International Journal of Enhanced Research in Medicines & Dental Care (IJERMDC),
ISSN: 2349-1590, Vol. 10 Issue 7, July 2023, Impact Factor: 7.125

Molecular Aspect of Pathogenesis of Periodontitis - Bridge Between Innate and Adaptive Immunity

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ABSTRACT

Early empirical theory held that periodontal tissue matrices were directly catabolized by periopathogenic bacteria, but significant molecular research has revealed that the host immune response is the main cause of periodontal tissue deterioration. The innate immune system has overcome this obstacle by recognizing pattern recognition receptors (PRRs), which are evolutionary conserved structures on pathogens that are absent in higher eukaryotes. These molecular patterns, sometimes referred to as pathogen-associated molecular patterns (PAMPs), are not vulnerable to significant mutation rates because they play crucial roles in the pathogen's capacity to avoid host defense. Pathogens all share PAMPs, but the host does not express them. Since the discovery of the toll-like receptors (TLRs), which have proven to be crucial for innate immune system recognition of microbes and for bridging the innate and acquired immune responses, it has become clear how the innate immune system functions even though many PRRs have been known for years.

Keywords: TLR, PAMP, Immune Responses, Microbe Recognition

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INTRODUCTION

A number of different factors contribute to the development and spread of the periodontitis lesion, including patient behaviors, medications, environmental factors, and genetic and epigenetic influences. Periodontitis is a complex disease with multiple component causes.

The understanding that maintaining or achieving clinical health involves a health-promoting biofilm in which symbiotic connections exist between bacteria and with the host response are significant developments in our perspectives of the infectious immunological disorder known as periodontitis. The latter can deliver essential nutrients through gingival crevicular fluid, and a host response that is both proportional and resolving is triggered by the different proteins and peptides generated by biofilm organisms.

The conditions within the biofilm begin to favor bacterial species, such as Fusobacterium nucleatum, that are able to sense and affect their environment by using chemical cues if the biofilm is not disturbed frequently and is allowed to build.

The emergence of these "quorum-sensing" organisms causes the host to react more strongly, which in turn can cause gingival inflammation and increase the availability of nutrients like heme that promote the growth of conventional pathogens like Porphyromonasgingivalis. In patients who are susceptible, incipient dysbiosis can set off an inappropriate, and frequently excessive, host response, leading to the production of too many cytokines, reactive oxygen species, and matrix metalloproteinases, which overwhelm their respective antagonists (such as antioxidants and tissue inhibitors of matrix metalloproteinases) and cause collateral damage to the periodontal tissues.

An inflammatory lesion becomes chronic as a result of the inability of innate mechanisms to resolve inflammation after release of damage-associated molecular peptides, which further spread the inflammatory response.

In order to cause "dysregulation" in the ordered nature of particular immunity, viruses also appear to play a role and are capable of stimulating inflammatory immune cells as well as subverting different signaling pathways within those cells.

Angiogenesis and fibrosis, which emerge concurrently with inflammation in the chronic inflammatory state, provide a rich nutritional milieu for sustaining the dysbiosis and subsequently the pathogenic biofilm.

The molecular peptides, which serve as a link between the innate and acquired (humoral) immune systems, appear to behave particularly destructively because of the dysregulation of chemotactic and microbicidal processes and the failure to release pro-resolving lipid mediators like lipoxins. Cells and neutrophils predominate in the active lesion. When periodontitis is advanced, intervention is required to remove biofilm to a point where healthy microbial species can re-establish themselves and contribute to a decrease in inflammation. This process, which has not been naturally triggered by pro-resolution pathways, also appears to be capable of restoring normal tissue structure and function when it is active.

The complex interplay between the health-promoting biofilm and the various signaling pathways that drive the host response appear to restore a balanced and well-regulated inflammatory immune repertoire. However, the degree of biofilm reduction necessary to reestablish symbiosis appears to vary from one person to the next.

Innate Immunity

Numerous mechanical, chemical, and microbiologic barriers that block pathogens from invading the body's cells and tissues make up the body's defenses against infection. The oral cavity's and the periodontium's underlying tissues are all shielded by saliva, GCF, and the epithelial keratinocytes of the oral mucosa.

Through effective competition for resources and ecological niches as well as by inducing protective immune responses, the commensal microbiota (such as that found in dental biofilm) may also be significant for supplying protection against infection by pathogenic microbes.

There is intimate integration between the innate and adaptive arms of the immune response, which prevents innate and adaptive immunity from working independently.

The cellular and molecular components of the innate immune response are activated when bacterial products penetrate the tissues.

Numerous cytokines, chemokines, and cell surface receptors coordinate innate immune responses, and the stimulation of innate immunity results in an inflammatory state.

The effector cells of adaptive immune responses (lymphocytes) are triggered if innate immune responses fail to eradicate infection. It is being increasingly understood that innate immunity and adaptive (antigen-specific) immunity cooperate as a network of interacting molecular and cellular elements to achieve a common goal.

Adaptive Immunity

Infections that overpower innate immune responses can be targeted and fiercely defended against by adaptive immunity. Since ecological, socioeconomic, and demographic changes—which modify susceptibility to both established and developing infectious microorganisms—outpace the biological systems' natural evolution, adaptive immunity is particularly crucial.

One of the biggest achievements in medical science, along with the discovery of antibiotics, is the invention of a successful vaccine. This achievement is founded on our understanding of the components and principles of adaptive immunity.

Regarding the dynamic of the underlying cellular and molecular responses, adaptive immunity differs from innate immunity: adaptive immunity is slower and dependent on intricate interactions between APCs and T and B cells. A key element is the antigen specificity of the responses that facilitates the specific targeting of a diverse range of effector elements, including cytotoxic T cells and antibodies.

Another facet is the ability of adaptive immune responses to improve during exposure to antigen and on subsequent reinfection events

Antigen-Presenting Cells

Antigen-presenting cells (APCs) interact with T cells to present antigen after detecting and capturing bacteria and their antigens. APCs may then move to lymph nodes.

