Microbes and Immunity: The Periodontal Battle Zone

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Abstract

Periodontal disease is known around the world as a significant health concern and a common cause of adult tooth loss. Periodontal diseases are common inflamed disorders such as gingivitis and periodontitis caused by the pathogens present in the biofilm which is seen subgingivally such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* that activate inflammatory, innate and adaptive responses of the immune system. The immune system plays a vital role in preservation from pathogens with the maintenance of commensal microbiota at the mucosal surface. This article is focused on discussing the role of microbes and the basis and concept of immunology in periodontal disease in brief.

Keywords: *Periodontitis, Pathogenesis, Immunology.*

Introduction

A chronic inflammatory disease of the periodontium known as periodontitis is characterized by loss of periodontal ligament and destruction of surrounding alveolar bone.[1] There is ample evidence that the presence of microorganisms and persistent inflammation in periodontium give rise to the development and/or initiation of many systemic inflammatory diseases like diabetes, obesity, metabolic disease, rheumatoid arthritis, chronic kidney disease, etc.

Many of the key pathways leading to systemic inflammation are the direct or indirect effects on certain body sites of circulatory bacteria, mediators of inflammation and/or immune systems from infected/inflamed tissues of the periodontium.

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Bacteremia may be caused by oral bacteria entering the bloodstream via mechanical processes involving periodontal debridement, flossing, and brushing techniques. Products from bacteria especially LPS from gram-negative bacteria can also contribute to systemic inflammation. The response of inflammation by bacteria of the periodontium in the inflamedtissue of periodontium is an origin of recurrent persistent systemic inflammation. P.*gingivalis* is considered to be the only bacteria capable of inducing enzymatic peptide citrullination with subsequent production of autoantibodies of the anti-citrullinated peptide.

During periodontitis, pro-inflammatory mediators and biomarkers are found to be substantially higher in serum and gingival crevicular fluid as compared to the periodontally well individuals. When defending against infections, the immune system plays an essential function when preserving commensal microbiota on the mucosal surface. Innate immunity provides a first line of protection against infection that creates a homeostatic network capable of detecting infectious microorganisms that cause immune reactions to remove them. Along with innate immunity, adaptive immunity cells and characteristic cytokines play a vital role in the pathogenesis periodontal disease involving $CD4^+$ T-cells (T-helper cells.[2]

Factors in causation of periodontal disease: Diseases of periodontium are usually inflaming disorders such as gingivitis and periodontitis occurs due to pathogens present in the biofilm which is seen subgingivally.

Such bacteria cause the gingival epithelium to develop cytokines and chemokines, resulting in molecular adhesion, elevate permeability in capillaries of the gingiva, and chemotaxis of polymorphonuclear neutrophils via junctional epithelium to the gingival sulcus.[3] Further continuation of this process results in inflammation that extends into the tissues and results in loss of alveolar bone and reinforcing connective tissue and also causes initiation of periodontal pocket. The inflammatory mediators and cytokines are capable of stimulating the demolition of the periodontium andthe destruction of collagen via matrix metalloproteinases extracted from tissues.

Various and distinctive ecosystems that are present in the human body provide a unique environment for the colonization of microorganisms. Typically the periodontal disease involving *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, and *Tannerella forsythia*are associated with a scanty bacterial typespresent in the biofilm which is seen subgingivally.

According to a study report,in most patients and active sites, the red complex types *P.gingivalis* and *T.forsythia* were found to be elevated in populations of persistent inflammatory disorder of periodontium in Chile, Spain and Colombia.[4] *P.gingivalis* were more often observed in patients of Chile (83.8%) and less commonly in Colombians (65.9%). *T.forsythia* were found to be lower in Spain (36.1%) than in Chile (16.2%) and Colombia (39%). This study also reports that the active sites of chronic periodontitis in Chileans have higher mean percentages of total anaerobic*P. gingivalis* and no major variations for *T.forsythia* have been reported.

Research on Latin American populations show a high prevalence of T.forsythia and P.gingivalis in Chileans with chronic periodontitis. A high incidence of T.forsythia and P.gingivalis were recorded in Brazilians (45% and 68% respectively) and Mexicans (97.4% and 100% respectively) using DNA-based method. **Botero** etal,in Colombian patients with chronic periodontitis using microbiological culture, reported high-frequency T. forsythia and P. gingivalis (50% and 75.4% respectively).[5]

Actinomycetemcomitans in chronic periodontitis use microbiological culture and DNA samples to detect this pathogen in a lower ratio than *T.forsythia* and *P.gingivalis* in the Chilean population. Some populations with aggressive periodontitis have been found to have a higher rate of P.gingivalis as oppose to those of chronic disease of periodontium by using microbiological culture of periodontal pathogens.

