Immunological Insights into Periodontal Disease Pathogenesis - A Review

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***Abstract*- Periodontal diseases are chronic inflammatory diseases of multifactorial etiology, common in all human populations, which may result in the gradual destruction of the supporting tissue of the teeth and ultimately tooth loss. Accumulation of plaque has been accepted as the primary initiator of periodontal disease, but it is not solely responsible for the destruction that may follow. The destruction seen in periodontal disease is a consequence of the interaction between the host and microorganisms present in plaque which results in the activation of the host's inflammatory and immune responses, leading to loss of collagen and bone supporting the tooth. In recent years, research has elucidated the contribution of host-microbe interaction to both disease initiation and disease progression. Epidemiological and clinical studies indicate variation in susceptibility to periodontal disease despite the long-term presence of the oral biofilm, in addition to increased susceptibility and greater severity of periodontal disease in patients with an impaired immune response. However, the immune response initiated by periodontal disease seems to be much broader. This review attempts to enlighten the various immune mechanisms involved in periodontal disease initiation and progression.**

Keywords: **Periodontitis, Immunology, Interleukins, Pathogens.**

Introduction:

Periodontal diseases are chronic inflammatory diseases of multifactorial etiology[1], common in all human populations, which may result in the gradual destruction of the supporting tissues of the teeth and ultimately tooth loss. The prevalence of periodontal disease as reported by the Adult Dental Health Survey (2008) is 45%, of which 9% suffer from the severe form of the disease[2].

The survey reported a marked reduction in moderate chronic periodontitis when compared to the 1998 survey, which was attributed to an improvement in oral hygiene practices and promotion of oral health. However, it is interesting to note that the prevalence of severe disease has increased from 6% to 9% which may be attributed to current diagnostic and management techniques which are limited in their ability to identify high-risk individuals, assess true periodontal status, and monitor response to therapy.

Accumulation of plaque has been accepted as the primary initiator of periodontal disease[3], but it is not solely responsible for the destruction that may follow. The destruction seen in periodontal disease is a consequence of the interaction between the host and microorganisms present in plaque which results in the activation of the host's inflammatory and immune responses, leading to the loss of collagen and bone supporting the tooth[4]. Although the role of bacteria is undisputed in the initiation of periodontitis, the quantity and types of bacteria have not been sufficient to explain the significant differences in disease severity between individuals[1].

The wide variations in susceptibility to periodontal disease seem to be associated with several secondary genetic, environmental, and behavioral risk factors[5]. All forms of the disease, however, have a common series of underlying events leading to tissue breakdown and loss of attachment. Although multiple factors have been cited as influencing the progression of periodontal disease, there is overwhelming evidence that it is the uncontrolled inflammatory and immune responses that largely drive tissue destruction and therefore the important role played by the host inflammatory and immune response has been the focus of much research in the last several years.

In recent years, research has elucidated the contribution of host-microbe interaction to both disease initiation and disease progression. Epidemiological and clinical studies indicate variation in susceptibility to periodontal disease despite the long-term presence of the oral bio-film[6], in addition to increased susceptibility and greater severity of periodontal disease in patients with an impaired immune response[7]. These variations may be attributed to an altered host response which is regulated by inflammatory mediators such as cytokines, which play a prominent role in amplifying the immune response resulting in degradation of the periodontal tissue and subsequent collection of host and bacterial products in the GCF.

Therefore, extensive research has been carried out in the last few years to identify biomarkers that have the potential to diagnose accurately disease activity, prognosis, and response to therapy[8,9]. However, our limited understanding of the

pathogenesis of periodontitis limits the implementation of markers as adjunctive diagnostic and prognostic tools in the management of patients with periodontal disease.

Innate Immunity in periodontal diseases:

The epithelial tissues play a key role in innate response because they are in constant contact with bacterial products. It is now recognized that epithelial cells also constitutively express a diverse range of antimicrobial peptides and their synthesis is upregulated in response to periodontal bacteria. These peptides belong to four families (α-defensins, β-defensins, cathelicidins, saposins) that have been found in humans[1] .