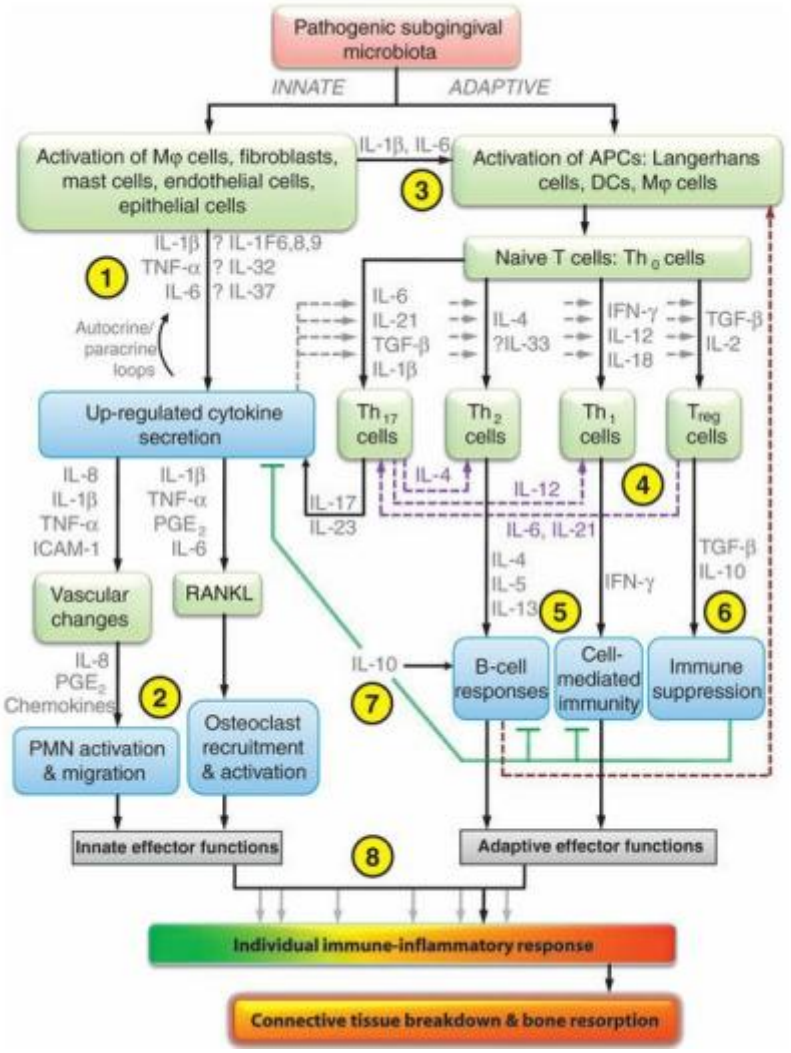
B cells, macrophages, and at least two different types of dendritic cells (dermal dendritic cells and Langerhans cells) are among the APCs seen in the periodontium. The expression of costimulatory molecules, which are essential for the interaction of APCs with T cells, rises in response to TLR activation.

T Cells

Functional T-cell subsets that leave the thymus are generally identified by the expression of certain T-cell antigen receptors (or) or cell surface molecules (CD4 or CD8). Through immunohistologic analyses of sick tissues, the part played by T lymphocytes in periodontal disease has been established.

The CD4 + T-cell effector subset that arises from naive T cells is influenced by the characteristics of APCs, which deliver Ag to cognate T-cell receptors on T cells, as well as the presence of particular combinations of cytokines and chemokines locally.

Cytokine networks in periodontal diseases. Schematic to illustrate the multiple interactions between cytokines and cellular functions in periodontal diseases



Antibodies

The result of B-cell activation is the production of certain antibodies in response to the bacterial assault in periodontal disease. Differentiated plasma cells start to show up concurrently with antibodies against bacterial antigens.

Antibodies are produced locally by plasma cells in periodontal tissues, and high levels of antibodies are seen in GCF. 8 Few IgM or IgA types of antibodies are generated in comparison to IgG antibodies against periodontal bacteria.

Numerous oral bacterial species cause a polyclonal B-cell response, which results in the development of antibodies specifically directed against those bacterial species. The creation of autoantibodies, such as those directed against collagen and connective tissue proteins, as a result of these reactions, however, may increase defenses against nonoral microorganisms and contribute to tissue deterioration in periodontal disease.

Complement System

Complement is addressed briefly in the current chapter due to its role in PRR signaling and the periodontal host immune defense. The periodontal host immune response is dependent on a functional complement system, which notably coordinates the recruitment and activation of immune cells, bacterial opsonization, phagocytosis, and lysis.

Complement–Pattern-Recognition Receptor Signaling In addition to PRR localization in plasma membranes (TLRs) and the cytoplasmic compartment (NLRs), some soluble PRR families are also secreted into the plasma as humoral proteins. Soluble PRRs include pentraxins, mannose-binding lectin (MBL), ficolins, and properdin, which represent the functional ancestors of antibodies. Soluble PRRs interact with circulating MAMPs and DAMPS to activate the complement system, ultimately resulting in the opsonization, phagocytosis, and lysis of microbes. Notably, complement interactions can amplify the host immune response through synergy with TLRs, another example of crosstalk among diverse PRR signaling pathways. Classical/Lectin/Alternative Pathways.

Microbe-Associated Molecular Patterns

Higher eukaryotes do not possess MAMPs, which are evolutionary conserved molecular motifs found in microbes. Molecular components of microbial cell walls, nucleic acids, and flagellin are examples of MAMPs. These substances act as ligands with specificity for homologous PRRs expressed by host cells.

The direct identification of MAMPs at PRRs by the host immune system allows it to distinguish between itself and the local oral flora (Table 1.1).

The immune response is triggered by MAMP recognition by the appropriate PRR, which causes host cell signaling and the production of cytokines and enzymes (Table 1.2). MAMP signaling immunomodulation leads to pathophysiologic tissue damage in chronic inflammatory disease states like periodontitis and is essential for the homeostatic control of colonized commensal bacteria in health.

The first line of defense against the colonizing oral bacteria is the periodontal innate immune response. Although the innate immune response was previously thought to be non-specific and unplanned, the discovery of PRRs made it clear that it is precise and planned. Proinflammatory cytokines, such as interleukin-1 beta [IL-1], IL-6, and tumor necrosis factor [TNF] as well as type I interferons (IFN-, IFN-), which are necessary for mounting a suitable innate immune response to colonizing or invading microorganisms, are stimulated by the recognition of MAMPs by innate immune cells (see Table 1.2).