For example- Chilean patients with chronic periodontitis have 76.4% frequency isolation of *P.gingivalis* compared to 86-100% in aggressive periodontitis.[6]

Besides, fimbriae is another clinical virulence factor that is the filamentous component on the surface of the cell, and its subunit protein fimbrillin (fimA) significantly performs on bacterial contact with tissues of the hosts by mediating adhesion of bacteria and target site colonization.[5] Several experiments have been conducted in the subjects of various disorders of periodontium at distinct geographical locations to evaluate the pervasiveness and dispersal of the fimA genotypes. Based on this finding, itcan be concluded that the type fimA genotypes are most common in patients of periodontitis whereas the second most prevalent genotypes were seen to type A, type B, or type Cdepending on the studied ethnic group. The subjects type A and type B fimA are more common in non-periodontitis cases.[7]

Moreover, a large molecule consisting of a lipid component (lipidA) and a polysaccharide component known as lipopolysaccharides which is a major component of the outer membrane of P.gingivalis and compares with most gram-ve bacteria.[6] Heterogeneous molecules from *P.gingivalis* lipid A induce an agonistic and antagonistic response of the innate immune system by one week. It is proposed that these mechanisms are critical for disrupting oral homeostasis and oral health.

Innate immunity in periodontal diseases: The response of the innate immunity system is a mechanism of homeostasis that provides the primary line of protection, and is alsocapable of recognizing invasion of pathogens as non-self, activating immune reactions to remove them. The responses of the innate immunity system are organized by a wide variety of chemokines, cytokines and receptors on the surface of the cell, and an inflammatory state created by innate immunity

stimulation. Adaptive immune strengthens the effector mechanisms of innate immune, providing a methodical loop for clearance of microbes, where activating actual innate systems guarantees a successful adaptive immune response that potentiates the action of these innate factors against periodontal bacteria.[8]

Polymorphonuclear neutrophils: Polymorphonuclear neutrophils after chemoattractants such as interleukin-8, connective fibroblasts, and immune cells are one of the first respondents of inflaming cells to move towards the site of inflammation of the periodontium.[7] Polymorphonuclear neutrophils are a characteristic of acute inflammation. Changes in their functions causeacute, serious, andgeneralized periodontitis in humans. Such modifications may include varying roles such as adhesion potential, reaction to chemotaxis and phagocytosisand thereby implication, the particular timing of the immune responses depends on polymorphonuclear neutrophils.

The cytoplasmic granules of polymorphonuclear neutrophils contain many enzymes that degenerate the structure of the cells of the tissues and the extracellular matrix when released. These are the brief-lived cells thatdemise in larger numbers at acute inflaming sites of periodontium due to interaction with pathogens and their toxins. The aggregation and huge death of polymorphonuclear neutrophils are the main causes of deterioration of tissue in developing disease of periodontium.[9]

Role of T-cell subsets and cytokines: In periodontal disease pathogenesis,the production of adaptive immunity mediated by T-cellis influenced by innate immune-associate cellspresenting antigen which, after antigen attack pass through a procedure of maturation and emigrate to the lymph nodes, where they generate different cytokine forms which cause successive polarization and activation of specific T $CD4^+$ lymphocytes.[7]

The $CD4^+$ T-cells are two subsets based on their cytokine development pattern, mainly T-helper 1 and T-helper 2. T-helper 1 cytokines are related to infectious inflammatory degradation of the bone, whereas T-helper 2 cytokines have been expressed to reduce loss of bone. The other regulatory subsets of T-cells are Tr1 and T-helper 3 which are identified respectively by the common development of transforming growth factor-b or interleukin-10. And additional subsets of T-helper just as T-helper 22 and T-helper 9 cells have played a vital role in the transition of inflammatory immune responses. T-helper 17 and T-regulatory cells together play a vital role in the organization ofresponses of the adaptive immunity and also have a significant impact on inflammatory and autoimmune diseases outcome.[9]

T- helper 1: T-helper 1 cells are produced under the interleukin-12/interleukin-1 signaling effect, which results in the incitation of the T-bet transcription factor. T-helper 1 cells primarily participate in the immunity mediated by the cells. Production of inflammatory cytokines, chemokines and activation phagocyte is correlated with interferon-$\gamma\gamma$, the T-helper 1 type response signature cytokine.According to a review by the **Gamonal and Garlet**, the interferon- $\gamma\ \gamma$ is highly present associated with progressive lesions or severe forms of periodontal diseases in humans and experimental periodontal lesions. Human and experimental evidencesuggest that theinterferon- $\gamma\ \gamma$ can give rise to the development of inflammation of periodontium by distinct mechanisms.[10]

T- helper 2: The activity of T-helper 2 cells depends primarily on interleukin- 4, the T-helper2 and the transcription factor GATA3. Interleukin- 4 has distinct suppressive and anti-inflammatory properties that are intervened by its ability to interrupt the transcription of pro-inflammatory cytokines and interferon- then suppress the polarization of T-helper 1 cells.[8] Interleukin-4 can hamper the development of factors causing tissue degradation including matrix metalloproteinases and the main osteoclastogenic factor RANKL. Interleukin- 4 can also induce regulation of its respective TIMPs and osteoprotegerin inhibitors in distinct tissues through their anabolic properties. In gingival crevicular fluid (GCF) the congregation of interleukin- 4 found to reduce from periodontal health to disease, indicating that this cytokine may intervene in the revocation of lesions of the periodontium.