Innate recognition of bacteria and their products by the host involves a sophisticated array of receptors providing specificity to pathogen detection. Through these receptors, cells can directly respond to conserved pathogen-associated microbial patterns (PAMPs) and host danger-associated molecular patterns (DAMPs). These molecular motifs are recognized by pattern recognition receptors (PRRs) on immune cell surfaces[10]. PAMPs associated with periodontal disease are bacterial lipoproteins, lipopolysaccharides, peptidoglycan, fimbriae, flagellin, heat shock proteins, and DNA. The toll-like receptor (TLR) family is the best-characterized class of PRRs and detects multiple PAMPs.

In the context of periodontal disease, TLR-2 and TLR-4 play important roles in bacterial antigen sensing[11]. TLR signaling occurs in a manner that is dependent on the adaptor molecule myeloid differentiation primary response gene (MyD88) or occurs independently via TIR-domain-containing adapter inducing Interferon-β (TRIF). All TLRs except for TLR-3 signal via MyD88; however, TLR-4 engages both MyD88 and TRIF signaling pathways[12]. Neutrophils are the first innate immune cells to migrate to the site of infection. Neutrophils utilize relevant Toll-like receptors to recognize and respond to different types of microbial challenges. Like neutrophils, macrophages/ monocytes also play a key role in host defense by recognizing, engulfing, and killing microorganisms.

Adaptive Immunity in periodontal diseases:

The adaptive immunity is activated when there is a breach in the epithelial barrier, with its antimicrobial peptides and other components of innate systems. The immune response in periodontal disease is governed by the net effect of T-helper 1 (Th1) and T-helper 2 (Th2) cytokines[13,14]. The differentiation of Th1 and Th2 T cell subsets is determined by antigen, nature of the antigen-presenting cell, and co-stimulatory molecules[15].IL-18, as a cofactor with IL-12, can enhance the maturation of naive T cells to Th1 cells. Th1 cytokines include interleukin-2 and Interferon-γ and promote cell-mediated immunity, while the Th2 cytokine, interleukin-4, suppresses cell-mediated responses and enhances humoral immunity[16].

However, there are controversial data about the Th1/ Th2 immune response in periodontal disease. Studies over the past decade or so have supported the hypothesis that Th1 cells are associated with stable lesions and Th2 cells are associated with disease progression[17]. However, other studies have reported a predominance of Th1-type cells or reduced Th2 responses in diseased tissues[18]. Recently, a new subset of T-helper cells, Th17 cells, characterized by the production of interleukin-17, has been described. This subset may have both destructive and protective effects in periodontal diseases[19].

On the other hand, the integrity of bone tissues depends on the interdependency between the osteoclasts and osteoblasts. The major regulatory mechanism of osteoclast activity is modulated by three novel members of the TNF family of receptors, RANK (receptor activator of nuclear factor-β), osteoprotegerin (OPG), and the RANK ligand (RANKL)[20]. RANK is expressed on osteoclasts and its precursors, while RANKL is expressed particularly on osteoblasts under homeostatic conditions. Interactions between RANK and RANKL are required for the differentiation and activation of osteoclast precursor cells to osteoclasts. OPG, a soluble decoy receptor produced by osteoblasts, marrow stromal cells, and other cells, strongly inhibits bone resorption by preventing RANK-RANKL interaction.

RANKL also induces the production of some substances, such as MCP-1/CCL2, which could contribute to bone resorption[21]. Osteoblasts are found to express chemokine receptors during synthesis, which can modulate their function through the binding of chemokines. Additionally, an osteoclast can produce important chemokines that are involved in the recruitment of neutrophils and different lymphocyte subsets, suggesting a role for osteoblasts in the development of the inflammatory immune reaction[22]. Furthermore, the production of chemokines, with the consequent chemoattraction of inflammatory cells, may contribute to the disruption of bone homeostasis, resulting in bone resorption.

