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"Toll-Like Receptors" A Recent Epoch In Periodontal Immunity

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ABSTRACT

Toll-like receptors (TLRs) are germline-encoded receptors that are central to innate and adaptive immune responses. Owing to their vital role in inflammation, TLRs are rational targets in clinics; thus, many ligands and biologics have been reported to overcome the progression of various inflammatory and malignant conditions and support the immune system. For each TLR, at least one, and often many, drug formulations are being evaluated. Ligands reported as stand-alone drugs may also be reported based on their use in combinatorial therapeutics as adjuvants. Despite their profound efficacy in TLR-modulation in preclinical studies, multiple drugs have been terminated at different stages of clinical trials. Here, TLR modulating drugs that have been evaluated in clinical trials are discussed, along with their mode of action, suggestive failure reasons,andwaystoimprovetheclinicaloutcomes.Thisreview presents recent advances in TLR-targeting drugs and provides directions for more successful immune system manipulation.

I. Introduction

Periodontitis is a chronic bacterial infection that affects the gingiva and bone supporting the teeth. Bacterial plaque stimulates the host inflammatory response leading to tissue damage. Bacterial plaque constitutes more than 700 distinct microbial species cultivated from dental plaque.[1]The host defense against periodontopathic bacteria comprises of innate and adaptive immunity. The interaction of the microorganisms with the host determines the course and extent of the resulting disease.

II. Innate immune system

Innate immune system recognizes the aggression made by microorganisms, toxins and chemical compounds. Primary challenge of immune system - Discriminate between large number of periodontal pathogens from host and host has limited number of cell surface receptors. Innate immune system met this challenge through recognition of evolutionary conserved structures on pathogens that are not present in higher eukaryotes, called as pattern recognition receptors. The first line of defense in is the recognition of conserved molecules characteristic of many microbes. These elicitors are also known as Microbe- or Pathogen-associated molecular patterns (MAMPs or PAMPs). Microbe-associated molecular patterns (MAMPs) or danger-associated molecular patterns (DAMPs) are recognized via the pattern recognition receptors (PRRs).[2]PRRs enable

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innate immune cells to instantly detect and respond to the presence of Danger and Microbe-associated molecular patterns (DAMPs and MAMPs). MAMPs are conserved microbial molecules that are not produced by mammalian host cells, such as nucleic acid structures that are unique to microorganisms, bacterial secretion systems and their effector proteins, and microbial cell wall components such as lipoproteins and lipopolysaccharides (LPSs). In contrast, DAMPs are a set of host-derived molecules that signal cellular stress, damage, or nonphysiological cell death. Innate immune receptors or pattern recognition receptors are classified into toll-like receptors (TLRs), retinoic acid inducible gene I-like receptors (RLRs), C-type lectin receptors (CLRs), absent in melanoma 2-like receptors (ALRs) and nucleotide-binding oligomerization domain-like receptors (NLRs).[3]

III. Discovery of toll-like receptors

The discovery of toll-like receptors has been evolved over the years.[4] It was named as toll-like receptors because of their similarity to the protein encoded by the toll gene which was identified in Drosophila.[4]First described as gene for Type I transmembrane receptor. Its role in dorsoventral development of Drosophila (fruit fly) embryo was given by ***Underhill et al*** **2002**. In 1989, theconcept of Pattern Recognition Receptors (PRR) came. In 1991, identification of NF-ƙB binding motifs in the promoters of *Drosophilia* was made. In 1994, first human TLR was discovered.[4] In 1996, TLR was mapped to a chromosome by ***Taguchi***.In 1996, Toll regulates *Drosomycin* expression and resistance to fungal infections.[4] In 1997,identification of human Toll orthologue regulating NF-ƙB was done. In 1998, TLR-4 as the receptor of LPS was identified. In 1999-2004,characterization of the family of Toll-like receptors and identification of TLR ligands were done. In 2004, TLR-4 deficiency was found to be associated with reduction of atheroscelorosis in apoE deficient mice. In 2007, identification of TLR-3 mutation in humans was done. In 2007-2009, 3D structure of TLR-ligand complexes was discovered. To date, 11 different toll-like-receptor molecules have been identified in human periodontal tissues, and their expression, distribution and ligand specificities have been characterized.[4]

