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CHRONIC INFLAMMATION IN PERIODONTAL DISEASES: IMMUNOPATHOGENESIS AND TREATMENT

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Abstract
Periodontal diseases generally progress as plaque biofilm and inflammation-associated destruction of the attachment apparatus. Certain host factors can predispose individuals to these diseases. This paper explores the host immunologic response to periodontal pathogens, which begins with an acute phase of inflammation, and includes an influx of neutrophils and plasma fluid proteins. Chronic inflammation appears to develop when this host response fails to properly resolve. Neutrophil production of excessive oxygen free radicals, as well as impaired neutrophil chemotaxis and phagocytosis, may play a role in the pathogenesis of various forms of periodontal disease (as well as in the pathogenesis of common risk factors such as diabetes and smoking). The genetic etiology of certain immune cell deficiencies may also play a role. Current treatment planning for periodontal disease recognizes the destructive role of chronic inflammatory conditions; resolvins and lipoxins may be a beneficial adjunctive approach to modulating the immune response at the cellular and molecular levels. Concomitant management of plaque biofilm and chronic inflammation may prove to be critical in optimum control of many forms of periodontal disease.

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Introduction

Periodontal disease refers to any disease of the supporting structures of the teeth, including the interface of epithelium, connective tissue, and mineralized tissue. Very briefly, the relationship of these supporting structures begins with the tooth, which is made up of a crown (visible in the mouth) and a root (embedded in the jaw bone, also known as alveolar bone or the alveolus). The tooth root is covered in cementum, an avascular mineralized bone-like tissue. Between the root and the alveolus is the periodontal ligament, comprised of parallel collagen fibers embedded in the cementum on one end, in the opposing alveolus on the other end. Thus the tooth is actually suspended within the alveolar bone. Where the tooth transitions from the bone to the oral cavity, the periodontal ligament ends and there is a narrow epithelial cuff attached to the cementum by hemidesmosomes. This epithelial cuff, known as the junctional epithelium, provides both a mechanical and biological barrier between the external environment of the oral cavity and the internal environment of the body. Near the crown, the cuff surrounds the tooth but is not attached; the space between the cuff and the tooth is called the sulcus. A deepening sulcus indicates conversion from health to disease; however, the hallmark of periodontal disease is the destruction of the periodontal ligament and subsequent loss of alveolar bone.

Periodontal diseases can be broadly classified as being plaque induced or non-plaque induced. The incidence and severity of plaque-induced periodontal diseases can be modified by an in-

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dividual's inflammatory response, systemic health, and various local factors that predispose to plaque accumulation.

Treatment strategies for periodontal disease currently include control of plaque biofilm and management of chronic inflammation with non-steroidal anti-inflammatory medications (NSAIDs). Novel classes of molecules may also promote resolution of inflammation; examples include resolvins and lipoxins, which have shown beneficial effects in animal models of periodontitis.

Immunopathogenesis of periodontal disease

In good health, the sulcus is colonized by mostly aerobic, non-motile, bacterial flora. The sulcular environment is bathed in a continuous flow of plasma that passes through the junctional epithelium. Contained within this sulcular fluid are neutrophils, which are drawn to the region by host and bacterial chemotaxins such as leukotriene B_4 (LTB_4) and f-Met-Leu-Phe (fMLP).[1] Once in the sulcus, the neutrophils kill bacteria in two ways: through phagocytosis and degranulation. Also contained within sulcular fluid are various inflammatory molecules, including prostaglandin E_2 (PGE_2), interleukin-1 beta (IL-1 β), tumor necrosis factor-alpha (TNF-α),[2] and gammaglobulin (IgG).[3]

