. Trevani AS, Chorny A, Salamone G, Vermeulen M, Gamberale R, Schettini J, et al. Bacterial DNA activates human neutrophils by a CpG-independent pathway. Eur J Immunol 2003;33:3164-74.

35. Galdiero M, de l'Ero GC, Marcatili A. Cytokine and adhesion molecule expression in human monocytes and endothelial cells stimulated with bacterial heat shock proteins. Infect Immun 1997;65:699-707