IV. Structure of TLR

It is a type-1 transmembrane protein and it has:[5]an extracellular domain, a transmembrane portion, and an intracellular domain. A horseshoe shaped solenoid Contains an extensive beta sheet on its concave surface.[5]

V. The TLR superfamily

Toll-like receptors, together with the Interleukin-1 receptors, form a receptor super family 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. They were classified as:[6]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, Proteins containing TLR domains consists of adaptor proteins that are exclusively cytosolic and mediate signalling from proteins of subgroups 1 and 2.

TLRs are expressed in innate immune cells such as dendritic cells (DCs) and macrophages as well as non-immune cells such as fibroblast cells and epithelial cells. TLRs are largely classified into two subfamilies based on their localization, cell surface TLRs and intracellular TLRs. Cell

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surface TLRs include TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10, whereas intracellular TLRs located in endosome includes TLR3, TLR7, TLR8, TLR9, TLR11, TLR12 and TLR13.[7]

VI. TLR ligands

Cell surface TLRs mainly recognize microbial membrane: lipids, lipoproteins, and proteins. TLR2 along with TLR1 or TLR6 recognizes a wide variety of MAMPs including lipoproteins, peptidoglycans, lipotechoic acids, zymosan, mannan, and tGPI-mucin. TLR4 recognizes bacterial lipopolysaccharide (LPS). TLR5 recognizes bacterial flagellin. TLR10 is pseudogene in mouse but inactive in humans. TLR3 recognizes viral double-stranded RNA. TLR7 recognizes single stranded (ss)RNA from viruses. TLR9 recognizes bacterial and viral DNA. TLR8 responds to viral and bacterial RNA.[7]

VII. Adaptor proteins

Adapter proteins of TLRs include:[8]

MyD88 (Myeloid differentiation factor 88)
TRIF (Toll/interlukin-1R domain containing adaptor-inducing interferon β)
TIRAP (or Mal) (MyD88 adaptor-like) (Toll interlukin-1 receptor containing adaptor protein)
TRAM (TRIF-related adaptor molecule)
SARM (sterile alpha and HEAT/ Armadilo motif protein)

VIII. TLR Signalling

Stimulation of TLRs by microbial components triggers expression of several genes that are involved in immune responses. The molecular mechanisms by which TLRs induce gene expression are now rapidly being elucidated through analyses of TLR-mediated signalling pathways[9]: Myeloid differentiation factor 88(MyD88) - dependent pathway and MyD88-independent pathway /TRIF-dependent pathway

IX. TLR Signalling - MyD88 dependent pathway

MyD88 functions as an adaptor linking TLRs/IL-1Rs with downstream signaling molecules that have DDs. It recognizes the conformational change in the TIR domain of the TLRs, binds to the new receptor complex, and transfer the signaling by amino (N)-terminal death domain (DD) interaction with IL-1R-associated kinases (IRAKs). These results a complex cascade with signaling invents that warns the cell of pathogen invasion. There are 4 IRAKs (IRAK 1, 2, 4, M). They contain an N-terminal DD and a central serine/threonine-kinase domain. IRAK1 and IRAK4 have intrinsic kinase activity, whereas IRAK2 and IRAK-M have no detectable kinase activity. IRAK4 activated by MyD88 and it continue to activate IRAK1. IRAK1 then activate the downstream TRAF6. TRAF6 is a member of the tumor necrosis factor receptor (TNFR)-associated factor (TRAF) family that mediates cytokine signaling pathways. Upon stimulation, TRAF6 is recruited to the receptor complex, and activated by IRAK-1 that binds to the TRAF domain of TRAF6. Then, the IRAK-1/TRAF6 complex dissociates from the receptor and associates with TGF-beta-activated kinase 1 (TAK1) and TAK1-binding proteins, TAB1 and TAB2. The complex of TRAF6, TAK1, TAB1, and TAB2 moves into the cytoplasm, where it forms a large complex with other proteins, such as the E2 ligases Ubc13 and Uev1A. The Ubc13 and Uev1A complex has been shown to catalyze the synthesis of a Lys 63-linked polyubiquitin

chain of TRAF6 and thereby induce TRAF6-mediated activation of TAK1 and finally of NF-kB.[9]

