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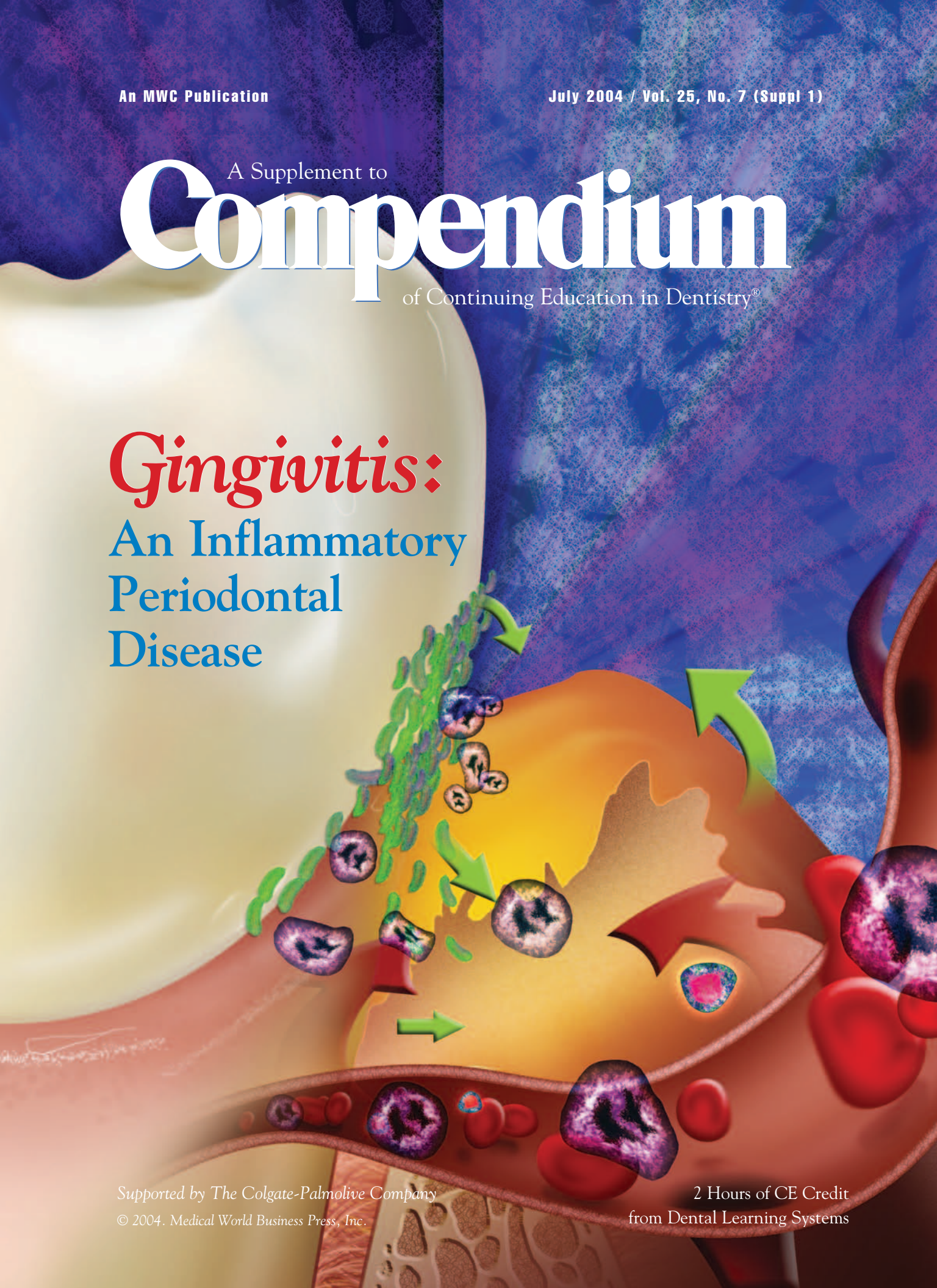
A Supplement to

Compendium

of Continuing Education in Dentistry®

Gingivitis: An Inflammatory Periodontal Disease



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Compendium

Dear Readers:

We are grateful to The Colgate-Palmolive Company for the educational grant that supports this issue because it deals with a process that affects most of our patients: inflammation in the oral cavity. Understanding the advances that have been made in identifying all of the reactions that occur with inflammation is of great importance to dentists, dental students, and dental hygienists. When the inflammatory lesion is recognized in our patients, it becomes imperative that a treatment plan to eliminate or reduce that process is implemented. The clinician also must be aware of the recent observations that indicate a possible oral–systemic link in patients with periodontal diseases who are pregnant; those at risk for cardiovascular disease; and those suffering from diabetes. Interestingly, the prevailing theory on coronary artery disease is the Ross Theory. Russell Ross,[a] who was a dentist and chaired the Department of Pathology at the University of Washington Medical School, wrote that atherosclerosis in coronary vessels was an inflammatory process. Today, more and more research studies are examining the possible mechanisms involved in linking inflammatory changes in oral tissues to systemic manifestations. Those in the profession today realize that the presence of gingivitis and periodontitis may have far greater implications on total systemic health than ever imagined before. This issue of *The Compendium* should be of great value to everyone involved in the treatment of patients with inflammatory changes in the oral cavity.

Sincerely,

Dr. D. Walter Cohen
Chancellor Emeritus
Drexel University College of Medicine

Dean Emeritus
University of Pennsylvania
School of Dental Medicine

Editor-in-Chief
The Compendium

Dear Colleagues:

One of my mentors during my periodontal residency at the University at Buffalo gave me keen insight to the phrase, "Let the [gingival] tissue speak." Careful visual observation would assist in describing the delicate tissue changes and begin to create a mental anatomy of the pathologies affecting the periodontium. Subsequent evidence-based therapeutic modalities led to the pathways toward optimum clinical health.

Fundamental to visual observations were the classical Latin terms included in this familiar medley: *calor* (heat); *dolor* (pain); *tumor* (swelling); *rubor* (redness); and *funcio laesa* (loss of function). This quintet of periodontal tissue characteristics provides visual language of the biology underlying the tissues through the stages of disease as well as in health. These terms, known from the earliest medical records, can, of late, be complemented with clinical research data from tissue messengers, also known as inflammatory mediators. Research observations give us a new vision and understanding of the mechanisms underlying what we can only "see" as clinicians.

We present this special issue of *The Compendium* as a reference manual to provide insight to this important field of dentistry and to better prepare you for optimum clinical care of your patients.

Sincerely,

C. Yolanda Bonta, DMD, MS, MS

Director of Technology
Professional Marketing/
External Relations
Academic and Professional
Relations
The Colgate-Palmolive Company

[a]Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med.* 1999;340:115-126.

Gingivitis:
An Inflammatory Periodontal Disease

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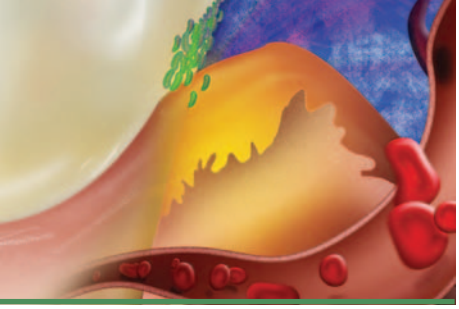


Inflammation, Periodontal Diseases, and Systemic Health

Abdul Gaffar, PhD
Vice President, Growth Technology

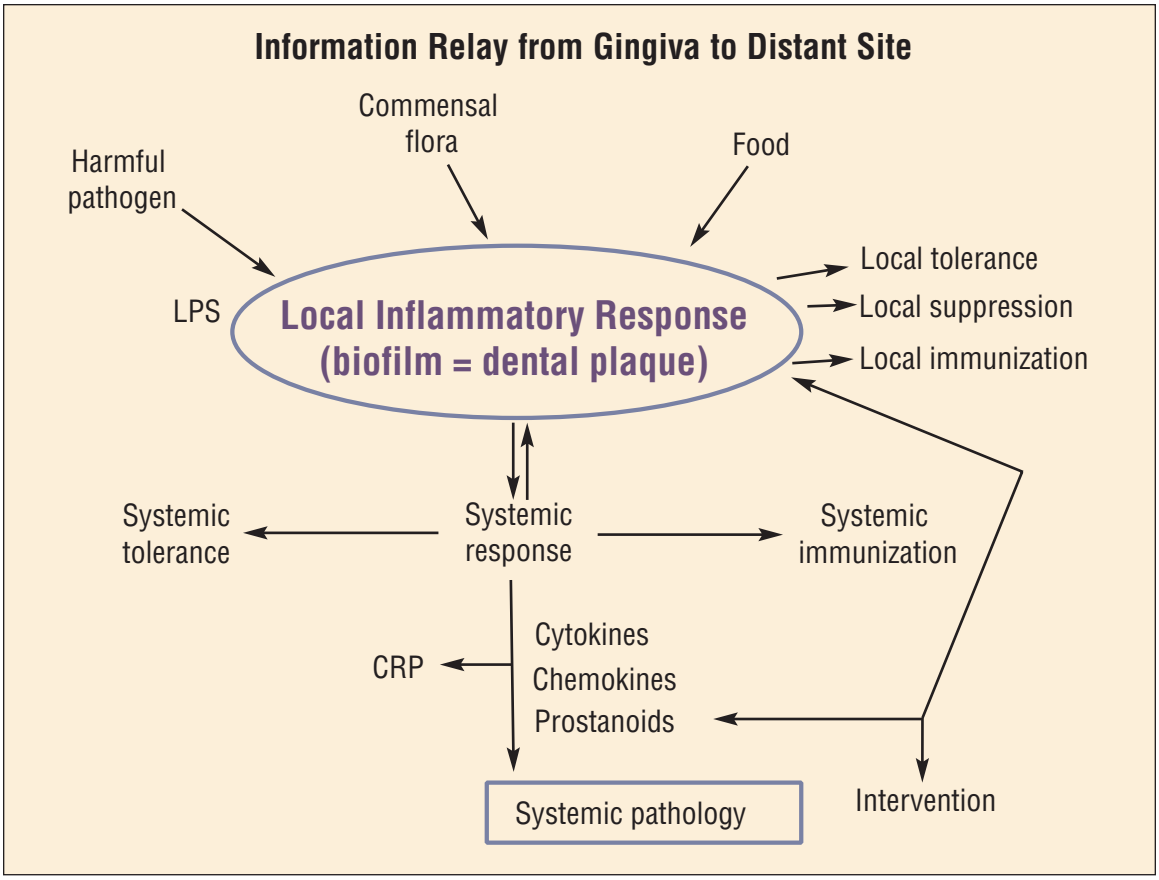
Anthony R. Volpe, DDS
Colgate Corporate Technology Group
Piscataway, New Jersey

A chronic inflammatory disease of the gingiva and periodontium results in destruction of gingival connective tissue, periodontal ligament, and alveolar bone. Clinically, inflammation is seen as redness, swelling, and bleeding upon probing. However, at molecular and cellular levels, the inflammatory process is defined by cellular infiltrates and the release of a variety of cytokines.[1] The main provoking factor that induces inflammation of gingival tissue is the presence of bacterial biofilm (dental plaque) on the teeth/gingival interfaces.[2] The products of biofilm bacteria, such as lipopolysaccharide (LPS) molecules, are known to initiate a chain of reactions in the tissue leading to host response as well as the destructive process (Figure).[3]

Current models of mucosal surfaces of oral, gut, lung, and skin tissue postulate that local bacterial antigens, derived from biofilms on surfaces, regulate local tolerance, local immune response, and systemic response by way of an "information relay system" through a series of nuclear factor-kappa beta pathways to synthesize and secrete cytokines and chemokines to regulate the inflammatory process at local as well as distant sites.[4] Evidence is also accumulating that the predominant cells of the periodontium, gingival fibroblasts, are capable of producing prostaglandins, interleukins (IL-1beta [β], IL-6, IL-8), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ).[5] It is hypothesized that these mediators modulate inflammation locally as well as at a distant site of infection.

Is there a rationale for linking the inflammation of gingival tissue and the pathophysiology of systemic chronic inflammatory diseases? In this supplement, two mechanisms are discussed. One presupposes the direct role of oral bacteria or their products in the pathogenesis of atherosclerotic plaque in myocardial infarctions. An alternative explanation is the possible role of mediators in inflammation initiated by periodontal pathogens in the development of chronic complications.[5-7] There is general agreement that chronic diseases, such as atherosclerosis, stroke, and diabetes, are multifactorial in origin. But there is growing evidence that these diseases are influenced by gingival inflammation and chronic periodontal infections. In a series of cross-sectional studies, a strong relationship has been found between acute-phase C-reactive protein (CRP) in serum and the severity of periodontal diseases.[8-11] CRP is triggered by infections, trauma, necrosis, and malignancy,[12] and is also linked to heart disease and diabetes. CRP is synthesized in the liver in response to proinflammatory cytokines such as IL-1α, IL-1β, and IL-6. TNF-α, IFN-γ, and transforming growth factor also participate in the production.

The current therapeutic strategy to control periodontal infections involves mechanical removal of deposits, both supra- and subgingival. This also could involve the use of topical and systemic antimicrobial agents. There are, however, very few studies to assess the effects of these therapies concomitantly with systemic health. Two pilot clinical studies with sub-antimicrobial administration of doxycyline indicated that these regimens



Figure—This illustration shows the local bacterial products that can influence the release of cytokines, which could moderate inflammation at a distant site. It also identifies two possible sites for intervention. Adapted from Hayday A, Viney JL.[4]

reduced the "marker" of periodontal diseases, as well as risk markers of acute coronary syndrome and diabetes.[12,13]

Can topical antimicrobials applied via an oral delivery system (toothpaste, rinse, or gels) also achieve such an outcome? It is believed that a triclosan/copolymer/fluoride dentifrice system could provide such a benefit. Triclosan is a broad-spectrum antibacterial agent that has been shown to kill oral pathogens at 0.3 ppm to 5 ppm. Because of a favorable partition coefficient, it can readily penetrate gingival tissue and reach the target subgingivally.[14] Relatively high concentrations of triclosan are retained in the plaque postbrushing (40 ppm after 2 hours and retained above minimum inhibitory concentration 12 hours postbrushing). It is an unusual antibacterial because it also has anti-inflammatory effects. For example, it inhibits both cyclooxygenase and lipooxygenase pathways at concentrations well below those retained in the dental plaque.[15] It has also been observed that triclosan inhibits the release of prostaglandin E_2 from IL-1β–stimulated gingival fibroblasts, as well as reduces the actual production of IL-1β and IFN-γ.[16] In long-term studies, topical effects of specially formulated triclosan have been shown to reduce or prevent periodontal disease in humans.[17]

> The main provoking factor that induces inflammation of gingival tissue is the presence of bacterial biofilm (dental plaque) on the teeth/gingival interfaces.

Recently, such a formulation was shown to significantly improve (P = .05) oral hygiene, gingival health, and periodontal status in a group of high-risk smokers for 24 months.[18] Based on the success of the agent as a topical antibacterial in the mouth, albeit in a specialized formulation, it stands to reason to evaluate the agent for its effect on periodontal inflammation concomitant to its effect on systemic inflammation. To confirm this hypothesis, long-term prospective studies will be needed.

