THE ROLE OF CYTOMEGALOVIRUS INFECTION IN THE PATHOGENESIS OF PERIODONTAL DISEASES

Nataša Beader[1] and Marija Ivić-Kardum [2]

[1]Department of Microbiology, School of Medicine, University of Zagreb, Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, [2]Department of Periodontology, School of Dentistry, University of Zagreb, Zagreb, Croatia

SUMMARY – The main characteristic of periodontal disease is chronic inflammation that leads to progressive destruction of the connective tissues and bone with subsequent tooth mobility and finally tooth loss. Traditionally, the pathogenesis of periodontitis was based on the infection caused by bacteria that colonize tooth surface and gingival sulcus. Accumulated evidence show that host response factors such as inflammatory reaction and activation of the innate immune system are critical to the pathogenesis of periodontal disease. Periodontal disease has been widely recognized as a chronic disease but the nature of chronicity remains unclear. The question is whether periodontal disease is a continuous process or consists of episodes of exacerbations and remissions. Maybe cytomegalovirus infection of the periodontium, depending on the latent or active phase of infection, can partly explain the episodic progressive nature of periodontal disease. Cytomegalovirus infection impairs periodontal defense and permits overgrowth of periodontopathogenic bacteria. Owing to advances in new technologies, experimental evidence show the influence and interrelatedness of genomic, epigenetic, proteomic and metabolic factors in the pathogenesis of periodontal disease. Data on the pathogenesis of periodontal disease are reviewed.

Key words: *Periodontal diseases – etiology; Cytomegalovirus*

Introduction

According to new data, clinical characteristics of periodontal disease are influenced by genetic and epigenetic factors such as bacterial accumulation, smoking, or diabetes. Even though the genetic basis of periodontal disease is considered essential for setting the inflammatory process, the epigenetic factors may strongly influence changes in tissue behavior[1]. On the other hand, infection and inflammation of periodontal disease could reach distant sites *via* bloodstream and contribute the systemic diseases such as atherosclerosis, diabetes and adverse outcomes in pregnancy[2,3].

Correspondence to: *Nataša Beader, MD, PhD*, Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, Kišpatićeva 12, HR-10000 Zagreb, Croatia
E-mail: natasaeli@gmail.com

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Over decades, periodontal disease has been recognized as an inflammatory disease initiated by oral microbial biofilm that elicits host response with resultant osseous and soft tissue destruction. Among 500 types of oral bacteria that have been studied to date, only a few of them are considered to be involved in the etiology of periodontal disease. Leaders in the field who gathered at the 1996 World Workshop in Periodontics agreed that these include *Aggregatiobacter actinomycetemcomitans (*previously *Actinobacillus actinomycetemcomitans), Porphyromonas gingivalis, Tannerella (T.) forsythia (*previously *T. forsythensis)* and *Treponema denticola*[4].

Now, it is understood that host response is playing an essential role in the breakdown of connective tissue and bone. The immune and inflammatory responses are critical to the pathogenesis of periodontal disease. Bacteria that colonize tooth surface and gin-

gival sulcus initiate local inflammatory reaction that activates the innate immune system. The initial localized response is amplified by the release of many various cytokines and mediators that propagate inflammation through gingival tissues. If the inflammation could not be encapsulated within gingival tissue, the immune response will be expanded to alveolar bone. Then the inflammatory process drives the destruction of connective tissue and adjacent alveolar bone. There are two critical factors that determine if the bone loss will occur in response to an inflammatory reaction. First, the concentration of inflammatory mediators present in gingival tissue must be sufficient to activate the pathways leading to bone resorption, and second, those mediators must penetrate gingival tissue to reach within a critical distance to alveolar bone[5]. Page and Schroeder have found out that the closer the cells of the inflammatory infiltrate are to the bone, the greater is the number of osteoclasts formed, hence the greater is the amount of bone degraded[6].

The Mechanisms of the Immune System and Inflammatory Response

The innate immune system provides immediate protection against infection and inflammation acting in several ways:

- recruitment of immune cells (phagocytic cells such as polymorphonuclear neutrophils, monocytes and activated macrophages);
- activation of complement system (induces the release of chemical mediators such as cytokines, e.g., tumor necrosis factor (TNF) or interleukins (IL);
- activation of adaptive immune system that represents more specific response to injury or inflammation (proliferation of antigen specific T and B cells with consequent antibody production, assisting macrophages and generating killing cells).