X. TLR Signalling - MyD88 Independent pathway

Both TLR3 and TLR4 utilize the TRIF-dependent pathway, which is triggered by dsRNA and LPS, respectively. For TLR3,dsRNA leads to activation of the receptor, recruiting the adaptor TRIF. TLR signalling ultimately leads to the induction or suppression of genes that augments the inflammatory response.[9]

XI. TLR4 Signalling Pathway

The MyD88 dependent pathway leads to subsequent activation of IRAK (IL-1R-associated kinase), TRAF6, and ultimately NF-kB and it is essential for cytokine induction.MyD88-independent pathway does not activate IRAK and leads to activation of NF-kB with delayed kinetics.This independent pathway requires different adaptor proteins, such as TIRAP, TRIF, and TRAM, and probably does not lead to cytokine induction. Another interesting aspect of MyD88-independent signalling is that it can induce dendritic cell maturation. Rather, it is related to interferon-β secretion and indirect upregulation of IFN-dependent genes.[10]

XII. TLR REGULATION

TLR regulators regulate the dependent and independent pathways and it includes:[11]RAK-M, SOCS1 (suppressor of cytokine signalling 1), MyD88s (MyD88short), SIGIRR (single immunoglobulin IL-1R-related molecule), ST2 (Sequestration of adaptor proteins -2)

XIII. TLR SPECIFIC TO IMMUNE CELLS

TLR IN INNATE IMMUNITY

Neutrophils, Monocytes/ Macrophages and Dendritic cellsexpress different Toll like receptorsthat triggers wide variety of immune response to specific pathogen.

Neutrophils:

First innate immune cell to migrate to the site of infection. Express TLR -1, TLR-2, TLR-4 to TLR-10. Neutrophils utilize TLR to recognize & respond to different types of microbial challenges. The main action of neutrophil is to recognize, engulf and kill the microorganism.[12]

Monocytes/Macrophages:

Also considered first line of defense. It Expresses TLR 1,2,4–8. Binding of MAMP to monocyte TLR tends to influence the type of adaptive immune response.Activation of TLR 2/1 leads to differentiation of monocyte into macrophages resulting in poor antigen specific Th 1 response.[13]

Antigen specific immune response

Development of specific adaptive immunity to pathogen controlled by activation of innate immune cells results in forming Antigen Presenting dendritic cells (APC). Professional APC are derived from bone marrow, developed throughout the body as immature cells. When an infection occurs, TLR of resident immature dendritic cells detect MAMP on/from invading microorganism

that activates dendritic cells, expresses co-stimulatory molecules and production of cytokines & chemokines.[14]

Dendritic cell:
CD 11c+ and CD11c-were found to express TLRs. CD 11c+ has myeloid appearance and express myeloid markers such as CD13, CD33 and expresses TLR1-6, TLR8 and TLR10.[15]Dendritic cells use TLR to distinguish between different pathogens and initiate appropriate, effective types of immune responses. Inflammatory cytokines can trigger maturation.Stimulation of TLR on immature dendritic cells leads to maturation of dendritic cells that induce T helper cell differentiation.[15]

XIV. TOLL-LIKE RECEPTORS IN PERIODONTAL TISSUE

Gingival epithelial cells
Protects underlying periodontal tissues (multilayered) and plays important role in innate immune response & homeostasis.[16] It is continuously exposed to large number of commensals & pathogenic bacteria. Express TLR 2, 3, 4, 5, 6 and 9.[17]Increased attachment and migration of leukocytes towards antigen on pocket,production of IL-8, MMPs. TLR 2 expression was found denser in spinous epithelial layer than basal layer.[17] TLRs was also observed in connective tissue subjacent to pocket epithelium. ***Uehara et al*** stated that epithelial cells expressed low levels of TLR 4, which can be enhanced by treating with Interferon γ.[18] TLR 3 & 9 were found to respond for both viral & bacterial nucleic acids.[17]*P. gingivalis* fimbriae & staphylococcus aureus peptidoglycans stimulate TLR 2 of epithelial cells and production of IL-8.[16]