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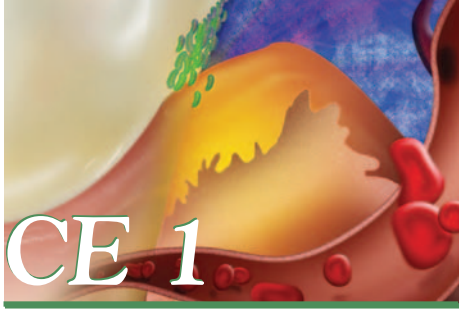
A Primer on Inflammation



Angelo Mariotti, DDS, PhD
Professor and Chair of Periodontology
The Ohio State University
College of Dentistry
Columbus, Ohio

Abstract: ***Inflammation is the localized, protective response of the body to injury or infection. The classic clinical signs that characterize inflammation are heat, redness, swelling, pain, and loss of function. During inflammation, cells and their secreted chemicals attempt to destroy, dilute, or wall off the injurious agent. A series of biochemical events cause the blood vessels to dilate and become more permeable, resulting in the activation of the complement, clotting, and kinin systems. The end result of inflammation is the return of function by the regeneration or repair of the affected tissue. In some instances, inflammation may continue for a prolonged period of time, producing untoward consequences for localized tissue as well as the entire body. The purpose of this article is to provide a basic and simplified understanding of how the inflammatory process functions in the human body.***

News about inflammation surrounds us. It is no longer confined to textbooks and dental classroom lectures. Today, even the lay press has suggested that our well-being is inextricably bound to an absence of inflammation. The interest in inflammation has been heightened because the etiology of many systemic diseases (once thought to be independent of the inflammatory process) has been associated with some component of the inflammatory process. Even in dentistry, inflammatory diseases can significantly affect tissues of the oral cavity, and, most recently, oral inflammatory processes have been implicated in affecting tissues distant from the mouth.

Although most of the recent press on inflammation has emphasized the deleterious effects it has on the body, it must be noted that without the inflammatory process, life as we know it could not survive. Inflammation involves the release of biochemical agents from cells located around vascularized tissues that defend the host against infection and facilitate tissue healing and repair.

The function and stages of the inflammatory process are not difficult to understand, but the response of cellular systems to biochemical mediators that regulate the various stages of inflammation is complex. The purpose of this article is to provide a basic and simplified understanding of how the inflammatory process functions in the human body.

Acute Inflammatory Response

Inflammation is a nonspecific and immediate cellular and biochemical response that begins after cellular injury. Cellular injury may occur as a result of trauma, genetic defects, chemical agents, radiation, microorganisms, etc. In general, the inflammatory process can be divided into mast cell responses, activation of plasma protein systems, and the release of cellular products (Figure 1).

Mast Cells

Mast cells are located in the connective tissues close to blood vessels and contain a variety of biochemical agents located in intracellular granules.[1,2] Immediately after cellular injury, mast cells release preformed biochemical

Learning Objectives

After reading this article, the reader should be able to:

- describe mast cell functions.
- discuss the three plasma protein systems associated with the inflammatory process.
- cite examples of inflammatory cellular products.
- list the features of chronic inflammation.
- explain the difference between regeneration and repair.

Table 1—Mechanisms Used by Neutrophils to Kill Microorganisms
Oxygen-independent mechanisms
Azuoocidin
Bacterial (permeability-inducing) protein
Cathepsin G
Defensins
Elastase
Lactoferrin
Lysozyme
Myeloperoxidase
Proteinase 3
Oxygen-dependent mechanisms
Chloramines
Hydrogen peroxide (H_2O_2)
Hydroxyl radicals
Hypochlorous acid
Singlet oxygen
Superoxide anion

mediators that include histamine, neutrophil chemotactic factor, and the eosinophil chemotactic factor of anaphylaxis (Figure 2). Histamine is a vasoactive amine that causes the rapid constriction of the smooth muscle cells of large blood vessels, dilation of postcapillary venules, and increased vascular permeability. This results in elevated pressure in the microcirculation, which increases exudation of plasma, blood cells, and platelets into the surrounding tissues (Figure 3). As plasma moves into the connective tissue, the remaining blood flows more slowly and becomes more viscous, thereby allowing leukocytes to attach to vessel walls and squeeze through the spaces created by endothelial retraction (Figure 3). Two chemical signals, the neutrophil chemotactic factor and the eosinophil chemotactic factor of anaphylaxis, cause the unidirectional movement of neutrophils and eosinophils. The first leukocytes at the inflamed site are neutrophils. These cells constitute almost 70% of the peripheral blood leukocytes and are responsible for killing microorganisms (Table 1) as well as the phagocytosis of dead cells and cellular debris. Because neutrophils have a short life span, another group of phagocytic cells (ie, monocytes and macrophages) perform similar functions to neutrophils but are present for longer periods of time. Eosinophils are also attracted to the inflamed site and, in addition to being phagocytic, can control the mediators released from mast cells. In addition to the release of preformed biochemical mediators, mast cells also synthesize leukotrienes, prostaglandins, and platelet-activating factor. Leukotrienes are produced from arachidonic acid and increase vascular permeability and neutrophil chemotaxis in a slower and more prolonged response when compared to histamines. Similar to leukotrienes, prostaglandins are derived from arachidonic acid and increase vascular permeability and neutrophil chemotaxis. Prostaglandins also induce pain and can suppress the release of histamine from mast cells. Platelet-activating factor is derived from plasma membrane phosphatidylcholine and enhances vascular permeability and activation of platelets.

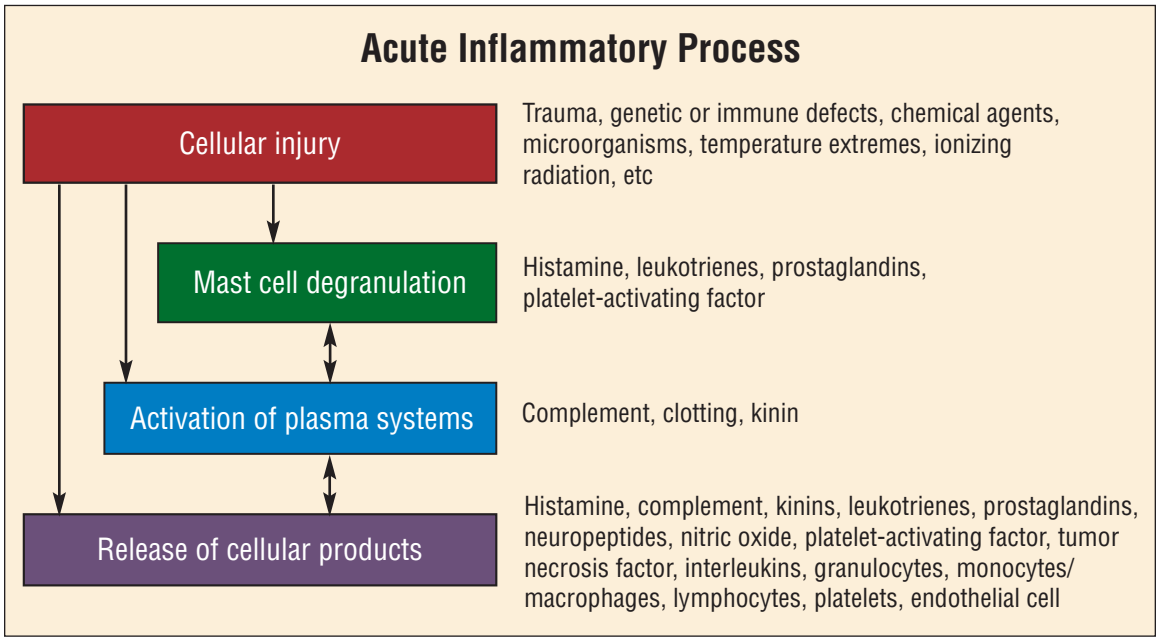


Figure 1—Relationship of mast cell plasma systems and cellular products during acute inflammation.

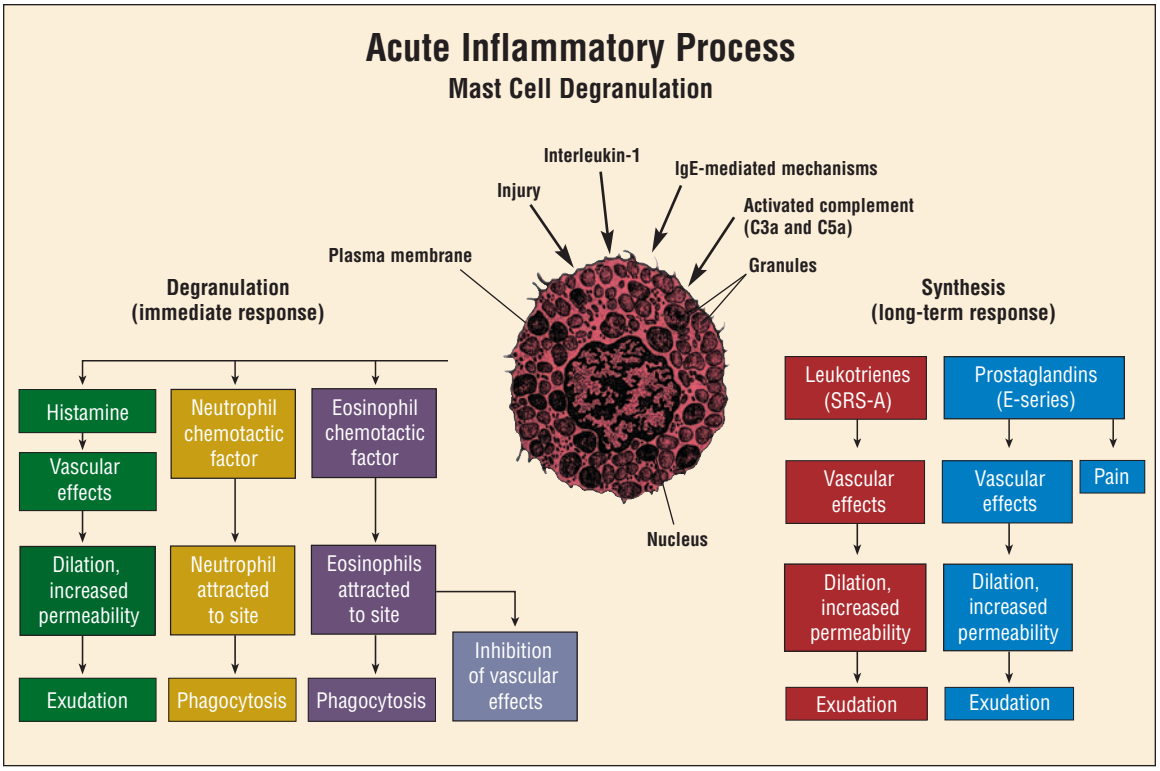


Figure 2—Effects of degranulation (left) and synthesis (right) by mast cells. Adapted from McCance KL, Huether SE, eds. *Pathophysiology—The Biologic Basis for Disease in Adults and Children.* 4th ed. Philadelphia, Pa: Mosby; 2002:200.

Plasma Protein Systems

Three important plasma protein systems mediate the inflammatory process. The *complement* system,[3] the *clotting* system,[4] and the *kinin* system,[5] all consist of a series of inactive enzymes that are converted through a cascade of steps to active enzymes. Most components of these systems have limited half-lives because they are rapidly inactivated by other proteins found in the bloodstream.

The 10 proteins that constitute the complement system comprise approximately 10% of all serum proteins in the circulation. The complement system can be activated by antigen–antibody complexes (classical pathway), bacterial components (alternative pathway), and specific plasma proteins (alternative pathway) (Figure 4). Many of the complement subcomponents are biochemical mediators that augment inflammation by leukocyte chemotaxis, degranulation of mast cells, opsonization of bacteria, and the creation of pores in plasma membranes. Similar to the complement system, the clotting system can be activated through two pathways called either the extrinsic or intrinsic pathway (Figure 5). The clotting system can be activated by substances (eg, collagen, proteinases, kallikrein, bacterial endotoxins, etc) that are released during tissue destruction. The functions of the clotting system during inflammation include: preventing the spread of infection and inflammation; localizing microorganisms and debris at the site of phagocytosis; clot formation to stop bleeding; clot formation to form a scaffold for repair and healing; chemotaxis of neutrophils; and increased permeability of the vasculature. Perhaps the least known of the plasma protein systems is the kinin system (Figure 6). The primary molecule of the kinin system is bradykinin. In low doses, bradykinin acts to increase vascular dilation and permeability, stimulate smooth muscle contraction, activate leukocyte chemotaxis, and induce pain.

> Three important plasma protein systems mediate the inflammatory process: complement, clotting, and kinin systems.

The complement, clotting, and kinin systems are all tied together through a mixture of interactions in which a protein from one system can activate one or both of the other plasma protein systems. The interconnections

between the three systems can occur through the effects of plasmin, kallikrein, clotting factor XI, Hageman factor (clotting factor XII), and complement fragments, as well as other molecules in the plasma protein systems (Figure 7). One example that demonstrates how the plasma systems are interrelated revolves around plasmin. More specifically, plasmin digests fibrinogen and fibrin, activates C1 to form C1 esterase (classical complement pathway), activates the Hageman factor (stimulates kinin production), and cleaves C3 to produce anaphylatoxic and chemotactic C3 fragments.

Cellular Products

Host cells produce a myriad of soluble proteins that act directly on proteins, cells, or invading microorganisms and, ultimately, contribute to the defense of the body.[6-10] The inflammatory factors have been identified by a numbing array of acronyms (eg, CSF, MAF, MIF, PDGF, ENA, MCP, IL, TNF, ICAM, INF, PAF, etc) and unless one is familiar with these proteins (and acronyms), understanding the inflammatory process can be confusing and frustrating. The diverse number of cellular products can be simplified into four categories: cytokines, hormones, mediators, or growth factors. A cytokine is a low-molecular-weight, biologically active protein that alters the function of the cell that released it (autocrine) or the function of adjacent cells (paracrine). Cytokines can be synergistic (the combined activity exceeds the sum of individual activities) or antagonistic (inhibitory). Individual cytokines are pleiotropic (diverse biological roles), whereas different cytokines may have similar biological activity. As a result, cytokines have a broad variety of actions combined with functional redundancy within the inflammatory system.[6-9] Examples of cytokines are the interleukins. Hormones are biologically active molecules that are released into the bloodstream and stimulate functional activity of cells distant from the site of secretion.[10] An example of a hormone is insulin. Mediators are proteins that are found in the plasma protein systems as well as agents released from mast cells.[1,2] An example of a mediator is histamine.

The interest in inflammation has been heightened because the etiology of many systemic diseases has been associated with some component of the inflammatory process.

Growth factors are a complex family of signal molecules that regulate differentiation, proliferation, growth, and maturation of cells.[7] An example of a growth factor is transforming growth factor.

With the baffling collection of inflammatory molecules, which of these molecules are necessary and required for the inflammatory process? A simple answer to this question is that all inflammatory molecules are important; however, a closer inspection of the inflamma-

Table 2—Inflammatory Molecules and Their Functions

Molecules	Functions*
Chemokines	Chemotactic, activate cells, ↑ cytokine release
Complement	Opsonize, chemotactic, anaphylatoxin
Intercellular adhesion molecules	Promote adhesion of cells to endothelium
Interferon	Antiviral protection, activation of macrophages
Interleukins	Chemotactic, mitogenic, cytokine release, ↑ immune/inflammatory cells
Kinins	SM contraction, ↑ permeability, chemotactic, pain
Leukotrienes	SM contraction, ↑ permeability, chemotactic
Lymphokines	Inhibits macrophage migration, ↑ phagocytosis
Platelet-activating factor	↑ permeability, leukocyte adhesion
Prostaglandins	SM contraction, ↑ permeability, chemotactic, pain
Transforming growth factor	Chemotactic, cytokine release, ↑ fibroblasts
Tumor necrosis factor	Cytokine release, ↑ phagocytosis, ↑ macrophages
Vasoactive amines	SM contraction, ↑ permeability, chemotactic

*Represents only a partial list of functions of cellular agents. SM = smooth muscle; ↑ = increased.