Complement System

In cases of periodontal disease, the host defense also is dependent on a functional complement system, which coordinates the recruitment and activation of inflammatory cells, bacterial opsonization, phagocytosis, and lysis[23]. In addition, complement can amplify the TLR4-mediated inflammatory response toward bacterial LPS challenge. The complement system is critical to the linking of innate and adaptive immunity by regulating the activation of both B cells and T cells, either directly or through effects on antigen-presenting cells. The activation of the complement cascade involves the sequential activation and proteolytic cleavage of a series of serum proteins via three distinct mechanisms,

namely the classical, lectin, and alternative[24]. Classical pathway activation occurs in response to antigen-antibody complexes that are recognized by the Clq subunit of C1.

However, the lectin pathway is triggered through the interaction of a secreted pattern-recognition receptor (mannose-binding lectin) with specific carbohydrate groups on the surface of a variety of microorganisms. Both the classical and the lectin pathways then proceed through C4 and C2 cleavage for the generation of the classical lectin C3 convertase (C4bC2b). The alternative pathway also serves as a positive feedback loop for the classical and lectin pathways.All three pathways converge at the third component of complement (C3), which upon activation by pathway-specific C3 convertases leads to the generation of key effector molecules. These include the C3a and CSa anaphylatoxins, which activate specific G-protein-coupled receptors and which mediate the mobilization and activation of leukocytes. Also important are the C3b opsonins, which promote phagocytosis through complement receptors, and the C5b-9 membrane attack complex, which can lyse targeted pathogens.

The complement systems have also been shown to provide a barrier against the spread of bacterial infections; to facilitate clotting mechanisms, to mobilize hematopoietic stem cells and progenitor cells from the bone marrow, to help replenish new leukocytes and to activate the differentiation of specific T-cell subsets. The dysregulation of complement activities may lead to a failure to protect the host against pathogens and amplify inflammatory tissue damage. In the context of periodontal inflammation, complement subversion appears to play a major role in periodontal pathogenesis[25].

Inflammatory responses in the Periodontium:

The molecules that play a role in the pathogenesis of periodontitis can be broadly divided into two main groups: those derived from the subgingival microbiota (i.e; microbial virulence factors) and those derived from the host immune inflammatory response.

1. Microbial virulence factors:

The subgingival biofilm initiates and perpetuates inflammatory responses in the gingival and periodontal tissues. The subgingival bacteria also contribute directly to tissue damage by the release of noxious substances. Their primary importance in periodontal pathogenesis is by activating immune-inflammatory responses that, in turn, result in tissue damage.

1a. Lipopolysaccharides:

Lipopolysaccharide (LPSs) are large molecules composed of a lipid component and a polysaccharide component. They are found in the outer membrane of gram-negative bacteria; they act as endotoxins. LPS is of key importance for initiating and sustaining inflammatory responses in the gingival and periodontal tissues[26].

1b. Bacterial enzymes and Noxious products:

Noxious agents such as ammonia and hydrogen sulfide as well as short chain carboxylic acids such as butyric acid and propionic acid are detectable in GCF and are found in increasing concentration as the severity of periodontal disease increases. Plaque bacteria produce proteases, which are capable of breaking down structural proteins of periodontium such as collagen, elastin, and fibronectin[27].

1c. Microbial invasion and Fimbriae:

Periodontal pathogens such as P. gingivalis and Aggregatibacter actinomycetemcomitans have been reported to invade the gingival tissues including the connective tissues[28]. P. gingivalis fimbriae stimulate immune responses such as IL-6 secretion, thereby modifying and stimulating immune -responses in the periodontium[29].

2. Host- derived Inflammatory mediators:

The inflammatory and immune processes that develop in the periodontal tissues in response to the long-term presence of the subgingival biofilm are protective by intent but can result in considerable tissue damage, thereby leading to the clinical signs and symptoms of periodontal disease[30].