T- Regulatory cells: In particular, regulatory T cells control the renewing, augmentation thereby regulating the outcome of many settings of immune systems varying from infective diseases to pathology of immune system and autoimmunity. The T- regulatory cells were initially identified as $CD4^+$,$CD25^+$ T cells. T regulatory cells serve the transcription factor forkhead box p3 (FOXp3), CD103, the glucocorticoid- inducible tumor necrosis factor receptor (GITR), inhibitory molecule cytotoxic

T-lymphocyte- related molecule 4(CTLA-4) and cell surface transforming growth factor-β1, in the middle of additional surface molecules. In some non-inflamed peripheral tissues, the T-regulatory cells are known to be in a relaxed state with minimum suppressor activity, but these cells are activated in inflammatory conditions and go through quick reprogramming to obtain helper/ effector functions.

According to a study paper, **Gamonal** et al demonstrated that T-regulatory cells- associated levels of cytokines have a negative association with RANKL levels, supporting their defensive role in active periodontal lesions against tissue destruction. In line with such theory, Garlet group was the first to recognize a reaction in an experimental model of periodontitis, in the association between the intensity of periodontitis via the ablation role of T-regulatory cells.

Role of prostaglandins: The transformation of arachidonic acid to prostaglandin catalyzed by the enzymes COX-1 and COX-2 cyclooxygenase. Prostaglandins in particular are the most significant inflammatory mediators especially, prostaglandin E_2 (PGE_2) which contributes to vasodilation and production of cytokines by different cell types. PGE_2 released remarkably in the periodontium by macrophages, fibroblasts from different cell types. PGE_2 is involved in the activation of MMPs and resorption of osteoclast and has an important role in tissue damage characterizing periodontitis.

COX-1 is a constituent enzyme involved in the initiation of physiologically active prostaglandins while COX-2 is an inductive enzyme mainly caused due to pro-inflammatory cytokines and results in the initiation of prostaglandin. Over the last years, the role of prostaglandin has been under investigation in the pathogenesis of periodontitis. PGE_2 and leukotriene B4 were present in GCF of localized aggressive inflammation of the periodontium, indicating a character for these molecules in the disease of periodontium. The use of a COX-2 inhibitor as an alternative to the periodontal therapy was investigated in several clinical trials. Such inhibitors increased the clinical results following periodontal therapy.[9] Thesestudies indicate that bone resorption induced by periodontitis may involve prostaglandin especially PGE_2.

Role of Osteoimmunology: The transformation of monocyte-macrophage precursor cells into osteoclasts induced by an increased level of RANKL mRNA and protein in periodontal tissue and alveolar bone loss caused by osteoclast maturation and survival has now been evident for days.[9]

RANK, RANKL, and OPG are TNF-α family cytokines. RANK is a receptor present on the surface of osteoclast precursor. A variety of other forms of cells produce RANKL primarily T-helper 17 and lymphocytes. Attachingto RANK without inducing any distinction, OPG competes with RANKL. The proportion of the expressions RANKL and OPG is essential in inflammation-induced resorption of bone including periodontitis. In order to determine the RANKL/OPG ratio, various clinical trials studied the congregations of RANKL and OPG in gingival tissues or GCF derived from individuals with periodontitis. This study shows that there is a rise in soluble RANKL in chronic periodontitis with or without a decrease in OPG compared with healthy individuals. The finding was typically that in people with periodontitis the RANKL/ OPG ratio was higher than in healthy individuals.[9]

Matrix metalloproteinases: Matrix metalloproteinases are the main proteases in destructive periodontal diseases. The relationship between inflammation of periodontium and chronic infection of bacteria controls the neutral proteinases manner and function. Especially the MMP family,which causes a continuous breakdown of the supporting tissues of the periodontium.[9]

Matrix metalloproteinase is a family ofhuman zinc-dependent endo-peptidases associated with a wide rangeof physiological and pathological processes includingthe growth of skeletal system and remodeling, healing of the wound, cancer andinflaming disease. MMPs are made up of an auto-inhibitory prodomain which is often involved in the identification and placing of MMP substrates for enzymatic latency, the catalytic domain, and the c-terminal hemopexin like domain.[10] MMP activity is closely regulated by gene expression, pro-enzyme activation, enzyme inhibition, and endogenous inhibitors. They are grouped particularly in collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs according to their substrate. MMPs are the master regulators of inflammation-related with a large variety of inflaming disorders. [10]

Conclusion

According to some evidence the disease

of periodontium has complex aggravation and revocationstates that can be defined as cycles of development and reversion of disease. Widespread forms of vital events of disease of periodontium can be related to dissimilarity in the population of supracrestal inflaming cells,where there are a substantially greater number of monocytes/macrophages and plasma cells inactive sites in comparison to inactive sites.

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