Additionally, co-stimulatory molecule production at innate immune cells is up-regulated by MAMP signaling, which is essential for the activation of adaptive immunity.

Because of this, PRRs are regarded as the link connecting the innate and adaptive immune systems. Researchers have discovered that PRRs are expressed by epithelial cells, extracellular matrix cells (fibroblasts, cementoblasts, osteoblasts), and adaptive immune cells (T lymphocytes, B lymphocytes) in addition to innate immune cells (neutrophils, monocytes, macrophages, dendritic cells, and natural killer cells) (see Table 1.1). Although it was previously believed that innate immune cells only came from the hematopoietic lineage, MAMP-PRR recognition at tissue extracellular matrix cells and epithelial cells has shown that both hematopoietic and mesenchymal cells play a crucial role in innate immune defense mechanisms that control colonizing or invading microorganisms.

The realizations that MAMPs aredirectly recognized by adaptive immune cells and do not requireinnate immune cell processing or priming provided insightdemonstrating that the innate and adaptive immune systems actmore as a continuum than as separate entities. Notably, theidentification of PRRs has significantly advanced ourunderstanding of innate and adaptive immunity.

The TLRs and NLRs are the two main PRR families that have been investigated the most in the periodontium. A wide variety of MAMPs produced from the oral microbiota are recognized by TLRs, which are transmembrane receptors, and NLRs, which are cytosolic receptors (see Table 1.1). More recently, it has been discovered that PRRs also recognize damage-associated molecular patterns (DAMPs), which are immunostimulatory by-products formed from damaged host tissues in addition to MAMPs. The chapter focuses on MAMPs researched in the context of periodontal bacteria (see Table 1.1), despite the fact that PRR identification of MAMPs and DAMPs is crucial for the host immune response and proper tissue remodeling.

Table 1.1: Host Cell Pattern-Recognition Receptor Ligand Binding of Periodontal Bacteria-Derived Microbe-Associated Molecular Patterns

Host Cells	PRRs	MAMPs	Periodontal Bacteria
Neutrophils, monocytes, macrophages, epithelial cells, fibroblasts, cementoblasts,	TLR-2	a) Lipoproteins b) Lipoproteins c) Lipoproteins d) Peptidoglycan e) Lipoproteins, lipoteichoic acid,	a) Porphyromonasgingivalis b) Tannerella forsythia c) Actinomyces viscosus d) Actinomyces naeslundii e) Streptococcus gordonii

osteoblasts, dendritic cells, T and B lymphocytes		peptidoglycan	
	TLR-4	a) LPS b) LPS	a) Porphyromonasgingivalis b) Aggregatibacteractinomycetemcomitans, Fusobacterium nucleatum
	TLR-9	a) CpG-DNA	b) Porphyromonasgingivalis, Tannerella forsythia
	NOD1 NOD2	a) iE-DAP b) MDP	a) Porphyromonasgingivalis, Aggregatibacteractinomycetemcomitans, Fusobacterium nucleatum

E-DAP, Gamma-D-glutamyl-mesodiaminopimelic acid; LPS, lipopolysaccharide; MAMP, microbe-associated molecular pattern; NOD, nucleotide-binding oligomerization domain; PRR, pattern-recognition receptor; TLR, Toll-like receptor.

Table 1.2: Periopathogenic Bacteria Microbe-Associated Molecular Pattern Induction of Biologic Mediators in Host Periodontal Tissue Cells

Host Cells	MAMPs	Biologic Mediators
Epithelial cells	LPS, fimbriae, bacteria cell wall extracts, gingipains	G-CSF, GM-CSF, βdefensin-2, MMPs-3/9/13, MIP-1α, IL-1β
Dendritic cells	LPS, CpG-DNA, fimbriae	IFN-α, IL-6, IL-8, IL-10, IL12, TNF-α, GM-CSF
Endothelial cells	LPS	IL-6, GM-CSF, ICAM-1
Gingival fibroblasts	LPS, CpG-DNA, gingipains, peptidoglycan	IL-1β, IL-6, IL-8, TNF-α, PGE2 , MCP-1, MMP-2
PDL fibroblasts	LPS	IL-6, IL-8, MMP-13, RANKL
Cementoblasts	LPS	OPN, OCN, RANKL, IL-6
Macrophages	LPS, CpG-DNA, leukotoxin	IL-1α/1β, IL-6, IL-12, TNF-α, MMP-1, NO
Osteoblasts	LPS	IL-1β, IL-6, TNF-α, RANKL, PGE2 , NO, MMP-2, MMP-9
Neutrophils	LPS, CpG-DNA	IL-8, MIP-1α
Monocytes	LPS, CpG-DNA, fimbriae	IFN-γ, IL-1α/1β, IL-6, IL-8, IL-12, TNF-α, LIF, RANKL, PGE2
B lymphocytes	CpG-DNA, cell sonicate extracts	IL-6, IL-10, IL-12, TNF-α
T lymphocytes	LPS, CpG-DNA, peptidoglycan	IFN-γ, IL-4, IL-10, IL-13

G-CSF, Granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LIF, leukocyte inhibitory factor; LPS, lipopolysaccharide; MAMP, microbe_associated molecular pattern; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; NO, nitric oxide; OCN, osteocalcin; OPN, osteopontin; PDL, periodontal ligament; PGE2 , prostaglandin E2 ; RANKL, receptor activator of nuclear factor-κB ligand; TNF, tumor necrosis factor.

Toll-Like Receptors

Toll was initially identified as a type I trans membrane receptor gene, playing a significant role in the dorsoventral development of the Drosophila (fruit fly) embryo. Additionally, it had become clear that a toll was genetically inadequate in their absence.