Properties of Key types of Inflammatory mediators in Periodontitis:

Cytokines	Proteins that transmit signals from one -cell to another. Bind to cell surface receptors to trigger production of protein by the cell. There are proinflammatory and anti-inflammatory cytokines. A key proinflammatory cytokine is IL-1β, which upregulates inflammatory responses and is produced by multiple cell types in the periodontium.
Prostaglandins	Prostaglandin E_2 (PGE_2) is a key inflammatory mediator, stimulating production of other inflammatory mediators and cytokine production. PGE_2 also stimulate bone resorption and plays a key role in periodontitis progression.

Matrix Metalloproteinases (MMPs)	MMPs include collagenases, which breakdown collagen. Key MMPs in periodontitis include MMP -8 and MMP -9, which are produced by neutrophils as they migrate through periodontal tissues, thus contributing to periodontal tissue breakdown.

Chair-side diagnostic kits to detect Inflammatory mediators:

The heterogeneity of periodontal disease onset, patterning, activity, and treatment response has hindered the accurate and reliable diagnosis of periodontal tissue infection. Efforts to develop a chairside biomarker assay kit for rapid periodontal disease diagnosis are continuing worldwide, examples of biomarker assay kits in the market are:

Biomarker Classification	Sampling From	Product Name	Detecting Target	Detecting Principle	Analyzing in
Biochemical assay Biochemical assay	GCF	Periocheck	Neutral proteases	Enzymatic digestion reaction (Colorimetric assays)	Chairside Chairside
	GCF	PocketWatch	AST	Enzymatic catalysis reaction (Colorimetric assays)	
	GCF	PerioGard	AST	Enzymatic catalysis reaction (Colorimetric assays)	
	Oral rinse	PerioSafe	aMMP-8	Lateral flow test with digital reader (OraLyzer®)	
	GCF	ImplantSafe			
	Oral rinse	SillHa ST-4910	Blood, leukocytes, and protein	Lateral flow test with dual-wavelength reflectometry	
Microbiological assay Microbiological assay	Subgingival plaque	Evalusite	*Aa, Pg, Pi*	Sandwich enzyme immunoassay (Colorimetric assays)	Chairside
	Subgingival plaque	BANA-Enzymatic test kit	*Pg, Td, Tf*	BANA hydrolysis reaction (Colorimetric assays)	
	Gums and plaque	OMNIgene ORAL/ OMR-110	Characterization of virus species of all genome type including *Aa,*	DNA hybridization	Company or research laboratory
	Saliva	OMNIgene ORAL/ OM-501, 505			

Biomarker Classification	Sampling From	Product Name	Detecting Target	Detecting Principle	Analyzing in
			Pg, Pt, Fn, Td, Ec		
	Subgingival plaque	Carpegen® Perio Diagnostik	*Aa, Pg, Tf, Td, Fn, Pi*	Real-time qPCR	
	Oral rinse	MyPerioPath®	*Aa, Pg, Td, Tf, En, Fn, Pi, Cr, Pm, Ec, Cs*	DNA hybridization	
	Microbiological samples/subgingival plaque	iai Pado Test	*Aa, Pg, Pi, Td, Tf, Fa*	DNA hybridization	
	Subgingival plaque	micro-IDent®plus11	*Aa, Pg, Pi, Tf, Td, Pm, Fn, Cr, En, Ec, Cs*	DNA hybridization	
Genetic assay	Cheek swab	PerioPredict™	genes for IL-1	DNA hybridization	Company laboratory
	Oral rinse	MyPerioID® IL-6 or IL-1	genes for IL-6 or IL-1	Genetic polymorphisms detection	

Conclusion:

In this review, a brief introduction to periodontal disease, focusing on the participation of the host immune system including both innate, adaptive, and complement systems which may interfere in the development and progression of the periodontal disease and the molecules that play a role in the pathogenesis of periodontitis i.e; microbial virulence factors and host immune-inflammatory responses along with the chairside investigations of inflammatory biomarkers are described. The available data show that the host response is critical in protecting the periodontium from the pathological sequelae of bacterial colonization and invasion. However, to date, no consensus regarding the pattern of the immune response in controlling periodontal disease has been documented. Further research is essential to fully comprehend the immunological basis of periodontal disease, paving the way for innovative treatment approaches and preventive measures.