Transition from health to disease. Regular oral hygiene measures mechanically disrupt the bacterial flora, supporting bacterial control by helping to maintain a relatively constant composition of healthy bacteria.[4] Clinically, this bacterial control correlates with an absence of visible inflammation, and healthy sulcus depths of 1-3 mm.[5] However, the hallmark of periodontal disease — the destruction of the periodontal ligament and subsequent loss of alveolar bone — is mediated primarily by osteoclasts and triggered by the proinflammatory molecule PGE_2.[6] At this point, the attachment of the periodontal ligament to the tooth is lost, and the epithelial attachment migrates into the space the periodontal ligament previously occupied, resulting in a deepening of the sulcus beyond 3 mm. A sulcus measuring greater than 3 mm is termed a periodontal pocket, and indicates a conversion from health to disease. Clinically this correlates with bleeding when pocket depths are measured by mechanical probing.[2]

Development of (plaque) biofilm. As the pocket deepens, it becomes increasingly anaerobic and less accessible to mechanical disruption by routine oral hygiene methods, e.g., brushing and flossing. When this occurs, a biofilm (plaque) forms. The biofilm is characterized by greater numbers of anaerobes, spirochetes and motile bacterial species.[4] Biofilms are bacterial communities composed of many different organisms existing in a communal state. An extracellular environment is created with an identifiable structured organization of bacteria, in which nutrients and defenses become shared. In this sense, the biofilm can be considered a single organism. Biofilms are characterized by impermeability and therefore resistance to host molecular and cellular defenses as well as to chemotherapeutics.[7] For example, the production of penicillinase by one organism benefits all susceptible organisms within the biofilm. In this environment, known periodontal pathogens are able to thrive and proliferate, and the disease progresses. Clinically, this correlates with increased clinical signs of inflammation and increased pocket depths.[7] At a microscopic level, this is characterized by an increase in certain inflammatory markers, cellular breakdown products, and sulcular fluid flow.

Clinical inconsistencies to plaque biofilm etiology. Historically, it was assumed that plaque-induced periodontal disease was a single disease caused by a non-specific accumulation of plaque whose destructive effects over time resulted in the progressive loss of periodontal attachment at a relatively constant rate. Thus, emphasis was placed on maintaining high standards of oral hygiene in all patients and the presence of disease was generally attributed to poor oral hygiene measures. However, several observations have challenged this view. Baer noted that in a small percentage of juveniles, periodontal destruction progressed very rapidly and was characterized by a relative absence of bacterial deposits.[8] This disease came to be known as juvenile periodontitis, and is characterized by specific bacterial infection and an altered immune response. Löe and colleagues conducted a long-term study in a community characterized by poor oral hygiene and a lack of access to professional dental care.[9-11] They found that despite similar accumulations of bacterial deposits, not all individuals suffered periodontal destruction at the same rate. Furthermore, a small subset suffered destruction very quickly compared with the rest of the group.

Contemporary concepts clearly recognize that periodontal disease is caused by the presence of specific pathogens, but that the loss of periodontal tissues is the result of a destructive *host response* to pathogenic bacteria.

Inflammatory molecules in periodontal tissue. Within the periodontal tissue, at the cellular level, are neutrophils, T-Cells, B-cells, and osteoclasts.[12] Also present, at the molecular level, are complement, LTB_4, IL-1 and interleukin-6 (IL-6), matrix metalloproteinases, prostaglandins, TNF-α, and immunoglobulins.[5] These cells and molecules are found in greater numbers in disease than in health, and can generally be regarded as inflammatory molecules. Directly and indirectly they initiate and mediate destruction of both the periodontal ligament and surrounding alveolar bone.[12]

Role of inflammation. Inflammation is defined as "a complex reaction to injurious agents such as microbes and

damaged, usually necrotic, cells that consists of vascular responses, migration and activation of leukocytes, and systemic reactions."[13] The function of inflammation is to defend the body against microbial infection, and to repair injured tissues. Nonetheless, inflammation does have the potential to cause harm. Inflammation is now believed to play a causative or contributory role in the pathogenesis of diseases such as atherosclerosis,[14] rheumatoid arthritis,[15] and gastric cancer.[16] It is increasingly evident that chronic inflammatory conditions in one organ or tissue of the body may produce systemic effects with deleterious consequences. For example, an increased level of C-reactive protein is a risk factor for atherosclerosis, and is correlated with levels of periodontal inflammation.[17] Some evidence now points to a contributory or causative role for periodontitis in the pathogenesis of atherosclerosis.[18]