Gingival fibroblast
Gingival fibroblast maintains tissue integrity by regulating collagen & proteoglycan metabolism. It express mRNA of TLR 2, 4 and 9.[16]Increased production of IL-8 and other cytokines were found and also other TLR related molecules such as CD14 and MyD88, MD-2 (co-receptor of TLR 4) were expressed.[16]

Periodontal ligament fibroblast
Periodontal ligament fibroblast express mRNA for TLR 2, TLR 4, CD 14, MD 2 & MyD88 compared to gingival fibroblast. Enhanced production of pro inflammatory cytokines,release of proteases causing destruction was observed. This differential expression of TLR 2, CD14 suggests different functions of both cell types in response to plaque bacteria.[16]

Cementum
Cementum is the mineralized cellular tissue of root surface. In periodontitis, cementum is invaded by biofilm and it was found to express mRNA for TLR 2, TLR 4.[16]

Osteoblasts

Osteoblasts are bone forming cells that modulate differentiation & activity of osteoclasts. It expresses mRNA of TLR 1, 4-6, 9and upregulates proinflammatory cytokines like IL-8,TNF–α and biologic mediators for bone resorption resulting in increased expression of NF-kB.[16]

The relationship between TLRs and alveolar bone resorption

Alveolar bone resorption appears to be closely associated with the receptor activator of NF-ƙB ligand (RANKL) expression, which is positively regulated by TLRs.[17]Alveolar bone loss in periodontitis might be regulated by TLR9. ***Lin et al*** found that TLR2 and TLR4 were associated with periodontal bone loss in a mouse model of CP by suppressing the expression of RANKL with antibody, bone loss was reduced.[19]

The involvement of TLRs in CP

LPS is an effective ligand for TLR4. ***Li et al*** **2014** discovered that the ability of human periodontal ligament stem cells (hPDLSCs) to differentiate into osteoblasts was impaired by LPS through a TLR4-mediated NF-kB pathway.[20]Besides lipoproteins, TLR2 in gingival tissue can be activated by LPS from *Porphyromonas gingivalis*. Tumor necrosis factor-a (TNF-a) was able to activate TLR2 in HGFs (human gingival fibroblasts) through the JNK and NF-kB pathways, leading to elevated TLR2 gene and protein expression.*Porphyromonas gingivalis* fimbriae can activate the TLR2 and TLR4 pathways, leading to excessive production of pro-inflammatory cytokines and chemokines in monocytic cells.[17]

The association between TLRs and endotoxin tolerance

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 TLR2 and TLR4 levels in human monocytes are elevated following the first stimulation with LPS and then downregulated after the second stimulation.Repeated stimulation with periodontopathic bacteria might induce endotoxin tolerance, which is modulated by TLR2 and TLR4. Therefore, either hyper-responsiveness or hypo-responsiveness to LPS stimulation in the periodontium can initiate CP.[17]

TLRs as potential biomarkers for chronic periodontitis (CP)

As TLRs play a pivotal role in periodontitis and abnormal expression of TLRs can be observed in subjects with periodontitis.[17] TLRs have the potential to serve as diagnostic or prognostic biomarkers for periodontitis. Saliva can serve as a diagnostic fluid in CP, and both TLR2 and TLR4 have been detected in saliva. ***Budunelietal***revealed that salivary TLR4 was elevated in a CP group relative to a control group.[21] In addition, TLR2 and TLR4 levels in the plasma of subjects with CP were markedly higher than those of periodontally healthy subjects.[22]

XV. CONCLUSION

It is clear that periodontal cells actively participate in the innate immune response against dental plaque bacteria. They express different types of Toll-like receptors and NOD-like receptors. Periodontitis, a chronic inflammation of the periodontium, provides a unique opportunity to investigate the host–microbe interactions. Down-regulation of Toll-like receptor expression and inhibition of intracellular signalling may represent the underlying mechanism of tolerance. This provides new insight into the role of the immune system in maintaining health and combating disease.

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