The recruitment of immune cells and releasing of proinflammatory cytokines lead to destructive processes. Several osteoclast related mediators such as metalloproteinases (MMPs) and cathepsins are responsible for the destruction of alveolar bone and the supporting connective tissues[7].

The main event in the inflammatory process is the infiltration of leukocytes in order to limit the level of bacterial invasion. Leukocytes are recruited by many factors such as bacterial products, cytokines, chemokines, and cross-talk between innate and adaptive immune responses, lipid mediators and complement. These phagocytic cells trigger the release of chemical mediators such as TNF and ILs, which in turn activate complement system and acute phase response in order to assist antibodies in clearing pathogens or mark them for other cells' destruction. Finally, activated adaptive immune response will run the proliferation of antigen specific T and B cells. T cells recognize foreign antigen and target it, and B cells produce specific antibodies. T and B cells together assist macrophages and help generate killing cells that mount a response. The role of the immune system is essential. However, if the immune response in the inflammatory disease becomes chronic, the tissues do not return to homeostasis.

The production of cytokines, which stimulate the inflammatory process that activates effector mechanisms, is considered the key feature between bacterial stimulation and tissue destruction. The cytokines have been recognized as cell products of different kinds of cells such as leukocytes, keratinocytes, resident mesenchymal cells (fibroblasts and osteoblasts) or their precursor cells, dendritic cells and endothelial cells. Cytokines are organized as innate and acquired immune cytokines or chemokines. Chemokines are chemotactic cytokines important in leukocyte recruitment and regulation of tissue infiltration with Th1 cells in periodontal disease (e.g., CXCl 10 chemokine)[8]. They are produced by many cell types in the periodontium, such as fibroblasts, endothelial cells, macrophages, osteoclasts, epithelial cells, polymorphonuclear leukocytes, monocytes, lymphocytes and mast cells. Based on the ligand structure, chemokines are divided into two major families: CC and CXC chemokines. Some of them can stimulate one or more steps of bone resorption by recruiting, differentiating or fusing of precursor cells to form osteoclasts or to enhance osteoclast survival[9]. Chemokines as neutrophil chemoattractants contribute to the process of destruction of mineral tissue during bone resorption because macrophages release MMPs. MMPs are structurally related endopeptidases usually divided into several subclasses according to their substrate specificities and physical structure: interstitial collagenases, gelatinases, membrane-type MMPs, and other kinds of

MMPs including stromelysins and metalloelastases. Their activity is directed against most extracellular matrix, pericellular and non-matrix macromolecules. They are involved in degradation process of different extracellular molecules such as collagen, elastin, proteoglycans and laminins. The study by Kubota *et al.* showed that gene expression of MMP-1, -3, -9 and -13 is simultaneously augmented in periodontitis affected gingival tissue[10]. MMPs are considered to be of great clinical importance in the pathogenesis of periodontal disease due to their ability to activate latent forms of effector proteins, e.g., antimicrobial peptides, chemokines and cytokines. Also, they can alter protein function like shedding of cell-surface proteins[7].

Accumulated evidence show that the effects of cytokines that promote osteoclast formation and bone resorption seem to be counteracted by other cytokines that are anti-inflammatory. IL-1, -6, -11 and -17, tumor necrosis factor-alpha (TNF-α), leukemia inhibitory factor, kinins, thrombin and different chemokines are well known as proinflammatory mediators. Opposite, IL-4, -10, -12, -13 and -18 as well as interferon-beta (IFN-β) and gamma (INF-γ) have anti-inflammatory and inhibitory action to bone resorption[5].

T and B lymphocytes are predominant mononuclear cells in periodontitis of gingival tissues. They can produce IL-1, -6 and -17, TNF-α and receptor activator of nuclear factor-kappa B ligand (RANKL). IL-1 and -6 are included in bone resorption *via* the induction of RANKL. RANKL binds to receptor activator of nuclear factor-kappa B (RANK), one of the most potent inducers of osteoclast formation and activity. This interaction can be stopped with osteoprotegerin (OPG), which binds to RANKL and inhibits the stimulation of RANK, if produced by lymphocytes[11]. In the initial phase of their study (less than 30 days from the onset of experimental disease), Garlet *et al.* showed the leukocyte count to have increased just prior to the rapid increase of alveolar bone loss. At the same time, the levels of proinflammatory cytokines, MMPs and RANKL were also elevated. Later when the bone loss rate was slower, the concentration of proinflammatory cytokines, MMPs and RANKL decreased and there was a dramatic increase in the concentrations of anti-inflammatory cytokines as well as tissue inhibitors of matrix metalloproteinases (TIMPs) and OPG[12]. According to these experimental data, it seems that the type of cytokines produced in periodontal tissues may determine the progression and severity of periodontal disease by controlling the breakdown of soft and bone tissues through the balance between MMPs/TIMP and RANKL/OPG expression in gingival tissues.