Inflammation generally begins with an acute phase characterized by extravasations of plasma fluid proteins and cells, in particular neutrophils. The function of these various factors includes the killing and elimination of microbes, debridement of necrotic tissue and eventually repair and possibly regeneration of damaged tissue. As the inflammation persists, changing from an acute to a chronic state, additional cellular players become involved, notably macrophages, mast cells, T-cells, B-cells, plasma cells, and eosinophils. Ideally, when the microbial insult is controlled or damaged, inflammation will subside and tissue is repaired. The resolution of inflammation requires leukocytes in the area of inflammation to undergo apoptosis, and then be cleared from the area (without additional leukocytes being drawn to the region during the resolution process).[19] When these conditions are not met, inflammation persists in a self-sustaining chronic state, and can lead to diseases such as periodontitis. However, it is well recognized that not all individuals are equally susceptible to developing periodontal disease, and that there are several forms of the disease.

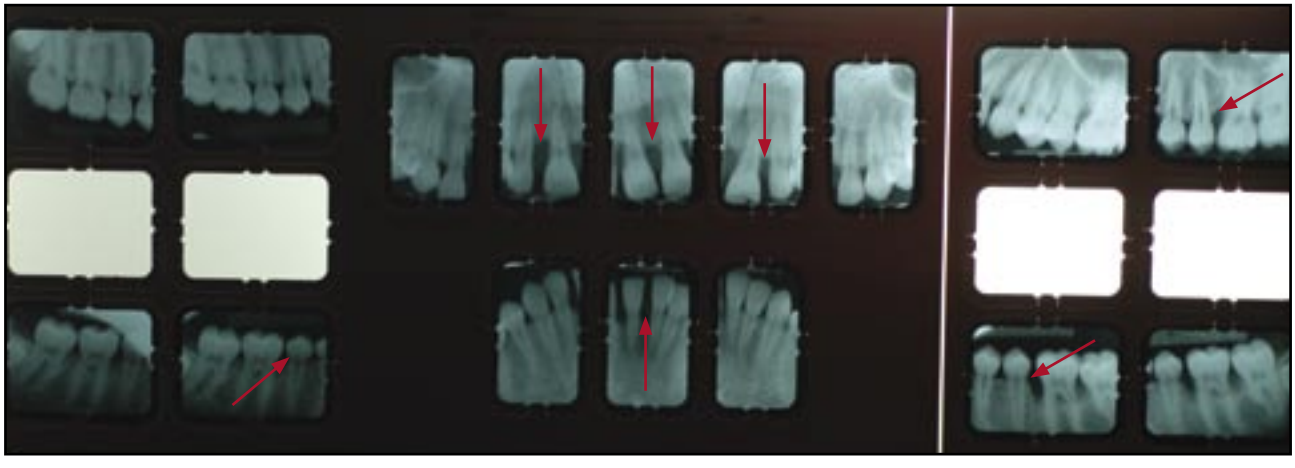
Periodontal disease classifications, characterization, and etiology

The American Academy of Periodontology (AAP) currently recognizes two distinct forms of plaque-induced periodontal disease: aggressive and chronic. Each form can affect only a few teeth or most teeth, and hence each form can be subclassified as either localized or generalized. A third classification recognizes periodontal disease induced by systemic conditions.[20]

Aggressive periodontitis. Localized aggressive periodontitis (LAP) (Figure 1) and generalized aggressive periodontitis (GAP) (Figure 2) are distinct diseases of the periodontium. LAP is well characterized and affects 0.2% of individuals.[21] However, its distinct profile has made it an ideal model for studying the underlying features and pathogenesis of periodontal disease. LAP is characterized by early onset, rapid progression, familial aggregation, relatively little plaque accumulation and infection with *Aggregatibacter (Actinobacillus) actinomycetemcomitans* (Aa).[22]