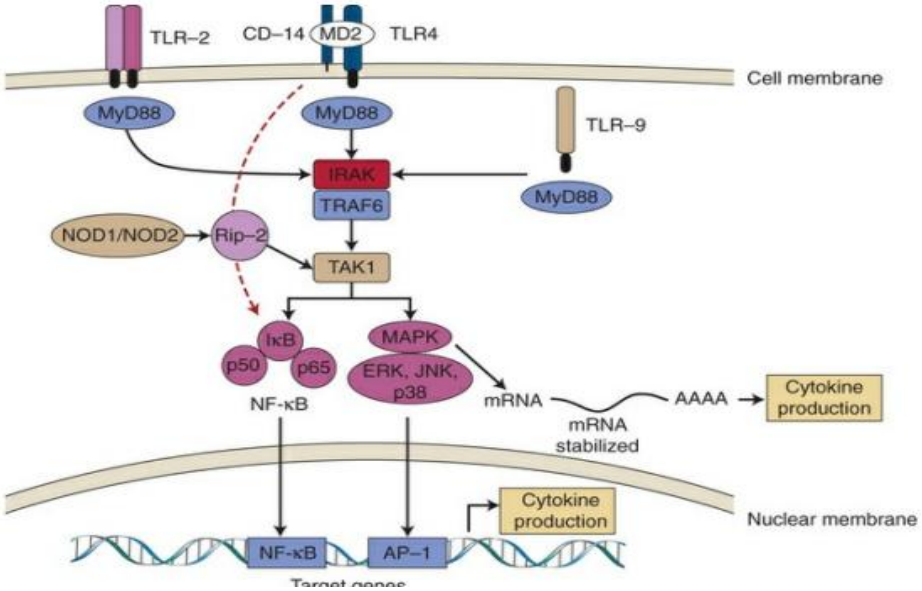
There are currently 10 functioning TLRs known to exist in humans, with TLR-10 being the only one with an unknown biologic function. The periodontium has been found to express TLR-1 through TLR-9 in both healthy and diseased conditions.

According to whether they are found on the plasma membrane (TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-10) or endolysosomal membrane (TLR-3, TLR-7, TLR-8, and TLR-9), TLR family members are typically split into two categories.

Notably, TLR-4 is special in that it can localize to the endolysosomal membrane in addition to the plasma membrane. Proinflammatory cytokines are expressed as a result of plasma membrane TLR signaling, whereas type I IFNs are

mostly expressed as a result of endosomal TLR signaling. TLRs that are localized to the plasma membrane (TLR-1, TLR-2, TLR-4, TLR-6) or flagellin (TLR-5) recognize external microbial cell wall components, whereas TLRs that are localized to the endolysosomal membrane (TLR-3, TLR-7, TLR-8, TLR-9) recognize microbial nucleic acids. Because these TLR family members have been investigated the most in the context of sensing periopathogenic microorganisms, MAMP ligand recognition at TLR-2, TLR_4, and TLR-9 (see Table 1.1).

The TLRs are single-pass transmembrane proteins (see Fig. 1.1) with an internal C-terminal Toll/IL-1 receptor signaling domain (TIR) and an N-terminal leucine-rich recognition domain. TIR domains of TLRs act as a scaffold to recruit different TIR domain-containing adaptor proteins, such as MYD88 and MYD88-adaptor-like protein (MAL) or TIR domain-containing adaptor protein inducing IFN- (TRIF) and TRIF-related adaptor molecule (TRAM), upon MAMP ligand recognition at the N-terminal domain and subsequent formation of a sustainable homodimer or heterodimer. All TLRs except TLR-3 interact with the MyD88 adaptor protein. The TRIF adaptor protein only interacts with TLR-3 and endosomal TLR-4. The signal transduction process is started when the adaptor molecules, IL-1 receptor-associated kinases (IRAKs), and TNF receptor-associated factors (TRAFs), are engaged in the TIR domain of TLRs (see Fig. 1.1).
The downstream activation of transforming growth factor-beta-activated kinase 1 (TAK_1) in the case of TLR-2 and TLR-4 localized to the plasma membrane activates both mitogen-activated protein kinase (MAPK) and nuclear factor-B (NF-B) signaling. Pro-inflammatory cytokine genes are expressed as a result of NF-B translocation to the nucleus and activation of AP-1 by MAPK cascades. TLR-4 activates NF_B and IFN-regulatory factor (IRF)-3 when it translocates to endosomes, which causes the production of type I IFN and proinflammatory cytokine genes.



The notable crosstalk of the TLRsignaling pathways (see Fig. 1.1) highlights the potential forsynergy or amplification effects modulating the host immuneresponse.

FIG. 1.1 Pattern-recognition receptor (PRR)/microbe-associated molecular pattern (MAMP) signaling. Toll-like receptor 2 (TLR-2), TLR_4, and TLR-9 are depicted as examples of TLRs expressed in cells of the periodontal tissues. After ligand binding, all TLRs (except TLR-3) recruit myeloid differentiation primary response gene 88 (MyD88) adaptor protein and activate the common upstream activator (interleukin-1 receptor-associated kinase [IRAK]/tumor necrosis factor receptor-associated factor-6 [TRAF6] and transforming growth factor-β activated kinase 1 [TAK1]) of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs). TLR-4 may also activate NF-κB independently of MyD88, with delayed kinetics (red dashed arrow). Nucleotide-binding oligomerization domain 1 (NOD1) and NOD2 are cytosolic PRRs that recognize peptidoglycan fragments of the bacterial cell wall, and they may amplify the TLR-induced activation of signaling pathways. Activated NF-κB and MAP kinases translocate to the nucleus and bind to their motifs (NF-κB and activator protein 1 [AP_1]) in the promoters of target genes (including early-response and inflammatory genes) and induce their transcription into mRNA, which will ultimately lead to increased cytokine production. p38 MAPK is also involved after the transcriptional regulation of proinflammatory genes (e.g., interleukin-6 [IL-6], cyclooxygenase-2 [COX-2]) via the modulation of mRNA stability in the cytoplasm. CD14, cluster of differentiation 14 molecule; ERK, extracellular signal-regulated kinase; I-κB, inhibitor of NF-κB; JNK, c-Jun N-terminal kinase; MD2, myeloid differentiation protein-2, RIP-2, serine/threonine kinase adapter protein.

INNATE IMMUNE RESPONSE AND TOLL LIKE RECEPTORS

The majority of infections can be successfully treated by the innate immune system, which consists of cellular and humoral components, but it is thought to be non-specific to pathogens that are entering the body.