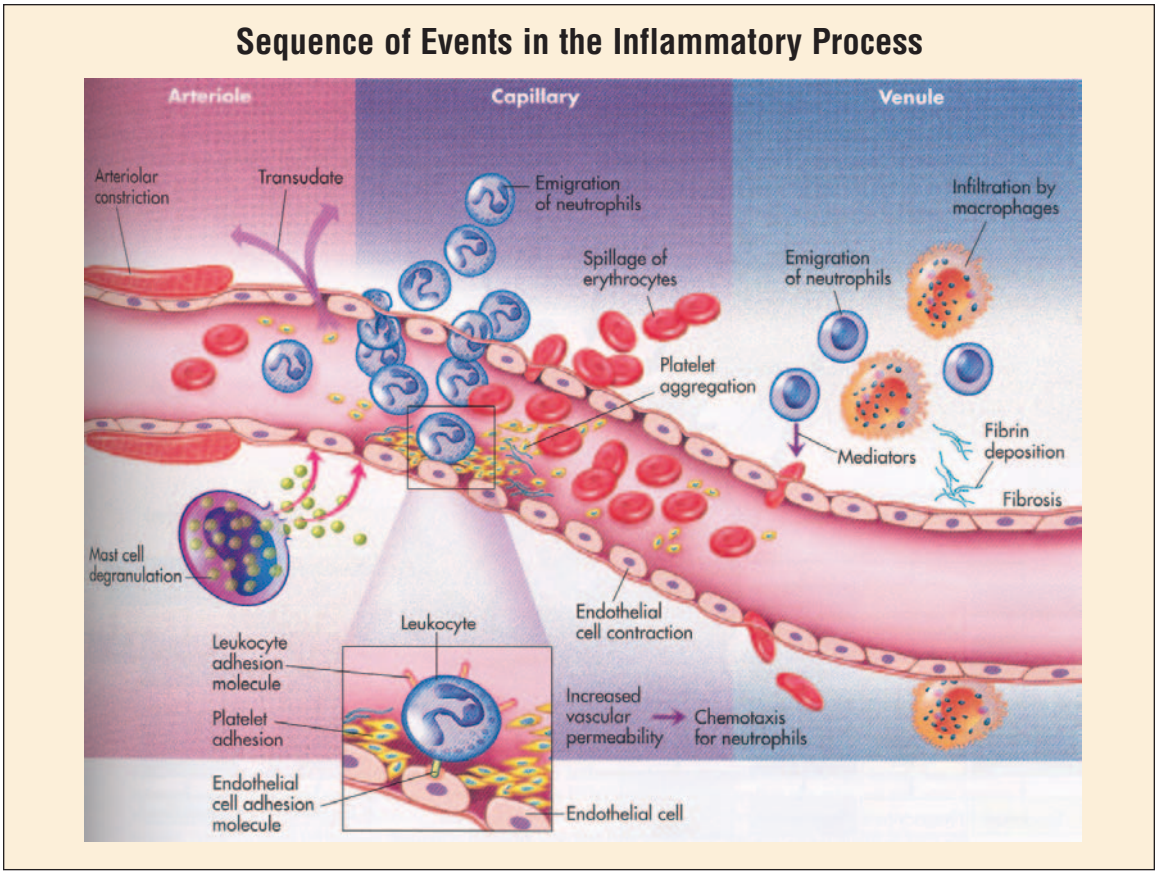


Figure 3—Sequence of events in the process of inflammation. Adapted from McCance KL, Huether SE, eds. *Pathophysiology—The Biologic Basis for Disease in Adults and Children.* 4th ed. Philadelphia, Pa: Mosby; 2002:199.

tory process suggests that this complex system is not fully understood, so therefore we cannot accurately answer the question. If one considers the functional redundancy of many cytokines as well as the fact that investigators have much to learn about the cellular, molecular, biochemical, and genetic components of inflammation, it becomes difficult to identify those molecules that are both required and necessary for a particular inflammatory event. Until the time comes when essential roles of regulatory molecules during inflammation can be recognized, a continued understanding of major categories of inflammatory molecules is necessary. Table 2 provides a partial list of inflammatory cellular products and their functions.

Chronic Inflammation

Chronic inflammation is generally considered an inflammatory process that lasts for a prolonged period of time (ie, weeks, months, or even years). Although there is no clear dividing line between acute and chronic inflammation, the extended time frame for chronic inflammation is a result of persistent stimuli from causative agents. For example, in the oral cavity, periodontal diseases are often considered chronic inflammatory processes because of the difficulty of eliminating oral bacteria via host defenses. Dental biofilms (ie, plaque) provide a shelter for microorganisms to grow and proliferate and, in turn, release bacterial products while continually provoking an inflammatory host-cell response.

In the oral cavity, periodontal diseases are often considered chronic inflammatory processes because of the difficulty of eliminating bacterias via host defenses.

The histological appearance in chronic inflammation is characterized by a mixed inflammatory infiltrate consisting predominantly of lymphocytes, macrophages, and plasma cells. In addition, there is a proliferation of fibroblasts and vascular elements in the inflamed tissues that result in an increased

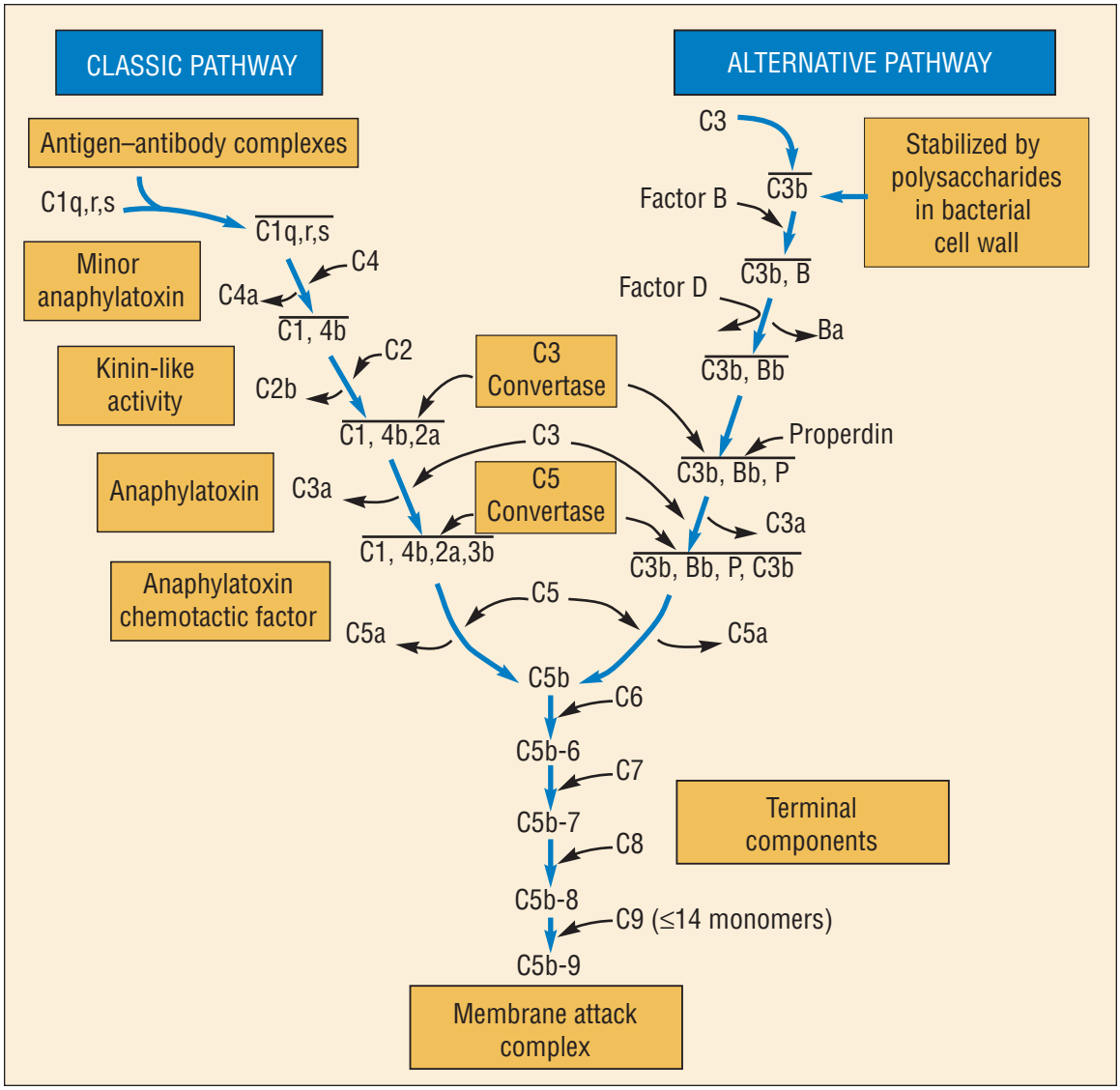


Figure 4—Pathways of activation of the complement cascade. Complement components are cleaved into fragments, or subcomponents (denoted by lowercase letters), during activation. Many of the subcomponents are biochemical mediators of inflammation. The larger activated fragment is usually converted into an active enzyme (indicated by the bar above the fragment) and forms a complex with the proceeding components in the cascade. The classic pathway usually is activated by antigen–antibody complexes through component C1, whereas the alternative pathway is activated by many agents, such as bacterial polysaccharides, through component C3b. Both pathways produce C3 convertases and C5 convertases, which are enzymatically active complexes that activate C3 and C5, respectively. Adapted from McCance KL, Huether SE, eds. *Pathophysiology—The Biologic Basis for Disease in Adults and Children.* 4th ed. Philadelphia, Pa: Mosby; 2002:203.

amount of connective tissue. Both T-helper cells and macrophages are important cells in initiating a chronic inflammatory response. During chronic inflammation, the activated macrophages will release numerous secretory products[11] (eg, neutral proteases, acid hydrolases, plasminogen activators, nitric oxide, interferons, complement components, fibronectin, interleukins, and angiogenesis factors) that, in combination with other host cells (ie, neutrophils, eosinophils, T-cells, fibroblasts, mast cells, epithelial cells, etc), may cause significant local tissue destruction. What ultimately results is a healing process that oscillates between tissue destruction and tissue regeneration, often ending in tissue repair instead of tissue regeneration. This incomplete wound healing may result in a permanent loss of function.

During chronic inflammation, the capacity to assemble this type of inflammatory environment places the host tissue under continual stress to destroy the target stimuli. Moreover, the continued production of chemical agents from host cells may not only affect local tissues, but may also affect tissues distant from the site where chemical mediators are being produced.

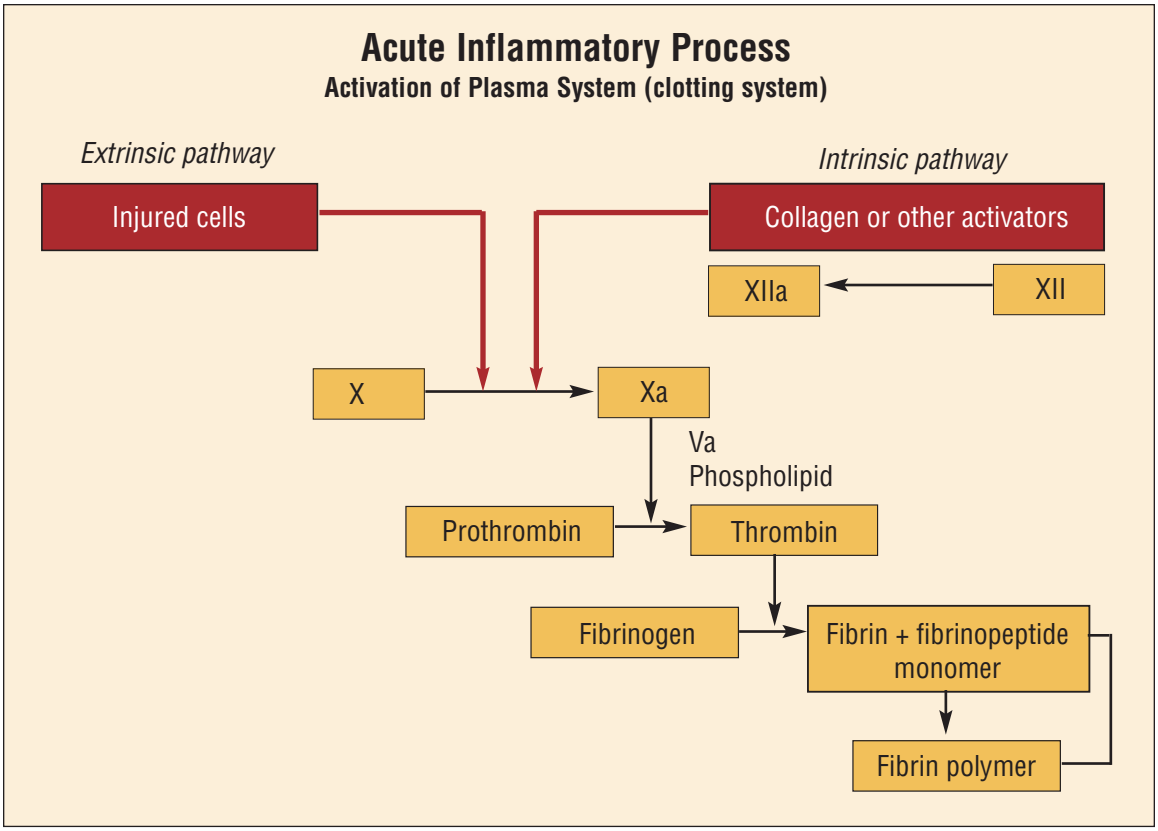


Figure 5—Coagulation cascade. During activation of the coagulation cascade, several components are converted from inactive to active forms. Adapted from McCance KL, Huether SE, eds. *Pathophysiology—The Biologic Basis for Disease in Adults and Children.* 4th ed. Philadelphia, Pa: Mosby; 2002:205.