REFERENCES:

1. Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. Periodontol 2000. 1997 Jun;14:9-11. doi: 10.1111/j.1600-0757.1997.tb00189.x. PMID: 9567963.
2. Kaur S, White S, Bartold PM. Periodontal disease and rheumatoid arthritis: a systematic review. J Dent Res. 2013 May;92(5):399-408. doi: 10.1177/0022034513483142. Epub 2013 Mar 22. PMID: 23525531.
3. LOE H, THEILADE E, JENSEN SB. EXPERIMENTAL GINGIVITIS IN MAN. J Periodontol (1930). 1965 May-Jun;36:177-87. doi: 10.1902/jop.1965.36.3.177. PMID: 14296927.
4. Van Dyke TE, Kornman KS. Inflammation and factors that may regulate inflammatory response. J Periodontol. 2008 Aug;79(8 Suppl):1503-7. doi: 10.1902/jop.2008.080239. PMID: 18673003.
5. Salvi GE, Lawrence HP, Offenbacher S, Beck JD. Influence of risk factors on the pathogenesis of periodontitis. Periodontol 2000. 1997 Jun;14:173-201. doi: 10.1111/j.1600-0757.1997.tb00197.x. PMID: 9567971.
6. Sissons, C.H., Wong, L., and Shu, M., 1998. Factors affecting the resting pH of in vitro human microcosum dental plaque and Streptococcus mutans biofilms. Archives of oral biology, 43(2), pp.93-102.
7. Wood NH, Blignaut E, Lemmer J, Meyerov R, Feller L. Necrotizing periodontal diseases in a semirural district of South Africa. AIDS Res Treat. 2011;2011:638584. doi: 10.1155/2011/638584. Epub 2011 Jun 21. PMID: 21755050; PMCID: PMC3132873.
8. Bakri I, Douglas CW, Rawlinson A. The effects of stress on periodontal treatment: a longitudinal investigation using clinical and biological markers. J Clin Periodontol. 2013 Oct;40(10):955-61. doi: 10.1111/jcpe.12142. Epub 2013 Aug 18. PMID: 23952266.