Any loss in host defense is likely to be repaired by subsequent activation of particular acquired immunity, which is mediated by T and B lymphocytes and is intended to eradicate the organism. To distinguish between a vast number of periodontal pathogens from the host with a finite number of cell surface receptors is one of the innate immune system's main hurdles.Because microbial infections can mutate as a means of evading host detection, this problem is made more difficult. The innate immune system has overcome this obstacle by recognizing pattern recognition receptors (PRRs), which are evolutionary conserved structures on pathogens that are absent in higher eukaryotes.

These molecular patterns, sometimes referred to as pathogen-associated molecular patterns (PAMPs), are not vulnerable to significant mutation rates because they play crucial roles in the pathogen's capacity to avoid host defense. Pathogens share PAMPs, but the host does not express them. Although many PRRs have been known for years, it was not clear how the innate immune system functioned until the discovery of the toll-like receptors (TLRs), which have proved to be critical for recognition of microbes by the innate immune system and for bridging the innate and acquired immune responses. Indeed, 10 different mammalian TLR shave been identified in humans to date. Despite the alleged nonspecificity of the innate immune response, it has long been known that cytokine release on stimulation with gram-positive or gram-negative bacteria showed important quantitative and qualitative differences.

There are a number of significant PAMPs for which the specificity of TLR recognition has been discovered, including peptidoglycan (PGN), bacterial lipoproteins, atypical LPS, and zymosan for TLR-2; double-stranded RNA for TLR-3; LPS and heat-shock proteins (HSPs) for TLR-4; flagellin for TLR-5; and CpG motifs for bacterial DNA for TLR-9.73 Periopathogenic PAMPs are included in Table A along with the TLR used for each specific PAMP. Due to their ability to detect gram-positive and gram-negative bacterial PAMPs, respectively, TLR-2 and TLR-4 have been the subject of the majority of investigations. TLRs all share an external leucine-rich domain and an intracellular domain that is conserved.

The intra-cellular tail of the receptor was shown to be homologous with the intracellular domain of the interleukin-1 receptor (IL-1R) type I, currently being designated as the toll/IL-1R (TIR) domain. The TLR-PAMP interaction results in the recruitment of specific adapter molecules such as MyD88 and Mal, which then bind the IL-1R-associated kinase (IRAK).

The signal is thereafter transmitted through a chain of signaling molecules, which is apparently common to all TLRs, involving tumor necrosis factor (TNF) receptor-associated factor-6 (TRAF6) and mitogen-activated protein kinases (MAPKs). Thereafter, activation of nuclear factor kappa B (NF-kB) and activated protein-1 (AP-1) leads to transcription of genes involved in the activation of the innate host defense, notably proinflammatory cytokines2.

TOLLLIKE RECEPTOR EXPRESSION AND MICROBIAL RECOGNITION IN PERIODONTAL TISSUES

Human gingival fibroblasts and human periodontal ligament fibroblasts are representative elements of periodontal tissues and are stimulated by oral bacterial LPS fractions from P. gingivalis, Prevotella intermedia, or Actinobacillus actin mycetemcomitans to produce a variety of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and IL-8. The membrane protein CD14 (cluster of differentiation, CD), which is crucial for pattern recognition of common bacterial cell surface components like LPS and PGN, is typically absent from fibroblasts. In the literature, there seems to be disagreement over CD 14 expression in gingival fibroblasts.

In contrast, others reported that human gingival fibroblasts expressed membrane CD14 and responded to P. gingivalis LPS in a membrane CD14-dependent manner. Part of this disparity in the literature may be caused by heterogeneity of membrane CD14 expression in human gingival fibroblasts, as indicated in studies where CD 14 expression is directly correlated with chemokine production.

This variance, ranging from 5% to 22.9% of CD14+ cells, has also recently been found in PDL fibroblasts.60 Gingival fibroblasts 156 and periodontal ligament (PDL) fibroblasts both constitutively express TLR-2 and TLR-4 in addition to membrane-bound CD14.

The hypothesis that gingival fibroblasts and PDL cells differ significantly was supported by the latter's higher expression of TLR-2 and lower expression of TLR-4. Recent research has revealed that in addition to CD-14, cementoblasts also express TLR-2 and TLR-4.

It has been suggested that TLR-4 mediates the response to LPS, whereas TLR-2 is involved in the response to other bacterial cell wall components and antigens, such as PGN and lipoprotein.

Gingival fibroblasts and PDL cells are equipped to respond to LPS stimulation. Nevertheless, the signaling pathways involved in LPS stimulation are only partially understood, especially in these cell types. Despite the similarity of the intracellular domain of TLRs and IL-1R, which is supported by activation of some common "upstream" kinases (e.g., IRAK-IL-1R-associated kinase, TRAF6-TNF receptor-associated kinase) as well as activation of a common "downstream" transcription factor (NF-κB), there isa great complexity in terms of the adapter proteins involved in signaling by TLRs.

The pathways implicated entail the activation of multiple MAPK cascades, including ERK-1 and ERK-2, JNK, and p38, as well as the recruitment of other adaptor proteins. In general, growth factors and hormones activate ERKs, whereas environmental stress and inflammatory cytokines activate JNKs and p38 MAPKs.

One of the primary bifurcation sites in LPS signaling through TLRs is close to the toll/IL-1R's cytoplasmic domain, where, during receptor dimerization, an adaptor protein named MyD88 is recruited.

The MyD88-dependent pathway leads to subsequent activation of IRAK, TRAF6, and ultimately NF-κB, and it is essential for cytokine induction. On the other hand, the MyD88-independent pathway does not activate IRAK and leads to activation of NF-κB with delayed kinetics. This pathway requires different adapter proteins, such as TIRAP, TRIF, and TRAM, and probably does not lead to cytokine induction.

It may result in the activation of NF-B via the inhibitor of nuclear factor kappa B (IKK) pathway or AP-1 via MAPK pathways.

The significance of multiple pathways being activated by a single receptor is unclear; it may be connected to compensatory mechanisms or to the specificity of cell response via the modulation of biologic cell response by pro- or anti-inflammatory cytokine production.

Toll-like Receptor-4–Lipopolysaccharide Recognition
The external membrane macromolecules of bacterial cell walls, which are exposed during oral bacterial contacts with host TLRs, play a significant role in these interactions. Lipoteichoic acid (LTA) and peptidoglycan are specific to the outer membrane of gram-positive bacteria, whereas lipopolysaccharide (LPS) is only present in the outer membrane of gram-negative bacteria.