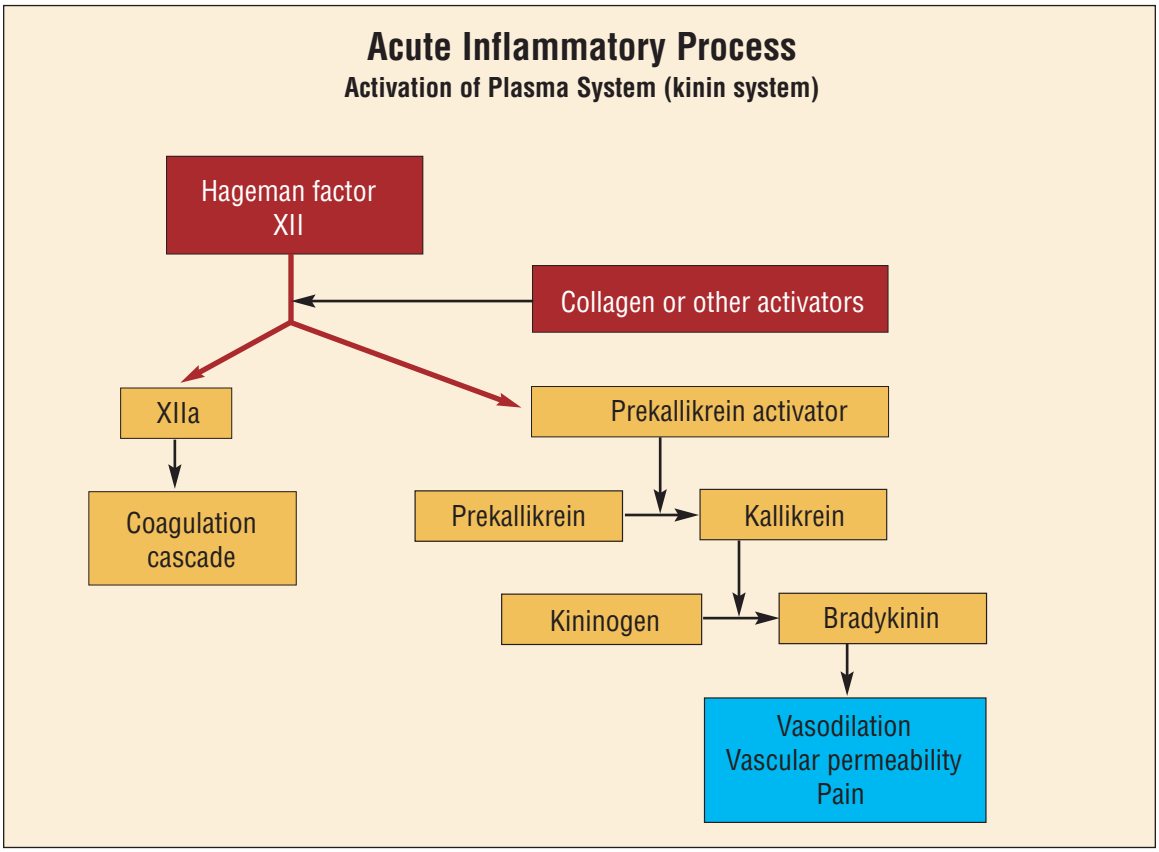


Figure 6—Plasma kinin cascade. Adapted from McCance KL, Huether SE, eds. *Pathophysiology—The Biologic Basis for Disease in Adults and Children.* 4th ed. Philadelphia, Pa: Mosby; 2002:206.

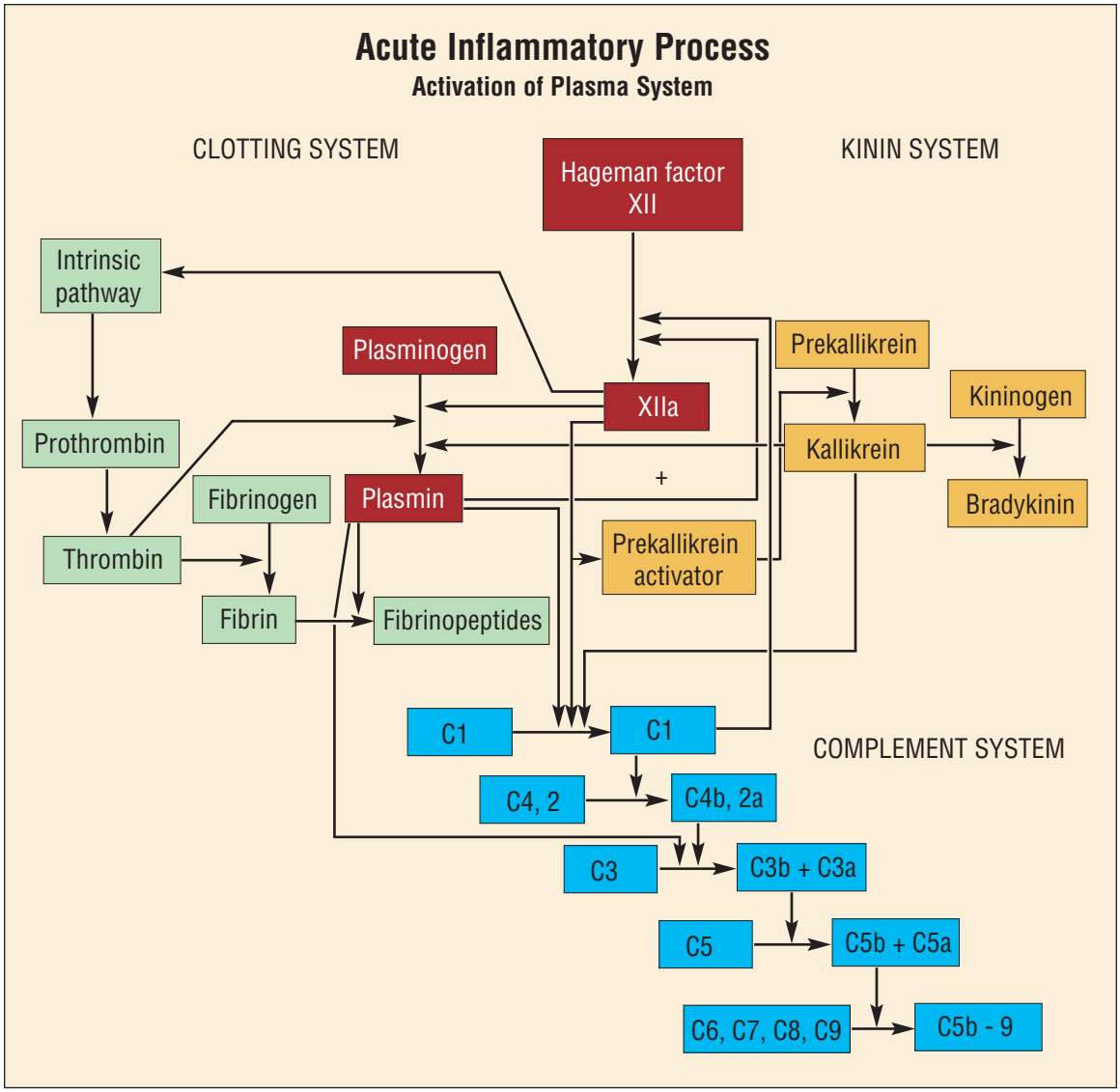


Figure 7—Interaction of the complement, clotting, kinin, and fibrinolytic (plasmin) systems. Adapted from McCance KL, Huether SE, eds. *Pathophysiology—The Biologic Basis for Disease in Adults and Children.* 4th ed. Philadelphia, Pa: Mosby; 2002:207.

Tissue Regeneration or Repair

Tissue destruction is a consequence of the inflammatory process, but it is followed by regeneration or repair. *Regeneration* is the reproduction or reconstitution of a lost or injured part of the tissue, and usually occurs if the tissue damage is minor. Tissue *repair* is characterized by wound healing that does not fully restore the architecture or function of the damaged tissue, and occurs when there is extensive tissue destruction. Repair concludes in scar tissue formation. Both regeneration and repair involve the dissolution of fibrin clots and the removal of toxic agents and particulate matter to prepare the lesion for wound healing. During the reconstructive phase of wound healing, granulation tissue contains fibroblasts and newly synthesized compounds (eg, collagens) of the extracellular matrix. After the reconstructive phase, collagen deposition, tissue regeneration, or scar formation and wound contraction define the maturation phase of wound healing.

Systemic Effects of Inflammation

The most common systemic effects of inflammation include fever and leukocytosis (abnormally high white blood cell count); however, in particular circumstances, inflammation can do more harm than good. For example, destructive forms of inflammation can affect joints (rheumatoid arthritis), the kidney (glomerulonephritis), blood vessels (vasculitis), cardiac blood vessels (atherosclerotic lesion development), the pancreas (diabetes mellitus), and the periodontium (periodontal diseases). It has been hypothesized that in the periodontium, the dentogingival

surface area of individuals with periodontitis (8 to 20 square centimeters[12]) is responsible for the release of cytokines that adversely affect tissues distant from the periodontium. Although recent data have suggested that periodontal inflammation may influence distant tissues, the functional relationship between periodontal inflammation and systemic disease remains to be elucidated.[13]

Conclusion

The inflammatory process occurs in vascular tissue and is a biochemical and cellular process that occurs approximately in the same manner regardless of the stimulus. On injury, blood vessels dilate and become more permeable, resulting in increased blood flow and leakage of plasma and cells into the extracellular matrix. The cells and platelets carry out their functions with the support of the three major plasma protein systems. Moreover, inflammation functions to destroy and remove noxious agents, confine the injurious agents for efficacious removal, limit systemic effects of cellular agents, enhance the immune response, and promote wound healing. Therefore, the biologic significance of inflammation is primarily associated with the defense of the body from injury and infection.

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Periodontal Inflammation: From Gingivitis to Systemic Disease?

Frank A. Scannapieco, DMD, PhD
Professor
Department of Oral Biology
School of Dental Medicine
State University of New York at Buffalo
Buffalo, New York

Abstract: ***There has been a resurgence of interest in recent years in the systemic effects of oral infections such as periodontal diseases. The study of the various means by which periodontal infections and inflammation may influence a variety of systemic conditions is collectively referred to as periodontal medicine. The periodontium responds to tooth-borne biofilm (dental plaque) by the process of inflammation. Dental biofilms release a variety of biologically active products, such as bacterial lipopolysaccharides (endotoxins), chemotactic peptides, protein toxins, and organic acids. These molecules stimulate the host to produce a variety of responses, among them the production and release of potent agents known as cytokines. These include interleukin-1 beta, interleukin-8, prostaglandins, and tumor necrosis factor-alpha. There is a spectrum of periodontal response to these molecules, from mild gingivitis to severe destructive periodontitis. These and other host products and responses may influence a variety of important disease pathways, including atherosclerosis, mucosal inflammation, and premature parturition. The purpose of this article is to review the possible biological pathways by which periodontal diseases may influence these disease processes.***

Learning Objectives

After reading this article, the reader should be able to:

- gain insight into the pathogenic mechanisms responsible for gingival inflammation.
- discuss plausible mechanisms that may explain associations between gingival and periodontal inflammation and systemic disease.
- describe oral therapeutic interventions that may positively influence systemic health.

There has been increasing attention paid in recent years to the possibility that oral bacteria and oral inflammation, particularly periodontal diseases, may influence the initiation and/or progression of several systemic disease processes. This, of course, is not a novel concept. Indeed, the focal-infection hypothesis, which grew from the principles of infectious disease first established by Koch and Pasteur in the mid-19th century, put forth the notion that the invasion of the bloodstream by bacteria from a localized infection (such as periodontal diseases) could spread to distant organs and tissues to cause disease.[1-3] In fact, this hypothesis was so convincing to practitioners of the time that tonsillectomy and full-mouth extraction enjoyed widespread implementation to treat many diseases, regardless of whether or not infection could be proven to be the cause. However, because it became clear that it was impossible to correlate with confidence a particular systemic disease with a preceding oral infection or dental procedure, the focal-infection hypothesis fell from favor by the middle of the 20th century. Yet, interest in the systemic effects of periodontal infection was reignited in the early 1990s by a series of case-control and other epidemiologic studies that demonstrated statistical associations between poor oral health and several systemic diseases. The goal of this article is to describe the biologically plausible circumstances that underlie these potential associations. The reader is further referred to recent definitive reviews on the pathogenesis of periodontal disease for specific details that are beyond the scope of this article.[4,5]

The periodontium responds to the tooth-borne biofilm, long known as dental plaque, by the process of inflammation. Plaque is composed of

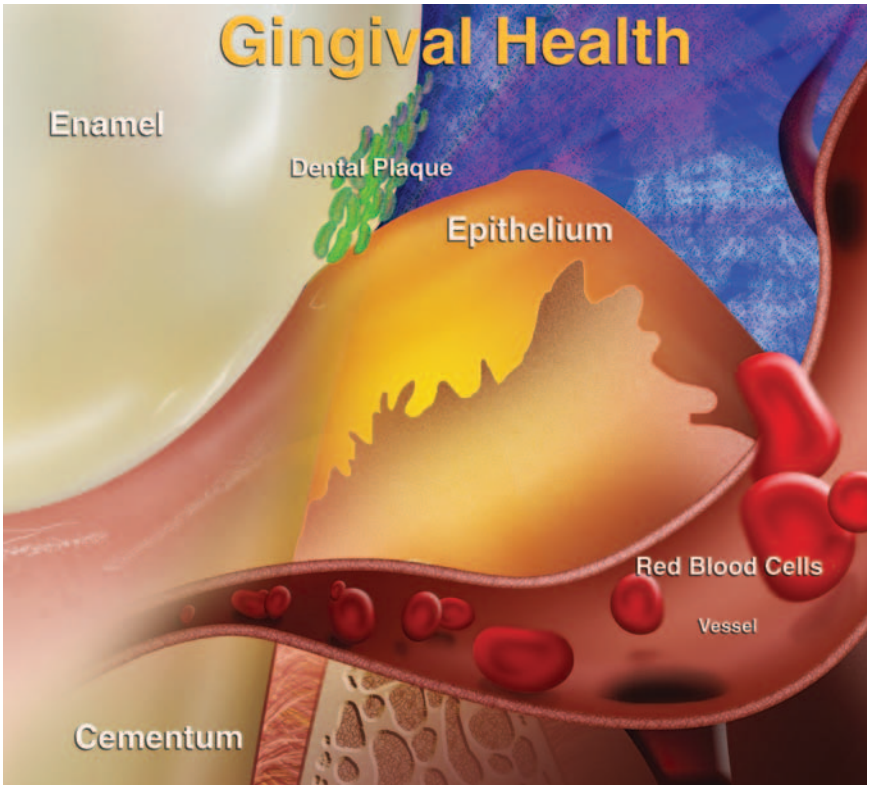

Figure 1—Biochemical events in periodontal disease. Pristine gingiva are not exposed to significant numbers of plaque microorganisms to yield a host response. Few signs of acute inflammation or cellular infiltrate are noted.

numerous bacteria, comprising over 400 species, which tenaciously adhere to the tooth surface.[6] Scientists are now beginning to understand the complex molecular interactions that occur, for example, between the bacteria and salivary pellicle that coats the tooth, and between gram-positive cocci of early plaque and gram-negative filamentous bacteria that populate the tooth as plaque matures.[7] Recent work has elucidated complex signaling pathways (referred to as quorum sensing) between bacteria, mediated by soluble chemicals produced by the bacteria that control biofilm development.[8] It is anticipated that this knowledge will eventually yield sophisticated strategies to limit the pathogenic potential of dental plaque.

Within a few hours of meticulous tooth cleaning, bacteria colonize the tooth surface primarily around the gingival margin and interdental spaces (Figure 1).[9] The developing biofilm releases a variety of biologically active products, including lipopolysaccharides (endotoxins), chemotactic peptides, protein toxins, and organic acids.[4] These molecules diffuse into the gingival epithelium to initiate the host response that eventually results in gingivitis and, in some circumstances, inflammatory periodontal diseases.[4] Clinically, gingivitis is characterized by a change in color—from normal pink to red—with swelling and, often, sensitivity and tenderness.[10] Gentle probing of the gingival margin typically elicits bleeding.[10] Because gingivitis is often not painful, it may remain untreated for many years.

Epidemiologically, the prevalence of gingivitis in non-Hispanic whites is approximately 50% of the population, with up to 63% in Mexican Americans showing clinical signs of the disease.[11] It is quite possible that this rate is somewhat understated because it is possible that gingivitis, in its most nascent form, is clinically undetectable. Periodontitis affects approximately 35% of dentate US adults 30 to 90 years of age, with 21% having a mild form and 12% having a moderate or severe form of the disease.[12] Thus, gingivitis is much more widespread than periodontitis in the US population.