So far, IL-1β, prostanglandin E_2, MMPs, neutrophil elastase and β-glucuronidase have been identified as potential biomarkers for the diagnosis of periodontal disease, as they are considered as measures of inflammation and mediators of the connective tissue breakdown[13,14].

However, it seems that there is no ideal marker that can predict the progression from gingivitis to periodontitis, or when periodontitis will turn to its active phase with consequent attachment loss[15].

CMV Infection and Immune Response in Periodontal Disease

Herpes viruses are found to be more frequently present in periodontal lesions and acute necrotizing ulcerative gingivitis lesions than in gingivitis or periodontally healthy sites. Most of the time, two herpesviruses are implicated in these lesions: Epstein-Barr virus (EBV) that infects periodontal B-lymphocytes and cytomegalovirus (CMV) that infects periodontal monocytes/macrophages and T-lymphocytes. Also, CMV infects salivary glands, epithelial and endothelial cells, and fibroblasts.

The seroprevalence of CMV infection in the world varies widely up to 95% of population depending on the geographic area (developed/developing countries)[16]. Very often, the infection starts early in the childhood, actually, early in gestation because placenta is pivotal in CMV transmission to the fetus[17]. Jaskoll *et al.* report that CMV infection involving dental papilla mesenchymal cells induced tooth defects such as amelogenesis imperfecta[18]. This pathogenesis appears to be mediated by the NFκB pathway.

Reactivation of CMV in periodontal lesions is considered to be associated with periodontal disease progression, especially in localized juvenile periodontitis[19]. The number of subgingival periodontopathogenic bacteria in these lesions is elevated probably because active viral infection impairs immune defenses of periodontal tissue. This might explain several hallmarks of periodontal disease such as:

- episodic progressive nature of periodontal disease (due to transient local immunosuppression depending on active or latent viral infection);
- localized pattern of tissue destruction (due to viral tissue tropism);
- some individuals carry periodontopathogenic bacteria and still maintain periodontal health (due to absence of viral infection).

In general, herpes viruses may give rise to periodontal pathology in two ways, first, by direct viral infection and replication, and second, as virally mediated damage to host defense and by evading the immune host surveillance. They may diminish tissue repair by direct cytopathic effect on inflammatory cells such as polymorphonuclear leukocytes, lymphocytes, macrophages, and other cells such as fibroblasts, endothelial cells, bone cells and others. In turn, this may lead to bacterial superinfection. Gingival herpes virus infection may promote subgingival attachment and colonization of periodontopathogenic bacteria. Even more, viral proteins expressed on eukaryotic cell membranes can act as bacterial receptors and generate new bacterial binding sites. It seems to be most important that viral infections can alter inflammatory mediator and cytokine responses. Concerning the pathology of periodontal disease, it appears that the crucial role belongs to CMV. Accumulated evidence show that CMV has not only developed many strategies to evade the host immune response but also to exploit the host immune response to achieve reactivation from latency and disseminated infection[20]. CMV uses NFκB to mediate an inflammatory immune response and to induce IE gene expression in order to drive viral replication[21]. CMV encodes the chemokines that draw susceptible monocytes and neutrophils to the site of infection. Consequently, the virus can induce production of IL-1 and TNF-α by macrophages and monocytes, which in turn may up-regulate MMPs and down regulate TIMPs. MMPs are the key proteolytic enzymes responsible for cleaving interstitial collagens, which make the highest protein part of the extracellular matrix in the periodontium[22,23]. In periodontal disease, they eventually degrade periodontal ligamentous attachments and bone matrix proteins, finally leading to bone destruction[10]. Analyses of gingival biopsies of CMV infected individuals with periodontitis demonstrated higher expression of MMPs than biopsies from CMV negative individuals[24].