Importantly, lipoproteins are frequently found in both gram-positive and gram-negative bacteria's outer membranes. A TLR-4 homodimer protein complex, which includes TLR-4, the coreceptor myeloid differentiation factor 2 (MD2), auxiliary proteins CD14, and lipopolysaccharide-binding protein (LBP), is used by mammalian cells to identify LPS (see Fig. 1.1). LBP converts LPS into CD14 and distributes it to it, making cells more receptive to LPS binding by the MD2-TLR-4 receptor.

Toll-likeReceptor-2–Lipoprotein/Lipoteichoic Acid/Peptidoglycan Recognition
TLR-2 has the ability to identify a variety of microbial macromolecules because it forms heterodimer protein complexes with other TLR family members (TLR-1, TLR-6), in contrast to the TLR-4 homodimer protein complex that is specific for LPS.

Lipoproteins, LTA, and peptidoglycan are TLR-2 ligands that are very important for oral flora interactions with host cells (see Table 1.1).

TLR-2/TLR-1 heterodimer complexes recognize triacylated lipoproteins, which are frequently expressed by gram-negative bacteria, whereas TLR-2/TR-6 heterodimer complexes recognize diacylated lipoproteins, which are typically expressed by gram-positive bacteria or mycoplasmas.

LTA and peptidoglycan, which are specific to the outer membranes of gram-positive bacteria, are identified by poorly known TLR-2/TLR-6 heterodimer complexes.

Toll-like Receptor-9–CpG-DNA Recognition
TLR-9 identifies MAMPs in endosomes, in contrast to TLR-2 and TLR-4 located to the plasma membrane, which do so at the cell surface (see Fig. 1.1).

The endosomal TLR family member TLR-9 has been investigated the most in terms of identifying intracellular microbial nucleic acids. Endosomal TLRs detect microbe-derived nucleic acids during infection, and this detection aids in developing a host immune response to eradicate the invading microbes.

Although TLR-9 may detect both bacterial and viral CpG-DNA, periodontal research has concentrated on TLR-9 because bacterial DNA contains many CpG motifs (see Table 1.1).

Nucleotide-Binding Oligomerization Domain–Like Receptors
There are now 22 family members of human NLRs that are expressed intracellularly. NLRs are found in the cytosol and are essential for detecting invasive pathogens and activating the immune system.

Both the specialist NLRs NOD1 and NOD2 and NLRP3, which is an illustration of how NLRs work as a component of inflammasome complexes, are capable of detecting bacterial peptidoglycan structures of encroaching pathogens in the cytoplasm.

NOD1/NOD2–Peptidoglycan Recognition
While NOD2 identifies muramyl dipeptide (MDP), which is present in peptidoglycan from all gram-negative and gram-positive bacteria, NOD1 recognizes gamma-D-glutamyl-mesodiaminopimelic acid (iE-DAP), a component of peptidoglycan present in most gram negative and some gram positive bacteria.

A serine/threonine kinase adapter protein called RIP-2/RICK is recruited to a caspase activation and recruitment domain (CARD) at the N-terminus as a result of peptididoglycan binding at NOD1 and NOD2 receptors, which induces their oligomerization.

Proinflammatory cytokine genes are activated by NF-B and MAPK-dependent up-regulation when RIP2/RICK recruitment occurs at the N-terminus (see Fig. 1.1).

NLRP3–Inflammasome Complex
An inflammatory form of cell death known as pyroptosis is regulated by inflammasomes, multiprotein complexes that identify a variety of inflammation-inducing stimuli, including foreign MAMPs and endogenous DAMPs.

The cytosolic NLRs and other PRR families participate in the formation of the inflammasome complex. The name "inflammasome" refers to the NLR proteins, which serve as the "core" of the multiprotein inflammasome complex. The proinflammatory cytokines IL-1 and IL-18 are processed and secreted in their terminal stages by the inflammasome complex known as NLRP3, which is the subject of this chapter and the most thoroughly studied inflammasome complex. Recognition of cytosolic MAMPs and DAMPs induces NLRP3 to acts as a recruiting scaffold for the inactive zymogen pro-caspase-1.

Pro-caspase-1 (which has a CARD) is recruited to the inflammasome complex through homotypic binding of CARD via a pyrin domain (PYD) and the adaptor apoptosis-associated speck like protein containing a CARD (ASC).

Pattern-Recognition Receptor(PRR)–Microbe Associated Molecular Pattern (MAMP) Ligand Recognitionin Periodontitis

PRRs	Localization	MAMP Ligand	Ligand Origin
TLR-2/TLR-1	Plasma membrane	Triacylated lipoproteins	G− bacteria
TLR-2/TLR-6	Plasma membrane	Diacylated lipoproteins	G+ bacteria
		Lipoteichoic acid (LTA)	G+ bacteria
		Peptidoglycan	G+ bacteria
TLR-4	Plasma membrane	Lipopolysaccharide (LPS)	G− bacteria
	Endolysosome		
TLR-9	Endolysosome	CpG-DNA	Bacterial and viral
NOD1	Cytoplasm	Gamma-D-glutamyl-mesodiaminopimelic acid (iE-DAP)	G+ bacteria
			G− bacteria
NOD2	Cytoplasm	Muramyl dipeptide (MDP)	G+bacteria
			G− bacteria

G+, Gram positive; *G−*, gram negative; *MAMP*, microbe-associated molecular pattern; *NOD*, nucleotide-binding oligomerization domain; *PRR*, pattern-recognition receptor; *TLR*, Toll-like receptor.

Role of NOD-like Receptors in Periodontitis
Strangely, there are no findings that indicate NOD1 or NOD2 expression levels in the human periodontium are altered by periodontal disease conditions. The crucial function of NOD1 and NOD2 in periodontal pathogenesis has received little attention in experimental studies of periodontitis in NLR-deficient mice.

Even though study procedures varied between investigations, it's interesting to note that there is no uniformity in the claimed study results.