In general, the incisor and first molar teeth are affected in LAP. GAP shares these features, although the entire dentition is at risk, and the bacteria *Porphyromonas gingivalis (Pg)* and *Tannerella forsythia (Tf)* may also be detected in significantly higher proportions than is found in the plaque of periodontally healthy individuals.[7] In addition to familial aggregation, African Americans, Hispanics, and Asians seem to be disproportionately affected.[23]

Figure 1. Full-mouth radiographs of a 15-year-old Hispanic female with localized aggressive periodontitis



Incisor and first molar osseous defects are noted. (Figure courtesy of Jon B. Suzuki, DDS, PhD, MBA)

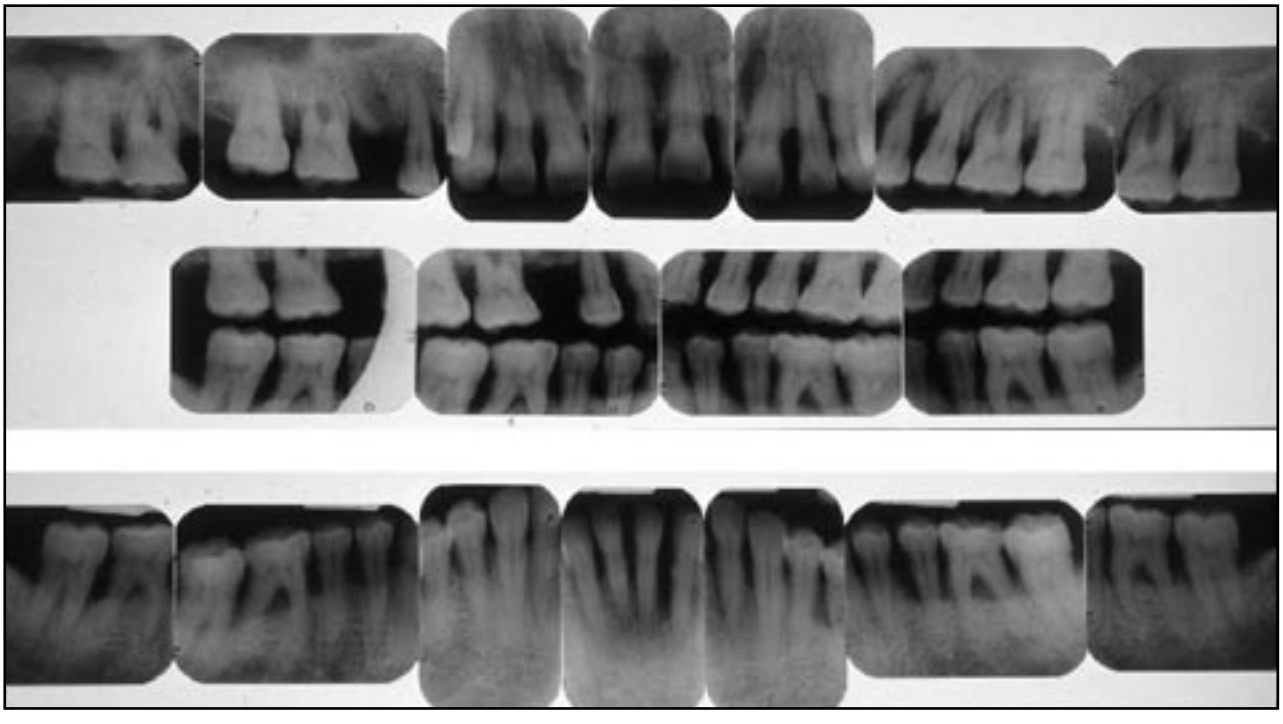
An underlying genetic etiology for LAP has been proposed, although no definitive genetic profile has been identified.[24] There is some debate whether the generalized form (GAP) is simply a continuation of the localized form (LAP), or a distinct disease. To the extent that they may be different diseases, it has been observed that localized cases may "burn out", i.e., the destruction stops.[25] However, precise mechanisms or triggering events have not been elucidated. The reason for loss of attachment at the incisors and molars may correlate more with early age of disease onset, and dentition present at that time, than with any specific characteristics of those individual teeth.[25]

The host response associated with LAP is well studied. LAP is characterized by neutrophils that exist in a primed state, and prone to degranulation and production of excessive oxygen free radicals.[26] At the same time, deficiencies of chemotaxis and phagocytosis are also observed. Oxidative stress (the increased production of free radical oxygen species) has been shown to result from excessive production by hyperresponsive neutrophils.[27] Oxygen free radicals are injurious to all classes of biologically important molecules and are associated with chronic inflammatory conditions including periodontitis, atherosclerosis, and diabetes.