Pathogenic Mechanisms of Gingival Inflammation

Histopathologically, gingival inflammation presents as a spectrum of severity in humans.[7] In a relatively small subset of the population, the gingiva are virtually devoid of inflammatory infiltrate, the so-called "pristine gingiva" (Figure 2).[7] These subjects practice impeccable oral hygiene and demonstrate no clinical signs of inflammation. More widespread would be the "normal healthy gingiva," which demon-

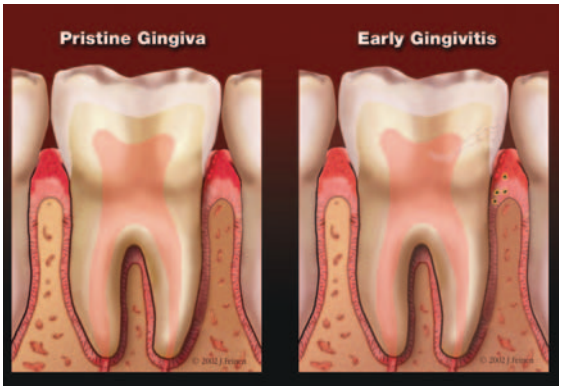

Figure 2—Left panel: Pristine gingiva is found in subjects with impeccable oral hygiene and minimal plaque. Gingival tissues are free of clinical signs of inflammation, and tissues are essentially free of inflammatory infiltrate. Right panel: Early gingivitis is found in subjects with some plaque formation. While the gingival tissues are free of clinical signs of inflammation, a mild inflammatory infiltrate is evident, consisting of vasculitis and the presence of neutrophils.

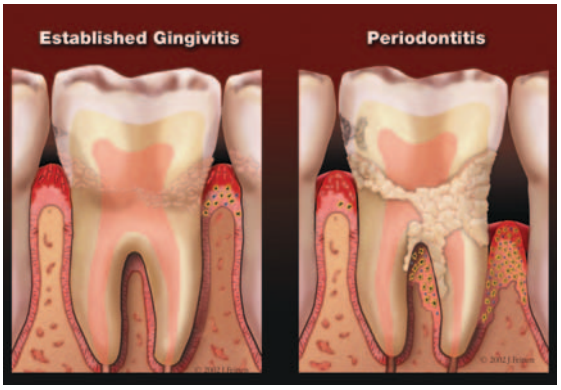

Figure 3—Left panel: In the absence of effective plaque control, a robust inflammatory response results in clinical signs of inflammation (redness, edema, bleeding) and a significant inflammatory infiltrate, including neutrophils, lymphocytes, and evidence of collagen breakdown. Signs of periodontal attachment loss or alveolar bone loss are not evident. Right panel: The inflammatory response results in marked collagen breakdown, periodontal attachment and alveolar bone loss, and clinical signs of inflammation.

strates a mild-to-moderate inflammatory infiltrate. Clinically, these two conditions would appear indistinguishable in that the tissues would appear quite healthy.

Probably most prevalent in the population is established gingivitis that is associated with a more widespread biofilm and clear clinical symptomology (redness, swelling, and bleeding), and histopathologically showing significant inflammatory infiltration (Figure 3).[7]

The most severe form of periodontal diseases results in the destruction of the periodontal ligament and supporting osseous tissue and, ultimately, exfoliation of the teeth. Periodontitis is associated with extensive formation of biofilm dominated by anaerobic, gram-negative bacteria and spirochetes.[13]

Established gingivitis, most prevalent in the population, is associated with more widespread biofilm and clear symptomology.

As mentioned previously, initial dental plaque bacteria (typically gram-positive cocci and filaments) release a variety of chemical compounds during their normal metabolism (organic acids, chemotactic peptides, etc). These products are soluble and penetrate the superficial layers of the sulcular epithelium. These substances signal the epithelium of the gingiva to produce a variety of biologically active mediators, most prominently cytokines such as interleukin-1 beta (IL-1β), interleukin-8 (IL-8), prostaglandins, tumor necrosis factor-alpha (TNF-α), and matrix metalloproteinases (Figure 4). These products influence a number of cellular processes, including the recruitment and chemotaxis of neutrophils to the site, with increased permeability of the gingival vessels that results in extravasation of plasma proteins from the blood vessels into the tissue. The epithelium also responds by induction of innate defense systems, which include the production of antimicrobial peptides, such as defensins, calprotectin, etc.[14] In addition, the salivary defense system works to limit bacterial growth through the flushing action of simple fluid flow that clears bacteria from the oral surfaces, bacterial-aggregation factors, antimicrobial proteins, etc.[15]

Should the dental plaque biofilm continue to grow and expand to populate the subgingival space, these noxious compounds will stimulate the epithelium to produce bioactive mediators, resulting in further recruitment of a variety of cell types, including neutrophils, T-cells, monocytes, etc (Figure 5). The resulting established or chronic gingivitis is the most prevalent type of gingival inflammatory lesion in the population as a whole. Thus, continued exacerbation of the process results in signaling of underlying cell types, including fibroblasts, to increase production of proinflammatory cytokines in the tissues. Host systemic responses to this insult also can be documented. For example, evidence of specific antibodies to oral organisms can be demonstrated in peripheral blood. Also, the acute-phase response is associ-

ated with gingival inflammation, including the production of C-reactive protein (CRP), fibrinogen, complement, etc, by both local cells and the liver.[16,17] These proteins not only possess biological activities that may further exacerbate the inflammatory response, they may also impact the initiation or progression of systemic disease processes, such as atherosclerosis.[18,19]

To this point, rigorous tooth cleaning and oral hygiene procedures would reverse the course of gingivitis and return the periodontium to a healthy state.[20] Unfortunately, however, many people fail to maintain adequate hygiene and so the process of inflammation often continues unchecked for years.

In some individuals, for reasons that are not entirely clear, the inflammatory process expands to involve the breakdown of collagen in periodontal ligament and bone resorption, resulting in periodontitis (Figure 6). The rate of breakdown varies between individuals. It has been suggested that there are underlying genetic mechanisms or other risk factors (eg, smoking, diabetes, stress, etc) that provoke these processes in certain people and not in others. We've heard lately of polymorphisms and various genes that control, for example, interleukin or fibrinogen synthesis. There is an ongoing scientific effort to determine the role of host genetics in the susceptibility to periodontal infection.[21]

Gingival Health and Bacteremia

A consequence of this inflammatory process is ulceration of the gingival sulcular epithelium, which allows bacterial translocation from the sulcus into the bloodstream. The surface area of the periodontal ligament has been calculated to cover about 75 square centimeters. Thus, a person having 50% horizontal bone loss and inflamed pocket epithelium would have a wound surface of approximately 30 to 40 square centimeters. Such a wound surface would likely increase the risk for bacterial translocation when compared to a healthy periodontium. In the most prevalent periodontal disease, established gingivitis, pockets of 4 to 5 millimeters may translate into a gingival wound surface area of 10 to 20 square centimeters. Considering that many people go a long time without having gingivitis treated, this chronic inflammatory condition may promote continuous, low-grade chronic bacteremia. Several studies have indeed shown that the incidence of bacteremia is elevated in subjects with increasing severity of gingival inflammation.[22,23] When using rather insensitive bacterial culture techniques, bacteremia could be detected even in subjects with clinically healthy gingiva. The

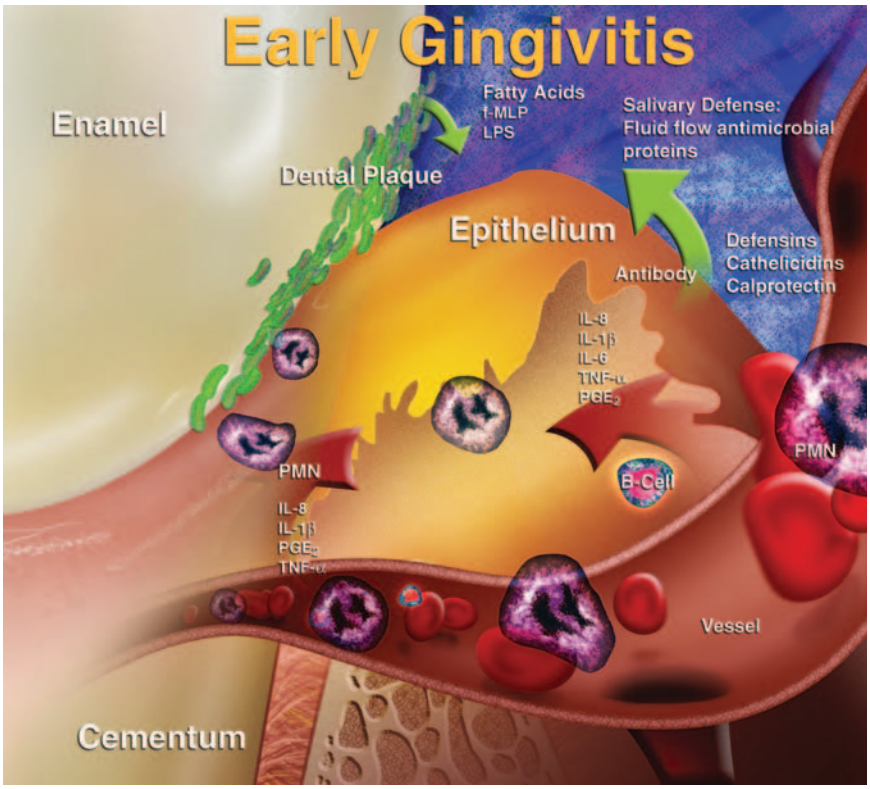

Figure 4—Bacteria in dental plaque release biologically active components, including lipopolysaccharides, chemotactic peptides, and fatty acids. These components signal gingival epithelial cells to release proinflammatory cytokines that diffuse into the underlying connective tissues to stimulate acute vasculitis, which leads to dilation of blood vessels and extravasation of plasma components into the connective tissue compartment. Chemotactic peptides signal white cells to interact with and stick to vascular endothelium, after which the neutrophils enter the connective tissues. In addition to the inflammatory response, the host attempts to clear itself of microorganisms by responding to these signals with epithelial production of antimicrobial peptides. Saliva also affords numerous antimicrobial mechanisms to protect the host.

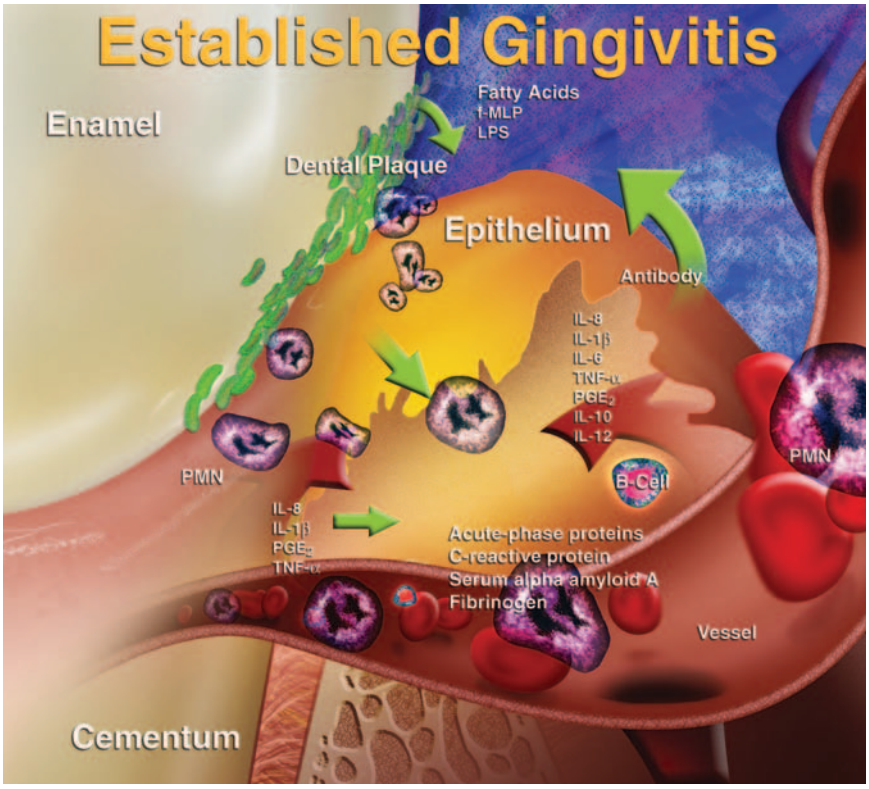

Figure 5—Increased numbers and increasing diversity of bacteria in dental plaque continue to release biologically active components that increase the intensity and spread of the inflammatory response. Increased numbers of neutrophils, monocytes, and macrophages infiltrate the tissues to release more diverse cytokines and prostaglandins that exacerbate the inflammatory response. Lymphocytes (T-and B-cells) and plasma cells also infiltrate, the latter releasing antibodies against the microorganisms that may also cross-react with the host tissues. The acute-phase response (including production of acute-phase proteins such as CRP, serum alpha amyloid A, and fibrinogen) also is evident.

use of more sensitive molecular techniques, such as the polymerase chain reaction,[24,25] would likely prove bacterial translocation from the periodontium to be even more common than presently appreciated. While most studies of dentally related bacteremia have centered around purposeful activities such as toothbrushing, periodontal probing, and tooth extraction, it is possible that while participating in daily activities (chewing, speaking, habits, etc), minor disruptions to gingival integrity occur in a significant number of individuals with gingival inflammation.

Gingival Inflammation: Pathways of Systemic Effects

Oral bacteria and gingival inflammation may theoretically influence systemic health through four potential pathways: bacteremia, systemic dissemination of locally produced inflammatory mediators, provocation of an autoimmune response, and aspiration or ingestion of oral contents into the gut or airway (Figure 7). Low-grade but persistent bacteremia may allow oral bacteria to aggregate platelets through receptor-ligand interactions. Studies have shown that infusing rabbits with aggregating bacteria caused significant hemodynamic changes, acute pulmonary hypertension, and cardiac abnormalities, including ischemia.[26] This very provocative work suggests that bacteremia of oral origin may have serious implications for systemic health.

Several inflammatory mediators can be measured as being elevated in peripheral blood in subjects with periodontal disease,[17] suggesting that periodontal inflammation either contributes directly to the elevation of the concentration of these substances in peripheral blood or signals distant organs (eg, the liver) to produce them. The liver could respond, for example, through the acute-phase response by producing CRP, fibrinogen, etc. These proteins may have deleterious effects on other target organs (eg, heart, brain) by modulating disease processes such as atherosclerosis.

Interest in the systemic effects of periodontal infection was reignited in the early 1990s by a series of case-control and other epidemiologic studies that demonstrated statistical associations between poor oral health and several systemic diseases.