Initial experimental periodontitis research using NOD1 and NOD2 knockout mouse models, which induced periodontitis by using ligatures, found that while mice lacking NOD1 showed reduced levels of alveolar bone loss when compared to wild-type control mice, mice lacking NOD2 showed comparable levels of alveolar bone resorption. The results of a second experimental periodontitis study in the NOD1 deletion mouse model, which produced periodontitis by injecting heat-killed gram-negative or gram-positive bacteria into the gingival tissue, were conflicting.

Increased osteoclast counts, worsened alveolar bone loss, and elevated pro-inflammatory cytokine production in cultured bone marrow macrophages were all seen in NOD1 mutant animals.

It is unclear whether NOD1 or NOD2 are crucial regulators of periodontal bone loss in light of the aforementioned conflicting study findings regarding NOD1 and an additional experimental periodontitis report showing that P. gingivalis inoculation caused less alveolar bone loss in NOD2 knockout mice compared to wild-type mice.

MAMPs can simultaneously activate TLRs and NOD1/2 signaling, which converge at the MAPK and NF-B signaling pathways, when they are present in the biofilm.

A colonizing oral biofilm may trigger downstream synergistic signaling effects that strengthen the host immune response. Early proof that periopathogenic biofilms may cause a destructive host immune response through concurrent activation of various PRRs is provided by a seminal study 167 showing that NOD1 and NOD2 activation has synergistic effects with TLR signaling to enhance the production of proinflammatory cytokines in cultured human periodontal ligament fibroblast (IL-1, IL-6, IL-8).

Periodontitis research has mostly concentrated on NLRP3 when it comes to NLR family inflammasome complexes. When compared to gingival biopsy samples from periodontally healthy regions, it was discovered that the expression of NLRP3 and its endogenous antagonist NLRP2 was higher in human gingival tissues affected by different types of periodontal disease.

According to earlier periodontitis gene expression research examining IL-1 and IL18 levels, gingival tissues impacted by periodontal disease states had higher levels of IL-1 and IL18 mRNA expression. The realization that NLRP3 levels were positively correlated with the IL-1β and IL-18 expression levels in periodontal disease–affected versus healthy gingival biopsy samples provides indirect evidence implying a role for the NLRP3 inflammasome in the pathogenesis of periodontal diseases states.

Additional evidence supporting the involvement of the NLRP3 inflammasome in the catabolic effects of periodontitis comes from in vitro studies indicating that bacteria from the dental biofilm altered the expression of the inflammasome, which was connected with the production of IL-1 and IL-18.

ROLE OF PATHOGEN ASSOCIATED MOLECULAR PATTERNS IN PERIODONTAL DISEASE

Because different PAMPs can stimulate a wide range of cell types, including resident cells that are typically present in the periodontal tissues in the absence of disease and nonresident or inflammatory cells drawn to the periodontal tissues as a result of the pathogenic process, host-pathogen interactions in destructive periodontal diseases are extremely complex.

Some PAMPs, on the other hand, have the opposite effect, decreasing the host response, which is thought to be a mechanism by which some microbial species increase their infectiousness by sidestepping the host immune system. For instance, P. gingivalis proteinases break down tumor necrosis factor alpha (TNF-), which can be produced by many PAMPs in various cells.

PAMP Effects on

- ✓ Resident Cells
- ✓ Nonresident Cells

PAMPEffects on Resident Cells

As the disease develops, cells typically found in periodontal tissues are the first to be exposed to PAMPs. These cells play crucial roles in both the formation and modification of the host response because they continue to be exposed to and stimulated by these substances as the disease worsens.

Leukocyte attachment and migration might be directed toward the antigen on the pocket lumen due to the interaction between these molecules. In response to LPS from different pathogens, these cells also produce interleukin-8, a

neutrophil chemoattractant and activator, whereas P. gingivalis LPS down-regulates both ICAM-1 and IL-8 production. After being stimulated by PAMPs, these cells create MMPs in addition to stimulating other host cells such endothelial cells, macrophages, dendritic cells, and neutrophils, which suggests a direct route of tissue destruction.

1. Dendritic cells

As soon as potential periodontal pathogens begin colonizing the subgingival region, these cells are also exposed to PAMPs. TLR-9 expression enables DCs to identify antigens, including PAMPs, and kick-starts the maturation process. In addition to being able to present antigens in an MHC class II-peptide complex, mature DCs can also create cytokines and costimulatory molecules (CD40, CD54, CD80, and CD86) that cause T cell activation and either Th1-type or Th2-type immunological responses.When invaded by fimbriated P. gingivalis and co-cultured with T lymphocytes, 83 DCs serve as a crucial link in the production and modification of the immune response to PAMPs; these cells incite a Th1 type of response.

The macrophages are highly efficient resident antigen-presenting cells (APCs) derived from peripheral blood monocytes and can produce various cytokines as well as other biologic mediators (e.g., MMP1, nitric oxide) when stimulated by CpG and LPS from different periodontal pathogens.

2. Fibroblast

The morphologies of gingival and PDL fibroblasts are different, and the alkaline phosphatase activity of the PDL fibroblast population is comparable to that of osteoblasts. In response to numerous PAMPs, such as LPS, PGN, and CpG DNA from various periodontal infections, gingival fibroblasts can release a range of proinflammatory cytokines and also express adhesion molecules.

Different PAMPs, however, can have conflicting effects, which explains how some bacteria manage to elude host immune responses.

However, PDL cells respond to PGN stimulation by producing more IL-8 than gingival fibroblasts, which may be associated to the higher levels of TLR-2 expression. Additionally, these cells have the capacity to release proteinases, which can directly degrade both soft and calcified tissues. Similar to gingival fibroblasts, cementoblasts triggered by LPS show increased production of OPG and osteopontin (OPN), as well as lower levels of the receptor activator of NF-B ligand (RANKL), which is also found in the periodontal ligament (PDL) region. Additionally, one study found that these effects were mediated by TLR-4 and potentially TLR-2, suggesting that they may be a defense mechanism against bone and root resorption.