CMV can produce tissue injury as the result of immunopathologic responses to virally infected cells such as:

- induction of cell-mediated immunosuppression by reduction of cell surface expression of major histocompatibility complex (MHC) class I molecules and interfering with T-lymphocyte recognition;
- causing metabolic abnormalities in monocytes and lymphocytes;
- suppressing antigen specific cytotoxic T-lymphocyte functions that results in a decrease in circulating CD4+ cells and an increase in CD8+ suppressor cells, which in turn might lead to global impairment of cell-mediated immunity.

In their study, Contreras and Slots showed the importance of CMV in the etiology and/or pathogenesis of human periodontal disease. They demonstrated the presence of nucleic acid sequences of CMV in juvenile and adult periodontal lesions; the association between viral infection and acute necrotizing gingivitis; mRNA late gene expression in adult and localized juvenile periodontitis lesions and association with progressive disease; increased frequency of periodontopathogneic bacteria in CMV positive periodontal lesions; detected viral nucleic acid sequences in inflammatory periodontal cells; demonstrated the effect of viral infection on periodontal defense cells; and viral ability to up-regulate the expression of tissue damaging cytokines in periodontal inflammatory cytokines[25].

According to these data, the main question might be: does active periodontal CMV infection initiate destructive periodontal disease or the disease reactivates latent CMV infection? It seems that the immune system is essential. Acute CMV infection is characterized by significant levels of viral replication and dissemination to multiple organs. Thus, the pathogenesis of acute infection shows a linkage between the levels of viral replication, organ dysfunction and disease in patients. In contrast, the pathogenesis of chronic infection is associated with a bi-directional relationship between viral gene expression and the host inflammatory response: viral persistence (reactivation) is facilitated by the host inflammatory response and the host

inflammatory response is stimulated by the presence of the virus. In this case, the disease may be attributed to both viral and host functions. Moreover, it seems that viral gene products that play the main role in chronic inflammation in the body are not needed for replication *in vitro*. If immune response becomes chronic due to many different factors in inflammatory disease, the injured tissues do not return to homeostasis. Consequently, CMV is being reactivated periodically resulting in transient immunosuppression that gives rise to overgrowth of periodontal pathogenic bacteria.

Conclusion

Although it has been considered that the pathogenesis of periodontitis is based on the infection caused by bacteria that colonize tooth surface and gingival sulcus, different studies have shown that the host response factors such as inflammatory reaction and activation of the innate immune system are critical to the pathogenesis of periodontal disease. CMV infection due to a variety of viral properties can impair periodontal defenses, thus permitting the overgrowth of periodontopathogenic bacteria. It seems that CMV infection in periodontal tissue depending on the latent or active phase of the infection can partly explain the episodic progressive nature of periodontal disease.

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Sažetak

ULOGA CITOMEGALOVIRUSNE INFEKCIJE U PATOGENEZI PARODONTNE BOLESTI

N. Beader i *M. Ivić-Kardum*

Glavna značajka parodontne bolesti je kronična upala koja vodi progresivnom razaranju vezivnog tkiva i kosti s posljedičnim klimanjem i na kraju ispadanjem zuba. Tradicionalno, patogeneza parodontitisa se temelji na infekciji uzrokovanoj bakterijama koje koloniziraju površinu zuba i gingivnog sulkusa. Dostupna literatura pokazuje da su činitelji obrane domaćina kao što je upalna reakcija i aktiviranje prirođenog imunog sustava presudno važni u patogenezi parodontne bolesti. Općenito se smatra da je parodontna bolest kronična, iako narav kroniciteta nije u potpunosti jasna. Pitanje je je li parodontna bolest trajan proces ili se sastoji od niza epizoda egzacerbacija i remisija. Moguće je da citomegalovirusna infekcija parodontnog tkiva, ovisno o latentnoj ili aktivnoj fazi infekcije, može djelomice objasniti progresivnu narav parodontne bolesti koja se javlja u epizodama. Citomegalovirusna infekcija smanjuje obranu parodontnog tkiva i tako omogućuje prekomjeran rast parodontopatogenih bakterija. Zahvaljujući napretku u razvoju novih tehnologija eksperimentalni podatci pokazuju utjecaj i međusobnu povezanost genomskih, epigenetskih, proteomskih i metaboličnih čimbenika u patogenezi parodontne bolesti. U ovom se radu daje pregled spoznaja vezanih za parodontnu bolest.

Ključne riječi: *Parodontne bolesti – etiologija; Citomegalovirus*