Recent studies have suggested a connection between chronic infections, such as *Chlamydia pneumoniae* infection or periodontal diseases, and atherosclerosis.[27] It has been suggested that immunity to bacterial pathogens plays a role in the atherosclerotic process and that this response may involve autoimmunity.[28] It has been observed that almost all humans have immune reactions against microbial heat-shock protein 60 (HSP60). The human version of this protein is highly homologous with bacterial HSP60. It is possible that the immune response generated against the microbial version of this protein could cross-react with human HSP60 on arterial endothelial cells to influence the course of atherosclerosis.[28] Bacteria thought to induce gingival inflammation may also stimulate an autoimmune response by presentation of cross-reactive epitopes that stimulate autoantibody or T-cell response reactive with host antigens, such as HSP60, to drive a proinflammatory response with cardiovascular effects.[29,30]

Dental plaque and/or periodontal inflammation may influence pathogenic processes occurring in distally contiguous mucosal surfaces, for example, in the respiratory or digestive tracts.[31,32] Salivary hydrolytic enzymes, observed to be elevated in patients with periodontitis, can promote the adhesion of pathogenic bacteria to the oral surfaces, thereby altering oropharyngeal colonization patterns. It is also possible that periodontopathic bacteria stimulate the periodontium to release proinflammatory cytokines that, when aspirated or swallowed, alter mucosal surfaces to promote adhesion of pathogenic bacteria that cause diseases such as pneumonia or gastric ulcers.[31,32] Finally, cytokines released from inflamed periodontal tissues may enter the respiratory tract in aspirated saliva, triggering the sequence of neutrophil recruitment, epithelial damage, and infection.[31]

Gingival Inflammation and Systemic Disease

Several case-control studies[33] published in the early 1990s found that patients with a history of myocardial infarction had worse oral health than control subjects (studies are summarized in the reference). This has led to a flurry of studies to verify these observations. While most of these studies support a modest association between periodontal diseases and the outcomes of atherosclerosis (of myocardial infarction, angina, or stroke), several studies have not supported this association. This is complicated by the absence of a standard definition or measures for periodontal diseases and that underlying mechanisms common to both periodontal diseases and atherosclerosis share common risk factors, such

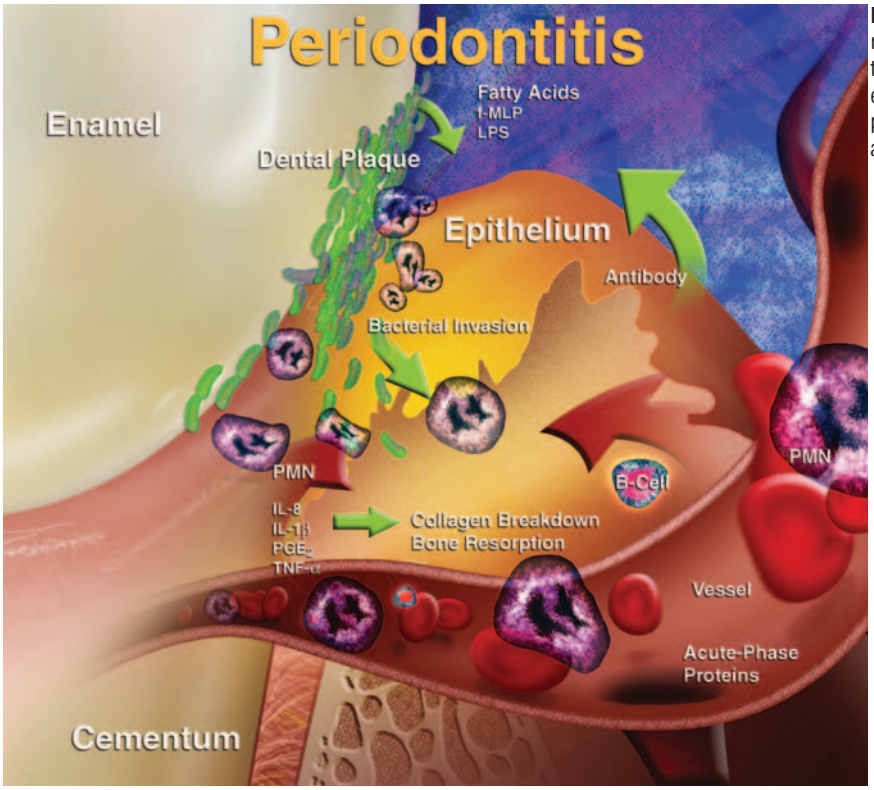

Figure 6—In some subjects, for reasons that remain unknown, the chronic inflammation of established gingivitis spreads to provoke periodontal ligament and alveolar bone destruction.

as lifestyle habits like cigarette smoking.

How might periodontal inflammation influence atherosclerosis? It is possible that dental plaque stimulation of cytokine production in the periodontium may elevate levels of cytokines in the peripheral blood. This may in turn stimulate hepatic production of acute-phase proteins, such as CRP. These proteins could then induce vascular injury, atherogenesis, cardiovascular disease, and stroke. Several studies have shown that patients with periodontal diseases demonstrate elevated levels of CRP and fibrinogen, as well as peripheral white blood cells.[17,34] Elevated levels of these proteins have been suggested to be risk factors for cardiovascular disease.[35-37] Additional evidence has been reported for the possible direct role of bacteria in atherosclerosis. It has been reported that chronic disease agents, such as C *pneumoniae*, play a role in atherosclerotic plaque development. Recently it has been reported that the DNA of oral bacteria could be amplified directly from atherosclerotic plaques. It is, therefore, possible that these pathogens may play a role in the development and progression of atherosclerosis leading to coronary vascular disease.

Lung diseases such as hospital-acquired pneumonia and chronic obstructive pulmonary disease (COPD) also have been associated with poor oral health.[31,38] It is possible that oral biofilms on the teeth may serve as a reservoir of infection for respiratory pathogenic bacteria. In subjects admitted to hospital intensive care units or nursing homes, bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and enteric bacteria have been shown to colonize the teeth. These bacteria may then be released into the oral secretions to be aspirated into the lower airway to cause infection. It is also possible that inflammatory mediators, such as cytokines produced by the periodontium, released into the secretions also can be aspirated to have proinflammatory effects in the lower airway.

Several epidemiologic studies have reported associations between poor oral health and COPD.[39,40] One interesting observation found that lung function measured through spirometry is associated with measures of periodontal

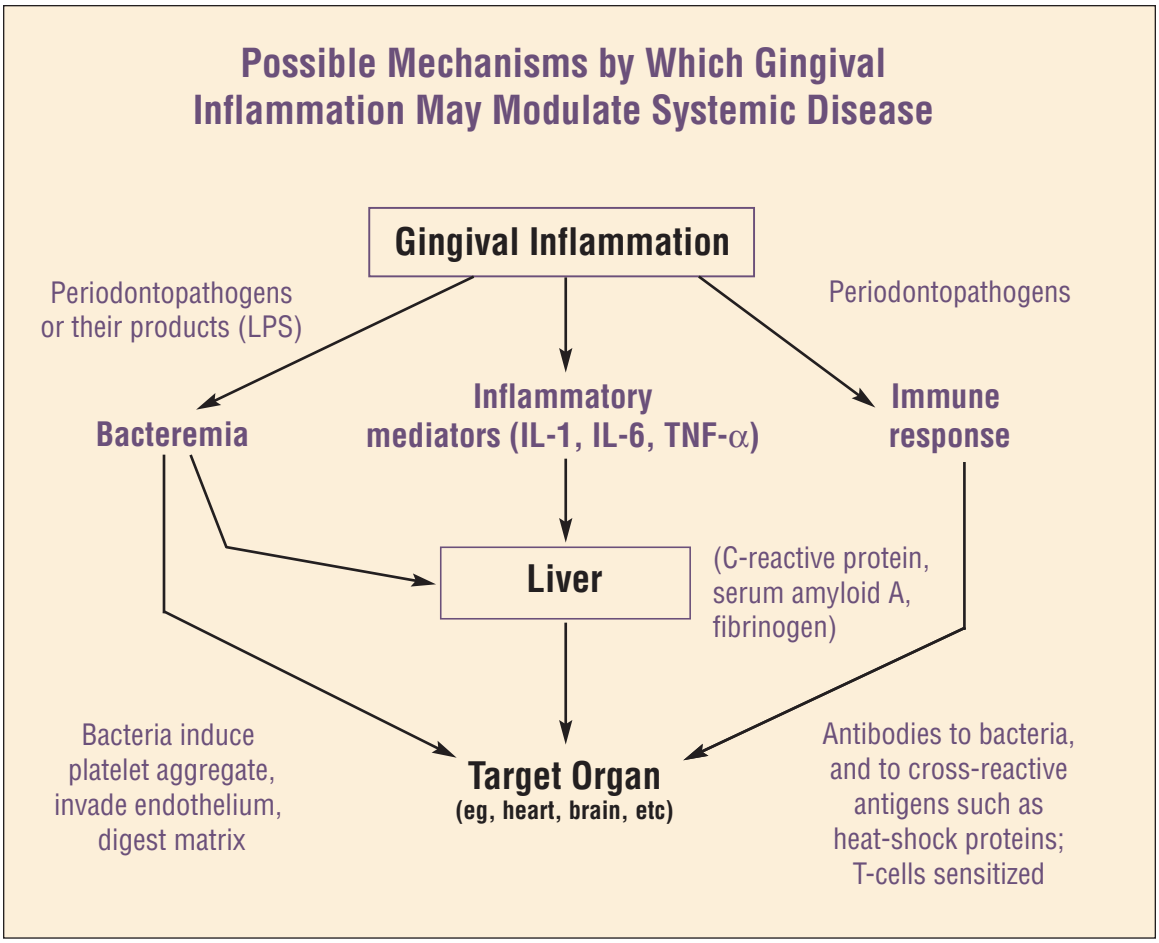

Figure 7—Theoretical pathways by which the gingival inflammatory response may impact systemic inflammation and systemic processes such as atherosclerosis.

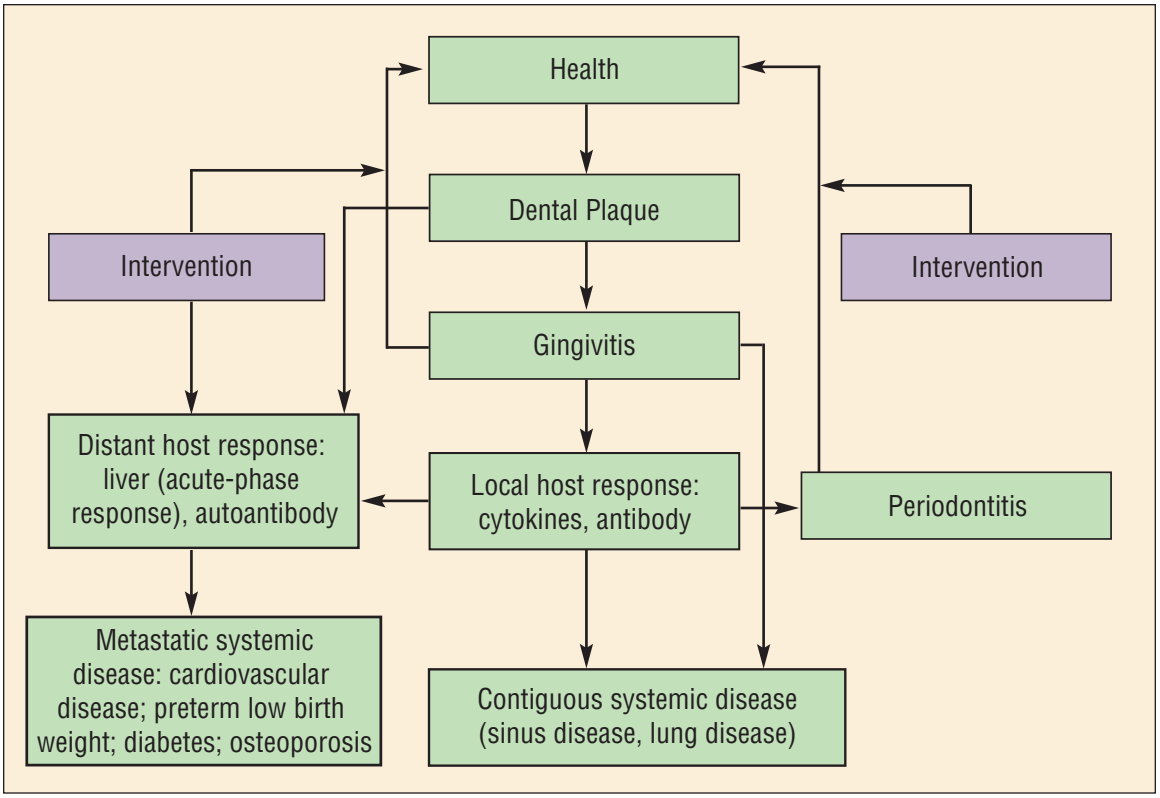

Figure 8—Suspected interrelationships between gingival inflammation, systemic disease, and response to periodontal therapy.

disease.[40] In subjects stratified by periodontal attachment loss, those with more severe attachment loss tended to demonstrate less lung function than those with less attachment loss. Further research is necessary to dissect the contribution of periodontal inflammation from those of established etiologies, such as smoking on lung function.

Scientists are beginning to understand the complex molecular interactions that occur between the bacteria and salivary pellicle that coats the tooth, and between gram-positive cocci of early plaque and gram-negative filamentous bacteria that populate the tooth as plaque matures.

There also has been interest in the association between periodontal inflammation and adverse pregnancy outcomes.[41,42] Unfortunately, adverse pregnancy outcomes, such as premature birth and low birth weight, are quite common events. This is a very significant public health problem in the United States, and has been associated with subclinical genitourinary or other infections. During parturition, the uterus is influenced by the hypothalamus through the production of oxytocin, which stimulates uterine contraction. Prostaglandins that are produced by the placenta also stimulate uterine contraction, which normally leads to birth in the third trimester (37 weeks). It is thought that chronic infections drive the inflammatory process, which leads to the release of inappropriate levels of prostaglandins and TNF-α, which prematurely stimulates uterine contraction to promote preterm birth.

It has been suggested that periodontal infection and the release of lipopolysaccharides and other biologically active molecules drive the process of inflammation, as described above. This results in the elevation of prostaglandins and TNF-α in the crevicular fluid. Lipopolysaccharides released from the oral cavity into the bloodstream may stimulate prostaglandins in the placenta, causing preterm birth. It is also possible, such as in atherosclerosis, that cytokines in the periodontium may lead to elevated peripheral blood cytokine levels and stimulate hepatic produc-

tion of acute-phase proteins that may influence the birth process. Very recent work has also found that periodontal pathogens, such as *Fusobacterium nucleatum*, may travel from the gingival sulcus to the placenta to cause preterm birth.[43] Thus, it is possible that these bacteria may enter the bloodstream from the oral cavity to directly effect the birth process.

Summary

Dental plaque drives periodontal inflammation, with gingivitis being the initial manifestation of this process. With appropriate intervention, this process can be reversed and the periodontium returned to a state of health (Figure 8). However, an exuberant local host response, including the synthesis of cytokines and antibodies, in some cases results in the destruction of periodontal ligament and supporting bone (periodontitis). Periodontitis is typically treated by removing the etiology (dental plaque) and returning the gingival tissues to health. Unfortunately, in many cases, periodontal disease goes untreated for many years. It is possible, then, for the systemic host response to this insult to contribute to disease processes that result in cardiovascular disease and stroke, respiratory disease, and adverse pregnancy outcomes.

What is the status of periodontal medicine today? While there are a number of preliminary studies that point to an association between periodontal inflammation and several systemic conditions, as mentioned above, the data are equivocal. In many cases, there has been an emphasis on linking periodontal attachment loss with systemic disease. It is possible that the use of this outcome measure, which represents "historical" evidence for the disease without indicating the temporal sequence or duration of disease activity, may cloud the role of periodontal inflammation in this process. Future investigations are needed that use better definitions for periodontal disease and measures of how gingival inflammation and tooth loss may best determine the role this localized, chronic disease process plays in the progression and severity of important systemic diseases.