Chronic periodontal disease. Chronic periodontal disease occurs mostly in adults, but also in younger individuals who are susceptible. From a clinical perspective, it appears to progress at a relatively constant rate over a person's lifetime. However, a more accurate description suggests short periods of intense disease activity followed by longer periods of relative quiescence. Chronic periodontitis is characterized by chronic plaque and calculus accumulation, gingival inflammation, and loss of attachment. At a microbial level, the bacteria most commonly associated with chronic periodontitis are *Pg, Treponema denticola (Td),* and *Tf*.[28] With an increase in plaque accumulation, attachment loss, and pocket depth, anaerobic bacteria increase as a component of plaque biofilm.

Although the incidence and severity of chronic periodontitis is correlated with composition and duration of plaque accumulation, individuals are not equally susceptible to chronic periodontitis. Michalowicz and colleagues, characterizing identical twins raised together and apart, have suggested that 50% of the susceptibility to periodontitis can be attributed to genetic factors.[29] To date there are no well-defined genetic determinants of periodontal disease. Although polymorphisms of Il-1A have been correlated with the progression of periodontal disease in northern

Figure 2. Full-mouth radiographs of a 34-year-old Caucasian female with generalized aggressive periodontitis



Osseous defects are noted throughout the dentition. (Figure courtesy of Jon B. Suzuki, DDS, PhD, MBA)

Europeans and particularly in smokers, this does not seem to extend to all population groups.[30] In a recent study of patients with severe chronic periodontitis, candidate genes were identified that may be markers or determinants of susceptibility to periodontitis, but these results are preliminary and additional studies are required.[31]

Periodontal disease induced by systemic conditions. Neutrophil disorders with systemic manifestations are known to result in periodontal destruction. Most of these conditions are very rare and because of their severe systemic manifestation, the periodontal aspects are not always well studied. Much of the information regarding the periodontal aspects of the diseases and periodontal management comes from case reports.

Neutrophil activity is characterized by migration, adherence, phagocytosis, and intracellular or extracellular microbicidal activity. Selectins are expressed on the surface of the endothelial (e-selectin) and neutrophil cells (p-selectin) and the interaction of these molecules with one another causes the neutrophil to adhere to the blood vessel wall. Once adherence is initiated, tighter binding occurs through the expression of intracellular adhesion molecule 1 (ICAM-1) on the neutrophil, as well as through beta 2 integrins on the endothelial cell surface. Migration to the site of injury is in response to LTB_4 and the inflammatory peptide C5a, as well as to molecules of bacterial origin. Once at the site of injury, phagocytosis may occur and is mediated by the presence of opsonins C3b and immunoglobulin.

Following phagocytosis, intracellular microbicidal activity occurs via non-oxidative and oxidative pathways. The non-oxidative pathway involves the fusion of the intercellular non-specific granules containing lytic enzymes with phagosome. The oxidative pathway involves the production of reactive oxygen species within the specific granules of the macrophage or monocyte, and subsequent fusion of these granules with the phagosome. Alternatively, bacteria may not be phagocytosed but killed instead by extracellular degranulation of specific and non-specific granules. The reactive oxygen species $O2^-$ and OH^- are microbicidal; when formed within the phagocyte vacuole, they are potent mechanisms for killing bacteria. However, when these oxygen species are released into the extracellular environment, significant tissue damage may result.