9. Leppilahti JM, Hernández-Ríos PA, Gamonal JA, Tervahartiala T, Brignardello-Petersen R, Mantyla P, Sorsa T, Hernández M. Matrix metalloproteinases and myeloperoxidase in gingival crevicular fluid provide site-specific diagnostic value for chronic periodontitis. J Clin Periodontol. 2014 Apr;41(4):348-56. doi: 10.1111/jcpe.12223. Epub 2014 Feb 17. PMID: 24382144.
10. Lien E, Sellati TJ, Yoshimura A, Flo TH, Rawadi G, Finberg RW, et al. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. J Biol Chem 1999; 274(47): 33419-25.
11. Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. J Immunol 1999; 285(5428): 732-6.
12. Sugawara Y, Uehara A, Fujimoto Y, Kusumoto S, Fukase K, Shibata K, et al. Toll-like receptors, NOD1, and NOD2 in oral epithelial cells. J Dent Res 2006; 85(6): 524-9.
13. Taubman MA, Kawai T. Involvement of T-lymphocytes in periodontal disease and in direct and indirect induction of bone resorption. Crit Rev Oral Biol Med 200; 12(2): 125-35.
14. Nakajima T, Ueki-Maruyama K, Oda T, Ohsawa Y, Ito H, Seymour GJ, et al. Regulatory T-cells infiltrate periodontal disease tissues. J Dent Res 2005; 84(7): 639-43.
15. Eastcott JW, Yamashita K, Taubman MA, Harada Y, Smith DJ. Adoptive transfer of cloned T helper cells ameliorates periodontal disease in nude rats. Oral Microbiol Immunol 1994; 9(5): 284-9.
16. Yamashita K, Eastcott JW, Taubman MA, Smith DJ, Cox DS. Effect of adoptive transfer of cloned Actinobacillus actinomycetemcomitans-specific T helper cells on periodontal disease. Infect Immun 1991; 59(4): 1529-34.
17. Aoyagi T, Sugawara-Aoyagi M, Yamazaki K, Hara K. Interleukin 4 (IL-4) and IL-6-producing memory T-cells in peripheral blood and gingival tissues in periodontitis patients with high serum antibody titers to Porphyromonas gingivalis. Oral Microbiol Immunol 1995: 10: 304-10.
18. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. Periodontol 2000 1994: 5: 112-41.
19. Gaffen SL, Hajishengallis G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. J Dent Res 2008; 87(9): 817-28.
20. Takahashi N, Udagawa N, Suda T. A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. Biochem Biophys Res Commun 1999: 256: 449-55.
21. Lorenzo J. Interactions between immune and bone cells: New insights with many remaining questions. J Clin Invest 2000: 106: 749-52.
22. Liu D, Xu JK, Figliomeni L, Huang L, Pavlos NJ, Rogers M, et al. Expression of RANKL and OPG mRNA in periodontal disease: Possible involvement in bone destruction. Int J Mol Med 2003: 11: 17-21.
23. Hajishengallis G, Lambris JD. Crosstalk pathways between Toll-like receptors and the complement system. Trends Immunol. 2010 Apr;31(4):154-63. doi: 10.1016/j.it.2010.01.002. Epub 2010 Feb 10. PMID: 20153254; PMCID: PMC2849859.
24. Hajishengallis G, Genco RJ. Downregulation of the DNA-binding activity of nuclear factor-kappaB p65 subunit in Porphyromonas gingivalis fimbria-induced tolerance. Infect Immun. 2004 Feb;72(2):1188-91. doi: 10.1128/IAI.72.2.1188-1191.2004. PMID: 14742573; PMCID: PMC321640.
25. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, McIntosh ML, Alsam A, Kirkwood KL, Lambris JD, Darveau RP, Curtis MA. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. 2011 Nov 17;10(5):497-506. doi: 10.1016/j.chom.2011.10.006. Epub 2011 Oct 27. PMID: 22036469; PMCID: PMC3221781.
26. Darveau RP, Pham TT, Lemley K, Reife RA, Bainbridge BW, Coats SR, Howald WN, Way SS, Hajjar AM. Porphyromonas gingivalis lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. Infect Immun. 2004 Sep;72(9):5041-51. doi: 10.1128/IAI.72.9.5041-5051.2004. PMID: 15321997; PMCID: PMC517442.
27. Kurita-Ochiai T, Amano S, Fukushima K, Ochiai K. Cellular events involved in butyric acid-induced T cell apoptosis. J Immunol. 2003 Oct 1;171(7):3576-84. doi: 10.4049/jimmunol.171.7.3576. PMID: 14500654.
28. Calkins CC, Platt K, Potempa J, Travis J. Inactivation of tumor necrosis factor-alpha by proteinases (gingipains) from the periodontal pathogen, Porphyromonas gingivalis. Implications of immune evasion. J Biol Chem. 1998 Mar 20;273(12):6611-4. doi: 10.1074/jbc.273.12.6611. PMID: 9506956.
29. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. Microbiol Mol Biol Rev. 1998 Dec;62(4):1244-63. doi: 10.1128/MMBR.62.4.1244-1263.1998. PMID: 9841671; PMCID: PMC98945.
30. Wang BY, Chi B, Kuramitsu HK. Genetic exchange between Treponema denticola and Streptococcus gordonii in biofilms. Oral Microbiol Immunol. 2002 Apr;17(2):108-12. doi: 10.1046/j.0902-0055.2001.00001.x. PMID: 11929558.