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Cardiovascular Disease and Periodontal Diseases: Commonality and Causation

Sheilesh Dave, DDS
Clinical Associate

Eraldo L. Batista Jr, DDS, MSc
Research Associate

Thomas E. Van Dyke, DDS, PhD
Professor
Department of Periodontology and Oral Biology
Goldman School of Dental Medicine
Boston University
Boston, Massachusetts

Abstract: ***Periodontal diseases have long been recognized as a public health problem. Awareness of the destructive nature of periodontal diseases and the importance of a tight control of bacterial plaque are basic concepts of periodontal treatment. In the past decade, there has been a conceptual shift from periodontal diseases as an oral problem to periodontitis having an impact on systemic health. Recent evidence suggests a strong relationship between periodontal inflammatory disease and systemic diseases, such as cardiovascular disease. It is now generally accepted that inflammation plays an important role in atherosclerosis, and factors that systemically amplify inflammation are under close investigation. This article reviews some of the emerging concepts for the inflammatory mechanisms of periodontal diseases and atherosclerosis and examines the potential role of local inflammation in systemic inflammatory disease.***

Learning Objectives

After reading this article, the reader should be able to:

- describe the major molecular and cellular mediators of inflammation in periodontal diseases and cardiovascular disease.
- list the possible biologic links between periodontal diseases and atherosclerosis.
- explain the additional evidence that will be required to firmly establish a causal link between periodontal diseases and atherosclerosis.
- identify risk factors common to both periodontal diseases and atherosclerosis.

Cardiovascular disease (CVD) accounts for about 50% of deaths in the United States each year.[1] CVD is characterized by the formation of intravascular, lipid-rich inflammatory plaques that may give rise to thromboses and, subsequently, to clinical events such as myocardial infarction (MI).[1,2] Currently well-known and well-accepted risk factors for the development of CVD include smoking, obesity, a high-cholesterol diet, and high blood pressure.[1-4] Interestingly, however, only about half of all coronary artery disease can be explained by these conventional risk factors,[5] which suggests that other undisclosed factors may be playing a role in atherogenesis.

Earlier attempts to understand the pathogenesis of atherosclerosis focused on lipid accumulation within the plaque, most notably cholesterol accumulation.[6,7] As scientific understanding of the disease has evolved over the past several years, it has become apparent that the atherosclerotic plaque, in addition to being an accumulation of lipids, is an inflammatory lesion.[8-11] Indeed, recent evidence suggests that in otherwise healthy individuals, markers of systemic inflammation, such as C-reactive protein (CRP), are more predictive of acute coronary events than are low-density lipoprotein (LDL) levels.[12-14] Considering the aforementioned evidence, it seems perfectly reasonable to hypothesize that chronic microbial infections may contribute directly or indirectly to the development of these inflammatory lesions. This concept has gained greater currency with opinion leaders during the past decade as new discoveries in the field have been made.[2,9] This has led to a search for possible infectious diseases that may contribute to systemic inflammation and, subsequently, the development of CVD and, specifically, atherosclerosis. It also has led to the hypothesis that atherosclerotic lesions are infected lesions and as such may be amenable to antimicrobial and/or antiviral therapies.[15-17] Thus, chronic inflammatory/infectious diseases, such as periodontal diseases, have come under closer scrutiny for their potential to contribute to both systemic inflammation and bacterial

seeding of atherosclerotic plaques.

Periodontal diseases are chronic inflammatory diseases of the tissues that support and attach the teeth to the jaws.[18] They are caused by gram-negative bacterial infections and are, for the most part, asymptomatic, although much of the actual destructive tissue changes observed clinically are a result of the inflammatory host response.[18] Historically, systemic diseases have been considered by the dental profession in the context of their influence on the severity of and predisposition to periodontal disease.[19-23] While the importance of periodontal health to dental health is not in dispute, its relationship to systemic disease is beginning to be investigated. Areas of ongoing investigation include the relationship between periodontal diseases and diabetes mellitus (DM), cerebrovascular ischemia, preterm birth, chronic obstructive pulmonary disease, institutional pneumonia, and CVD.[24-28] As will be discussed, possible systemic links include metastasis of infection injury and inflammation.

Cardiovascular Disease

Early Inflammatory Events in Atherosclerosis

Early atherogenic events occur in the endothelial cell layer lining the large, elastic arteries. Normally, these cells remain in a "resting" state. When systemic and, later, local inflammatory mediators are released (most notably as a result of dietary lipids among other things[11]), endothelial cells undergo important changes, which cause them to become "activated."[29] One of the hallmarks of the early atherosclerotic lesion is the recruitment and adhesion of neutrophils,[30] then monocytes and lymphocytes, to the site of endothelial damage.[31] A number of surface molecules temporally mediate this process, but, especially in the early stage, the interaction of circulating leukocytes with the activated endothelial cells seems to be mediated by E- and P-selectins[32] and, later, in a more stable fashion, by the intercellular adhesion molecule-1 (ICAM-1) and the vascular cell adhesion molecule-1 (VCAM-1).[11,33] P-selectins have a broader array of recognizing cell subsets, binding neutrophils, monocytes, activated lymphocytes, and platelets, acting in concert with other molecules expressed in the very early stages of atherosclerosis.[32] Polymorphonuclear (PMN) leukocytes initially "roll" over the vascular endothelial lining as a result of the less-than-stable adhesion provided by selectins. Firm adhesion occurs later at the expense of CD11a/CD18 molecules expressed on PMN and ICAM-1 expressed on endothelial cells. It has been shown that adhesion of PMN to endothelial cells pretreated with the cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) was substantially increased, also producing a massive increase in superoxide production by PMN.[34] The same study also showed that the cytokine-activated endothelial cells released granulocyte-macrophage colony-stimulating factor, a powerful activator of PMN. PMN's role during atherogenesis does not seem to be restricted to directly triggering lesion formation by interacting with the endothelium. Recent evidence shows that PMN can cause oxidation of LDL by the production of myeloperoxidase-mediated acid, a powerful oxidant.[35] VCAM-1 seems to be more specific for monocytes, and has gained particular attention because deficient mice do not demonstrate normal atherogenic lesion development with an atherogenic diet.[36] VCAM expressed on endothelial cells is recognized by the very-late antigen-4 molecule expressed on monocytes and lymphocytes, allowing these cell subsets to migrate into the vessel wall. Within the intimal layer, leukocytes encounter proinflammatory molecules, and monocytes present in this early lesion are then induced to become macrophages, which can take up modified lipoproteins[37] and become lipid-laden "foam-cells."[38] At this point in the pathogenesis, not only are systemic factors feeding the process, but both the recruited cells and the endothelial cells sustain the local inflammation by secreting cytokines and chemokines (Table 1),[10,39] most notably IL-1β, TNF-α, and monocyte chemotactic protein-1 (MCP-1). T-cells are stimulated to produce proinflammatory cytokines, such as tumor necrosis factor-beta (TNF-β) and interferon gamma (IFN-γ). Along with factors secreted by the recruited inflammatory cells, other molecules also may contribute to the pathophysiology of atherosclerosis. For instance, oxidized LDL has been shown to stimulate production of fibroblast growth factor, a key element in fibrotic tissue formation by endothelial cells.[39] At this point in lesion development, the atherosclerotic lesion is a bulge of the luminal wall, and as the lesion progresses, the extracellular matrix is degraded by macrophage-derived proteolytic enzymes.[40,41] The

Table 1—Select Cytokines Related to Pathogenesis of Periodontal Diseases and Atherosclerosis

Cytokine	Main Sources	Effects on Periodontium	Effects on Atherosclerosis
IL-1β	Monocytes/macrophages	Bone resorption	Expression of VCAM-1/ICAM-1/selectins
	Fibroblasts	Collagen degradation	by endothelial cells
	Epithelial cells	Activation of endothelial cells	Attenuation of vasodilation
	Endothelial cells	(ICAM-1/VCAM-1)	IL-6 production by monocytes
	Epicardial adipocytes	Enhanced IL-8 production by	
		gingival fibroblasts	
		Expression of MCP-1 by	
		gingival fibroblasts	
TNF-α	Monocytes/macrophages	Bone resorption	Expression of VCAM-1/ICAM-1/selectins
	T-cells	Collagen degradation	Attenuation of vasodilation
	SMCs	Activation of endothelial cells	Expression of IL-1/IL-6
	Myocytes	(ICAM-1/VCAM-1)	Affects T-cell proliferation
	Epicardial adipocytes	Enhanced IL-8 production by	Up-regulates growth factors (PDGF)
		gingival fibroblasts	Compromises contractile function
		Expression of MCP-1 by	
		gingival fibroblasts	
IL-6	Monocytes/macrophages	Bone resorption (increase in	Up-regulation of acute-phase proteins
	Endothelial cells	osteoclastic activity)	(CRP/serum amyloid A)
	Fibroblasts	Late B-cell development	Increased procoagulant activity of
	Epithelial cells		monocytes
	SMCs		Stimulation of LDL receptor gene in
	T-cells		hepatocytes
	Epicardial adipocytes		
	Adipocytes		
IL-8	Epithelial cells	Recruitment of leukocytes	Recruitment of leukocytes
	Endothelial cells		
	Fibroblasts		
	Neutrophils		
MCP-1	Macrophages	Recruitment of mono-	Recruitment/activation of mono-
	Endothelial cells	cytes/macrophages	cytes and macrophages
	Epicardial adipocytes		Induction of IL-6 production by SMCs
	Fibroblasts		
IFN-γ	T-lymphocytes	Amplification of monocytic	ICAM-1 expression of endothelial cells
	Natural killer cells	respiratory burst activity	Proliferation of SMCs
		ICAM-1 expression by	Decrease in plaque stability
		endothelial cells	Increase in LDL uptake by macro-
		Down-regulation of IL-8	phages (foam-cell formation)
		production by gingival	Up-regulation of TNF-α expression by
		fibroblasts	monocytes

IL-1β = interleukin-1 beta; TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin-6; IL-8 = interleukin-8; IFN-γ = interferon gamma; SMCs = smooth muscle cells; CRP = C-reactive protein; ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1; MCP-1 = monocyte chemotactic protein-1; PDGF = platelet-derived growth factor; LDL = low-density lipoprotein.

INF-γ produced by lymphocytes can inhibit collagen production by smooth muscle cells,[42] thus causing the fibrous lesion to weaken and become susceptible to rupture.[43] When these events occur, the subendothelial tissue is exposed to procoagulatory factors in the bloodstream, which then may give rise to thromboses and ensuing clinical events, such as occluded blood flow to the heart or other organs.[44] The consequence of these events, eventually leading to acute MI, ultimately depends on a number of other factors, such as fibrinolytic and coagulation factors.[1]

Classic Inflammatory Agents/Traits and Atherosclerosis

Oxidation of LDL

Atherosclerosis has long been linked to the consumption of a fat-rich diet and to increased levels of LDL.[8] Although it is not proven defin-

itively, oxidized lipoproteins have been regarded as fundamental to the development of atherosclerotic disease,[45] and the so-called oxidation hypothesis is still the most reasonable and acceptable one. According to this theory, LDL is deposited in the intima—possibly through binding to proteoglycans—and then undergoes oxidative modifications leading to the formation of carbonyl compounds, lysophospholipids, and hydroxyperoxides, among others.[3] The influence of oxidized LDL on inflammation is supported by the fact that it seems to mediate monocyte binding to human aortic endothelial cells through the platelet-activating factor receptor.[37] In a rabbit model, lipid lowering by means of a controlled diet drastically reduced the macrophage content within atherosclerotic lesions, as well as proteolytic activity, which accounts for lesion disruption and thrombosis.[46]

Good and Bad Free Radicals, Hypertension and Endothelial Dysfunction

Recent evidence points to an important role for different reactive oxygen species in the pathogenesis of atherosclerosis.[47] Reactive oxygen species are generated when electrons are enzymatically transferred to peroxide, producing free, highly reactive radicals such as superoxide, hydrogen peroxide, and hydroxyl radical. These are capable of causing direct damage to other biological molecules, including proteins and cell membrane lipids.[48] Not all free radicals are expected to cause damage; for example, nitric oxide has been shown to have anti-inflammatory properties, among them the down-regulation of VCAM-1.[49] Furthermore, it has been recognized as a key signal-transducing molecule with important modulating properties on blood pressure, as it has been shown to regulate vascular tone by inducing vasodilation.[50,51] Hypertension has been recognized as a risk factor for atherosclerosis, possibly by inducing endothelial inflammatory responses.[52] Hypertensive animals have a substantial increase in the production of superoxide by endothelial cells, suggesting that poor vascular tone control could induce changes leading to atherosclerosis.[53] Recently, Cuzzocrea and coworkers[54] provided sound evidence that the increased production of superoxide in hypertensive rats, possibly by endothelial cells, scavenges the nitric oxide necessary to regulate blood pressure. These very results seem to be supported by findings in human clinical trials where deficits in endothelial nitric oxide release have been observed in coronary and brachial circulations.[55,56] These findings point to additional important implications in the early inflammatory events leading to atherosclerosis; nitric oxide is produced by PMN in response to bacterial challenge, which suggests that innate immune response also could affect endothelial function. This seems to be supported by a related finding in rabbits, where PMN products secreted in response to a diet rich in cholesterol induced endothelial constriction.[57] Additional evidence demonstrating that neutrophils can modify LDL by oxidation—thus leading to its rapid incorporation by macrophages—suggests a role in the acceleration of atherosclerosis, particularly following cardiac reperfusion injury.[47]

Diabetes

A link between diabetes and cardiovascular alterations has been suggested by the inflammatory trait observed in both diseases.[58] Poor diabetic control results in the nonenzymatic glycation and oxidation of proteins, called advanced glycation end products (AGE).[59] Binding of AGE to its receptor, RAGE, activates endothelial cells to express VCAM-1 and other surface molecules, which take part in lesion formation,[60] suggesting an important role for poor glucose control in the amplification of inflammatory events leading to atherosclerosis.

Hypertension

This hypothesis is based on the fact that angiotensin II can induce intimal inflammation in addition to causing vasoconstriction. Proinflammatory factors that are induced include superoxide anion, MCP-1 and interleukin-6 (IL-6) from smooth muscle, and VCAM-1 from the endothelium.[61]

Markers Associated With Increased Systemic Inflammation

Because the atherosclerotic lesion is fundamentally inflammatory, many efforts have focused on identifying inflammatory markers that correlate with disease prognosis. Acute coronary syndromes (ACS) are positively correlated with inflammatory mediators, such as CRP, TNF-α, serum amyloid A, IL-1β, and IL-6.[12-14] One of the most consistent markers of sys-

temic inflammation and unfavorable cardiovascular prognosis is the acute-phase protein CRP.[12,14] It is produced by hepatocytes and released into the circulation, being positively correlated to IL-6.[59] CRP activates complement,[62] induces expression of adhesion molecules,[63] induces production of MCP-1 by endothelial cells,[64] and accounts for LDL uptake by macrophages.[65] More recently, another putative marker, CD40 ligand, also has been correlated with a higher risk of death and recurrent MI.[66,67]

Infectious Vectors Associated With Atherosclerosis

The role of infectious elements—bacterial or viral—in the pathogenesis of atherosclerosis is not certain. These elements could directly amplify the inflammatory response, or they could act as the sole vector leading to lesion formation. It has been hypothesized that bacteria or viruses may directly infect atherosclerotic plaques, thus contributing to the observed inflammatory process.[68] It has also been hypothesized that distant infections may increase the level of systemic inflammation either through the release of toxins and by-products or the leakage of inflammatory mediators into the circulation.[68] Epidemiologic and in situ associations between atherosclerosis and cytomegalovirus, *Helicobacter pylori*, and *Chlamydia pneumoniae*[69] have been shown, but causality has not been convincingly established.[70] Likewise, the presence of infectious elements in atherosclerotic plaques, including oral pathogens,[71] have been shown but, again, causality has not been established. Nevertheless, in light of the present knowledge, the connection between infections and inflammation leading to atherosclerosis is highly plausible. While a variety of studies have demonstrated possible molecular mechanisms by which bacterial infection may exacerbate atherosclerosis, none has been definitively established.