Biologic Mediators Elicited by PAMPs in Resident and Nonresident Cells Involved in Pathogenesis of Destructive Periodontal Disease:

- ✓ Epithelial cells
- ✓ LPS,
- ✓ fimbriae,
- ✓ glycoprotein,
- ✓ whole bacteria,
- ✓ cell wall extracts
- ✓ IL-8, G-CSF, GM-CSF, β-defensin-2, MMP-3, MMP-9
- ✓ Fimbriae, LPS, CpG DNA, DNA
- ✓ IFN-α, IL-6, IL-8, IL-10, IL-12, TNF-α, GM-CSF
- ✓ Endothelial cells LPS, heat-shock proteins
- ✓ IL-6, GM-CSF, ICAM-1
- ✓ LPS, peptidoglycan, CpG DNA
- ✓ IL1β, IL-6, IL-8, TNF-α, PGE2, MCP-1
- ✓ LPS
- ✓ IL-6, IL-8, MMP-13
- ✓ LPS
- ✓ OPN, OCN, RANKL
- ✓ LPS, CpG DNA etc.

3. Endothelial cells

It is possible for LPS to directly stimulate endothelial cells lining blood arteries as well as other host cells that release IL-8 through TLR-4 and the p38 MAPK pathway, activating and increasing monocyte adherence. The enhanced expression of the adhesion molecules E-selectin, ICAM-1, and VCAM-1 caused by PAMPs and the activation of cytokine production are both factors in this impact. With the expression of both MHC class I and MHC class II, these cells can also serve as APCs.

4. Osteoblasts

Osteoblasts are also sensitive to PAMPs; in particular, LPS can induce production of multiple proinflammatory cytokines and biologic mediators involved in bone resorption, as well as inhibit expression of the bone-protecting factor OPG.

Interestingly, if these osteoclast precursor cells are primed with RANKL, LPS synergistically increases differentiation, and this effect is influenced by autocrine stimulation with LPS-induced TNF-α and prostaglandin E2 (PGE2)

PAMP's Effects on Nonresident Cells

Neutrophils

Neutrophils respond to cytokines secreted by activated host cells, such as IL-8 released by epithelial cells, and to the adhesion molecules expressed by stimulated endothelial cells.

PAMPs may also directly stimulate these cells, inducing chemotaxis, shedding of the adhesion molecule L selectin, and cytokine production, effects mediated by TLR-2 expression.

Monocytes

Inflammatory cytokines are produced by monocytes induced by PAMPs, and their proliferation and adherence to endothelial cells are also increased. Importantly, even in the absence of osteoblasts, LPS stimulates the differentiation of monocytes into osteoclasts, and the upregulation of RANK L expression is likely to be a key factor in this process. P. gingivalis LPS significantly boosts monocyte and T cell production of IL-12 and IFN- in the presence of IL-12, indicating a positive-feedback mechanism increasing the Th1 immune response.

In contrast, P. gingivalis LPS does not cause the development of proinflammatory cytokines on monocytes in the absence of co-stimulatory factors; instead, it causes the expression of antiinflammatory IL-10, which can reduce the levels of IL-12 and cause the immune response to change to a Th2 pattern.

B lymphocytes

B lymphocytes are also directly stimulated by PAMPs, specifically CpG DNA, because they lack expression of TLR-4 while also expressing TLR-9. This leads to proliferation, antibody production in plasmocytes, expression of co-stimulatory factors (e.g., MHC class II, CD80, CD86), and production of inflammatory cytokines, including IL-12, in the presence of co-stimulatory factors.

T lymphocytes

T lymphocytes usually are activated through interaction with other cells and their biologic mediators, resulting in differentiation of either a Th1 or a Th2 type of immune response.

However, LPS can also directly induce proliferation and secretion of cytokines, but this effect depends on the microorganism species and probably is also influenced by the strain within the same species.

LPS from E. coli was demonstrated to induce both CD4+ and CD8+ T cells to produce IFN-γ, whereas P. gingivalis LPS resulted in higher levels of Th2 cytokines. These effects of LPS on T cells are thought to be dependent on the presence of viable monocytes and soluble co-stimulatory factors. Interestingly, no difference was observed for the proliferative effects of LPS between naive and memory CD4+ T lymphocytes.

PATHOBIOLOGY OF LIPOPOLYSACCHARIDE MEDIATED BONE DESTRUCTION

A local host response that includes the activation of osteoclasts, the recruitment of inflammatory cells, the production of prostanoids and cytokines, and the creation of lytic enzymes is launched in gingival tissues as a result of LPS-induced illness.

Particularly, LPS enhances the production of RANKL, IL-1, PGE2, and TNF in osteoblasts, each of which is known to stimulate osteoclast activity, viability, and differentiation.

IMMUNOMODULATORY THERAPIES

Targeting the host response to LPS-mediated tissue damage has resulted in the development of numerous treatment plans. Scaling and root planing or surgical therapy have both been used in conjunction with matrix metalloproteinase (MMP) inhibitors, such as low-dose doxycycline formulations.

The systemic delivery of MMP inhibitors has also been advantageous for high-risk patient populations, such as diabetic individuals and patients with persistent periodontal disease.

Using soluble TNF- and IL-1 antagonists that were locally administered to periodontal tissues in nonhuman primates, encouraging outcomes were seen. Inhibiting the signal transduction pathways connected to inflammation is one of the

other therapeutic approaches under investigation. Pharmacologic inhibitors of NF-κB and p38 MAPK pathways are actively being developed to manage rheumatoid arthritis and inflammatory bone diseases, and they have been applied in periodontal disease models with noteworthy accomplishments.

With the application of this novel approach, proinflammatory cytokines (like IL-1, TNF, and IL-6), MMPs, and other inflammatory mediators would be inhibited at the level of the cell-signaling pathways necessary for the activation of transcription factors necessary for inflammatory gene expression or mRNA stability.

In tiny animal models of periodontal disease progression, it is true that targeting RNA-binding proteins that mediate the effects of inflammatory cytokines has therapeutic efficacy.

These therapies might be the chemotherapeutic adjuvants of the future for the management of chronic periodontitis. There are emerging therapeutic strategies that concentrate on either CR3 or CR5 in the complement system (see Fig. 1.2). Blockade at this level is a feasible strategy for treating complement-associated illnesses, including periodontitis, as C3 is a key component of all three activation pathways. It has been demonstrated that inhibiting CR3 via topical application prevents P. gingivalis-induced alveolar bone loss.

The promise of this strategy has been demonstrated by novel defensin analogues, which exhibit even greater antibacterial activity than the natural -defensins 1 and 3 do without having any harmful effects on host cells.

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