Abnormalities and dysfunction at any step of the neutrophil response to infection can result in serious disease. Disorders of chemotaxis, phagocytosis, myeloperoxidase function, defective opsonization, and adherence are all observable. These diseases, while relatively rare, result in susceptibility to infection including periodontal infection. Furthermore, cyclic neutropenia and chronic agranulocytosis are characterized by reduced numbers of circulating neutrophils and increased susceptibility to infection including periodontal diseases. These conditions underline the importance of neutrophil function to periodontal health. They are characterized by diminished neutrophil function, and in contrast to hyperfunction, they illustrate the complexity of the inflammatory response.

Additional risk factors for periodontal disease: diabetes and smoking. Diabetes is a defined risk factor for chronic periodontal disease, and, in turn, periodontal infections may compromise glycemic control.[35] Possible mechanisms for this relationship have been investigated. Altered neutrophil and monocyte function in patients with diabetes are characterized by increased oxidative stress and impaired chemotactic and phagocytic capability.[23] In this respect, the profile of phagocytes in these patients is very similar to that seen in patients with aggressive periodontitis. The stimulus for priming of neutrophils and monocytes in people with diabetes seems to be mediated by advanced glycation end products (AGEs) binding to an extracellular receptor for advanced glycation end products (RAGE). This, in turn, initiates an intracellular cascade involving a protein kinase C (PKC) intracellular pathway whose final product is $O2^-$. This effect can be stopped by blocking the receptor for advanced glycation end products. The primed neutrophil state is characterized by several additional abnormalities: up-regulation of oxygen free radical species production; diminished phagocytic and chemotactic capability; and an increased predisposition to degranulation of catabolic and proinflammatory molecules.[27]

Smoking is also a well-defined risk factor for periodontal disease.[30] While there is no genetic component, the impact of smoking on neutrophils and monocytes affects periodontal health. Neutrophils taken from smokers, as with patients suffering from LAP or diabetes, demonstrate an increased oxidative burst but impaired phagocytosis and chemotaxis. Levels of both PGE_2, TNF-α, neutrophil collagenase and elastase are increased in the gingival crevicular fluid. Furthermore, monocytes taken from smokers demonstrate an increase in production of PGE_2 in response to stimulation with lipopolysaccharide. In many respects, the neutrophils of smokers appear primed.[33]

Treatment strategies

Management of aggressive periodontal diseases. Current management of aggressive periodontal diseases is focused primarily on eliminating the bacterial load and shifting the subgingival flora to a more favorable aerobic profile. This has been accomplished mainly with aggressive mechanical debridement with or without surgery, and often with the use of adjunctive antibiotic therapy. Protocols for antibiotic treatment vary widely but often involve the concomitant use of amoxicillin and metronidazole. Other

chemotherapeutics that have been studied include the tetracyclines, clindamycin, and the quinolones. Each has its relative advantages and disadvantages.

Management of chronic periodontal diseases. Current therapeutics recognize the destructive role of chronic inflammatory conditions. Therefore, contemporary treatment planning should also address management of inflammation.[34] The proinflammatory role of lipids is well known, and current NSAIDs target lipids specifically by inhibiting the conversion of cyclooxygenase 1 and 2 (COX-1 and COX-2) into prostaglandin; these medications have been shown to slow or halt periodontal destruction.[34] Unfortunately NSAIDs have undesirable side effects that can be severe in nature, and include gastric bleeding or gastrointestinal irritation; their safety in long-term use is debatable. Many of their negative effects stem from the fact they could be considered relatively blunt instruments, being used to modulate the very complex process of inflammation. In the process, these medications interrupt both the harmful and beneficial effects of inflammatory molecules. More recently, the role of lipid mediators in the resolution of inflammation has been elucidated. Two endogenously produced classes of molecules that promote the resolution of inflammation are lipoxins and resolvins.

Lipoxins are produced through cell-cell transmembrane interactions. It is known that aspirin triggered lipoxins (ATL) are produced through acetylation of the cyclooxygenase molecule. Indeed it was the discovery of ATL that, in turn, lead to the discovery of endogenous lipoxins. These were the first class of lipid molecules known to promote the resolution of inflammation. Lipoxins are known to inhibit neutrophil chemotaxis in response to both LTB_4 and fMLP, to inhibit angiogenesis, promote macrophage phagocytosis of apoptotic leukocytes, regulate cellular function of fibroblasts and endothelial cells and to inhibit the activity of TNF-α.[19] The beneficial effects of local and systemic application of these agents have been demonstrated in murine and rabbit models of periodontitis.[7]

Resolvins are a second recently described class of molecules derived from omega-3 fatty acids whose production is also triggered by aspirin. A recent study[19] demonstrated the beneficial effect of topical application of resolvins in a rabbit periodontitis model. It was shown that neutrophils from individuals with LAP produce as much as 10-15 times more superoxide than neutrophils from healthy individuals; this excess superoxide is damaging to the host. One study[35] reported that while lipoxins abrogate as much as 90% of superoxide production from healthy individuals, neutrophils from LAP healthy individuals are refractory to its effects; and conversely, that resolvins down regulate the production of superoxide by neutrophils from both healthy and LAP individuals.

Other recent studies are revealing new information about the impact of lipoxins and resolvins on periodontal disease. The authors have demonstrated that *Pg*-induced experimental periodontitis in rabbits can be arrested by topical application of synthetic lipoxins on the gingiva.[19] Briefly, periodontal disease was initiated in rabbits by the local application of *Pg*-soaked ligatures around the necks of the teeth. This destruction can be largely attenuated by the topical application of lipoxins or resolvins at the necks of the teeth.[36] Lipoxins and resolvins possess no antibacterial properties. The most logical explanation for their effect is that they inhibit the progression of inflammation. It should be noted that although tissue destruction was largely mitigated, there was no evidence that the rabbits suffered from uncontrolled local or systemic bacterial infection. Thus, Van Dyke and Dave[30] reported that, while lipoxins and resolvins modulate the inflammatory response, they do not interrupt it completely. Vital protective mechanisms remained largely intact in this model, though some of the rabbits in this study were susceptible to periodontal destruction consistent with current concepts regarding differences in human variation in susceptibility. Currently, lipoxins and resolvins are not available for clinical use. The results described suggest that they have the potential to fundamentally change future treatment strategies for periodontal disease.

Limited clinical impact has resulted from our understanding that the host response is the primary destructive agent in periodontal disease. Alternatives to mechanical debridement with or without antibiotic therapy have included the administration of sub-antimicrobial doses of doxycycline (SDD), which have been shown to inhibit the activity of collagenase but have no significant effect on microbes.[30] The SDD dose of 20 mg twice a day in conjunction with scaling and root planing has been shown to result in a statistically significant reduction in periodontal destruction over a one-year period.[37] However, the clinical significance of this effect is debatable, and to date this protocol has not achieved widespread utilization in the periodontal community.

Conclusion

Our present understanding of periodontal disease leads us to believe that while bacterial pathogens colonizing the sulcular environment are the primary etiologic agents, it is the destruction by the host response that plays a primary role in determining the extent of tissue destruction observed. On the basis of the recent studies of lipoxins and resolvins, as well as evidence from the successful use of NSAIDs for the treatment of periodontitis, we believe that the observed destruction is primarily caused by an excessive host inflammatory response to periodontal pathogens, and a failure of that response to properly resolve.

Regarding the current availability of effective and safe host immune modulation for treatment of periodontitis, while NSAIDs are effective, they have clear and significant risks. The sub-antimicrobial dose of doxycycline has been shown to be quite safe and clinically significant in clinical trials; however, this treatment modality has not gained widespread adoption. In the future, novel classes of molecules that promote the resolution of inflammation, including resolvins and lipoxins, may become clinically available, with the potential for tremendous impact on clinical management of periodontitis and other inflammatory conditions.

Disclosures

Thomas Van Dyke, DDS, PhD is a professor at Boston University, which is assigned patents on resolvins that are licensed for clinical development and are subject to consultant agreements.

Jon B. Suzuki, DDS, PhD, MBA serves on the scientific advisory boards of Biohorizons and Philips Oral HealthCare.

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