Periodontal Diseases

Periodontitis is caused by a multifactorial process triggered by infection of the periodontium with gram-negative bacteria. The organisms thought to play the most important role in the pathogenesis of periodontal diseases are *Porphyromonas gingivalis*, *Tannerella forsythensis* (formerly designated *Bacteroides forsythus*), and *Actinobacillus actinomycetemcomitans*.[72] In addition, periodontitis is characterized by the formation of a pellicle-adherent, subgingival bacterial biofilm. Biofilms are defined as matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces.[73] The organisms within the biofilm form a primitive yet complex system of communication, intercellular transport, and commensalism. Bacteria within biofilms are often inaccessible to host defense mechanisms and therapeutic measures such as antibiotics. Estimates of the number of different organisms that may comprise this biofilm range as high as 300.[72] Although the presence of specific gram-negative bacteria is necessary for periodontal disease to occur, it is not sufficient. Indeed, as is well known, many individuals who harbor periodontopathic organisms do not necessarily develop periodontal diseases.[74] Thus, host or other unknown factors play a decisive role in the pathogenesis and progression of periodontal diseases. Other important risk factors include, but are not limited to, smoking—for heavy smokers, the odds ratio of developing periodontal diseases is increased between two and six times—DM, poor oral hygiene, socioeconomic status, and race.[18] Thus, many of the risk factors for developing periodontal diseases also predispose the individual to developing CVD. Among the virulence factors possessed by periodontal pathogens are the ability to evade host defenses, the ability to attach to and invade host tissues, and the ability to elaborate enzymes capable of destroying host tissues.[72] For instance, *P gingivalis* possesses fimbriae that allow it to attach to host epithelial and endothelial cells,[75] and it produces proteases that degrade collagen and other proteases that degrade immunoglobulin and complement.[76] As mentioned previously, although only a restricted number of organisms have been thought to play a significant role in the pathogenesis of periodontal diseases, a significantly larger number of organisms colonize the subgingival plaque. These may play a role in the development of the atherosclerotic lesion as they gain opportunistic entry to the systemic circulation through the inflamed periodontal pocket.

Host Responses

The response of the host to most periodontal infections is chronic inflammation. The tooth/periodontium transgingival connection

constitutes a unique relationship; the root is an avascular mineralized surface, which emerges from the bone socket into the mouth, a contaminated cavity. Separating the "inner" and "outer" environments is the junctional epithelium at the base of the gingival sulcus.[77] Under normal conditions, neutrophils continuously migrate into the gingival crevicular space where they encounter bacteria and phagocytose or otherwise inactivate them.[18,78] In addition, immunoglobulin G, nonsecretory immunoglobulin A, and complement are present in the gingival crevicular fluid and also participate in the defense against periodontal pathogens. In most individuals, these host defenses, together with adequate oral hygiene, are enough to prevent the development of periodontal disease. If, however, the primary host response is quantitatively or qualitatively insufficient, a biofilm will develop subgingivally. As the biofilm persists, it continues to elaborate noxious substances that both directly and indirectly recruit monocytes and lymphocytes to the periodontium.[79] The host attempts to "wall off" the infection by destroying the tissues immediately proximal to the site of infection and, at the same time, inducing a fibrotic response farther away to prevent the spread of the infection.[78] This response has both local and systemic inflammatory manifestations.[80]

Chronic inflammatory/infectious diseases, such as periodontal diseases, have come under closer scrutiny for their potential to contribute to both systemic inflammation and bacterial seeding of atherosclerotic plaques.

Local Inflammatory Manifestations of Periodontal Disease

Once a biofilm has developed in the periodontal pocket, it is capable of persisting and thriving there. In addition to harboring potentially invasive species, such as *P gingivalis* and A *actinomycetemcomitans*, inflammatory substances such as lipopolysaccharides (LPS) and formyl-methionine-leucine-phenylalanine are continuously being elaborated.[81] This causes inflammatory cells and molecules to congregate in the affected periodontal tissues. These mediators include molecules such as IL-8 and MCP-1,[82] which recruit more neutrophils and monocytes, respectively. IL-1β and TNF-α, both possessing potent inflammatory effects, also are produced.[83] IL-1β is able to stimulate production of proinflammatory mediators such as prostaglandin E_2 (PGE_2)[84] and stimulate the expression of inflammatory adhesion molecules on endothelial cells. Interestingly, both IL-1β and TNF-α are expressed at increased levels during phases of active periodontal destruction.[83] It is possible that these molecules may gain transient access to the systemic circulation during periods of exacerbation in periodontal diseases. In addition to the above-mentioned cytokines, IL-2, -4, -5, -6, and -10 also are produced and may play roles in this process.[85] These molecules are responsible for regulating the activity of T-cells and B-cells. As mentioned, PGE_2 also is produced in response to gingival inflammation. Recent evidence suggests that host-modulating therapies that, in part, depress PGE_2 production may have an application in the treatment of periodontal diseases.[86] Although in the preliminary stages, these and related investigations being conducted in the authors' and affiliated laboratories have demonstrated that local administration of lipoxin analogs—potent, naturally occurring, anti-inflammatory arachidonic acid derivatives—can significantly suppress the progression of periodontal disease in animal models.[87]

Systemic Markers of Periodontal Disease

Periodontal diseases are associated with an increase in CRP levels.[88,89] This is significant because CRP is a widely accepted measure of the level of systemic inflammation, and increases in CRP levels are associated with an increased risk of ACS. In patients with both atherosclerosis and periodontal disease, CRP levels were elevated above the level seen with only one disease or the other.[90] Periodontal disease also may lead to transient increases in circulating levels of IL-1β, TNF-α, and PGE_2. This may be the first step in the contribution of periodontal diseases to systemic inflammation.

Periodontal Diseases and Atherosclerosis

The recognition of the relationship between periodontal diseases and atherosclerotic events

is relatively recent and mostly based on the inflammatory hypothesis of atherosclerosis, considering that periodontitis is an infection-triggered inflammatory alteration. However, some evidence points to a direct infectious relationship as well. The first associations were based on epidemiologic data; in a randomized controlled study, Mattila et al[91] analyzed 100 patients with acute MI and controls from the same community, correlating dental scores for carious lesions, missing teeth, periapical lesions, pocket depth (PD) measures, and pericoronitis. Their results showed that overall poor oral/dental health correlated positively with coronary heart disease (CHD) after variables such as age, cholesterol, high-density lipoprotein (HDL), triglycerides, hypertension, diabetes, and smoking had been adjusted. A survey involving National Health and Nutrition Examination Survey data obtained from nearly 10,000 individuals indicated that those with periodontitis had a 25% increased risk of CHD compared to those with minimal or no detectable periodontal inflammation.[92] Importantly, in the latter study, confounding variables such as age, gender, educational and social status, blood pressure, diabetes, and cigarette smoking, among others, also were analyzed. In a more directed case-control approach, it has been pointed out that atheromatosis and oral infections, age, and triglycerides were associated.[93] To date, no interventional study showing a decreased incidence of CVD after successful treatment of periodontal diseases has been shown, although this will be critical to establishing causality. Nevertheless, some evidence emerging from controlled studies begins to establish important connections. Different mechanisms have been proposed to explain the link between oral infections and systemic effects (Figure 1). These are metastatic spread of infection from the oral cavity as a result of transient bacteremia; metastatic injury from the effects of circulating oral microbial toxins; and systemic inflammation caused by immunologic injury induced by oral microorganisms. This is not to say that a single mechanism is solely responsible for amplifying atherogenesis, because most probably they work in concert.

Metastatic Infection

The incidence of bacteremia, both anaerobic and aerobic, after dental procedures such as tooth extraction, endodontic treatment, periodontal surgery, and root planing has been well documented. In some studies, anaerobes have been detected more frequently than facultative anaerobic bacteria.[94-96] If the disseminated microorganisms find favorable conditions, they may settle at a given site and perhaps proliferate. The DNA of *Streptococcus sanguis* and the periodontal pathogen *P gingivalis* may be amplified from atheromatous plaques.[71,97] Thus, the epithelial lining of the periodontal pocket, which frequently becomes thin and even ulcerated in disease, may then provide an avenue for subgingival bacteria periodontally significant (or insignificant) to gain access to the underlying host tissue and eventually to the vasculature. The fact that *P gingivalis* is capable of attaching to and invading both epithelial and endothelial cells[75,98] is of particular significance in this regard. Evidence for this derives from the high frequency with which bacteremias are observed in periodontal patients after manipulation of periodontal tissues.[99-102] A crucial aspect relates to the severity of the periodontal disease; although not well established, factors used to determine the severity and risk of periodontal disease progression—such as the number of moderate and deep pockets presenting active inflammation, bone loss, bacterial profiling, and antibody titers to periodontal pathogens—could provide potential indicators of additional risk for atherosclerosis.

Metastatic Injury

Some gram-positive and -negative bacteria have the ability to produce diffusible proteins, or exotoxins.[103-105] Exotoxins have specific pharmacologic actions and are considered powerful and lethal poisons. Conversely, endotoxin is part of the outer membranes released after cell death. Endotoxin is compositionally an LPS that, when introduced into the host, gives rise to a large number of pathological manifestations.[106] LPS is continuously shed from periodontal gram-negative rods during their growth in vivo. Recent evidence that this may be relevant comes from Kiechl et al,[107] who have shown that polymorphisms in the toll-like receptor 4, known to be a key factor in the inflammatory response to certain types of LPS, confer protection against atherosclerosis by attenuating the inflammatory response to gram-negative endotoxin.[107] Another emerging feature is the association between antibodies to specific periodontal pathogens and heart dis-

ease, suggesting that the host response to oral bacteria plays a role in atherogenesis.[108] An interesting finding was an inverse correlation between antibody response to periodontal pathogens and serum HDL cholesterol, which implies reduction in the antiatherogenic properties of HDL.[108,109] In another clinical trial, it has been shown that, along with increased systemic inflammation, patients with advanced periodontal disease present evidence of endothelial dysfunction.[110]

Metastatic Inflammation

According to the theory of metastatic inflammation, inflammatory products resulting from oral infection could trigger systemic alteration, notably production of acute-phase proteins mainly by hepatocytes, such as CRP and serum amyloid A.[111] Recently, Li et al[112] showed that *P gingivalis*, a known periodontal pathogen, directly inoculated into the tail vein of mice, caused an acceleration of the atherosclerotic process as observed in immunohistochemistry and histopathology. Another interesting finding was the fact that IL-1β and serum amyloid A, an acute-phase protein known to be the analog of CRP in the mice, was positively correlated. Jain et al[113] observed an increase in the

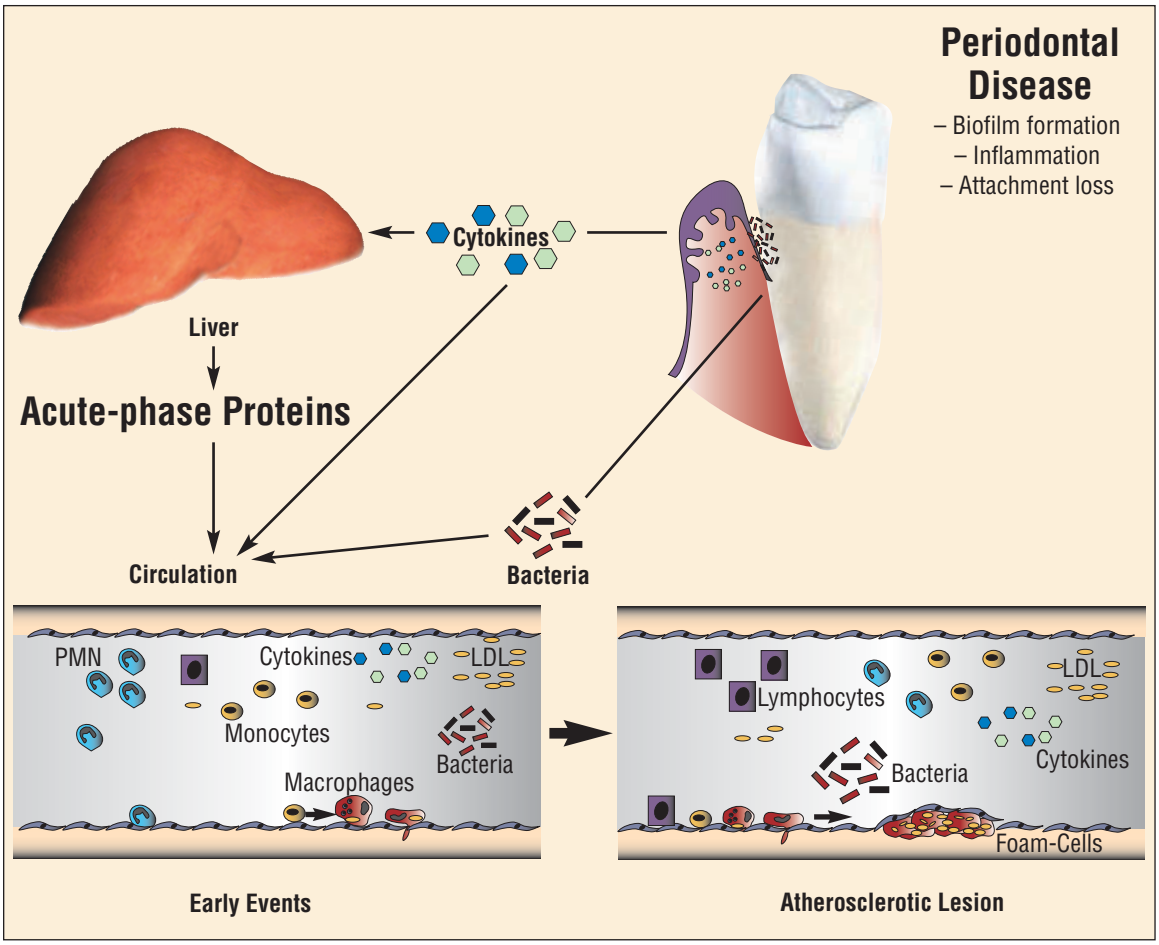

Figure 1—Proposed connection between periodontal disease and atherosclerosis. Biofilm formation leads to periodontal inflammation and eventual attachment loss. The biofilm covering the avascular root surface "feeds" the local inflammatory process and sustained production of cytokines by fibroblasts, epithelial cells, and recruited leukocytes and monocytes. Likewise, the biofilm itself may be sheared by physical vectors, causing bacteremia. Both components, bacteria and host products in response to infection, may directly or indirectly cause and/or amplify systemic inflammatory events leading to atherosclerosis. According to the evidence provided thus far, in the early stages of lesion formation, inflammatory vectors, most notably LDL but also (possibly) circulating cytokines and bacteria, activate endothelial cells lining the arteries, causing them to express surface molecules (please refer to text). These molecules have their counter-receptors on inflammatory cells, which then stably attach to the endothelium. In this very early phase, neutrophils (PMN) initially bind to E- and P-selectins and, later, in a more stable fashion to ICAM via CD11/CD18. Once damage occurs, the endothelium produces chemoattractants (IL-8, MCP-1), which recruit more leukocytes and monocytes, thus amplifying the process. Next, monocytes are attracted by the expression and release of MCP-1, binding to the molecule VCAM-1 expressed on endothelial cells. Once activated, monocytes are converted into macrophages, whose expression of scavenger receptors allow them to uptake LDL, becoming in later stages "foam-cells," a hallmark of atherosclerosis. In later stages, lymphocytes also take part in the pathogenesis of atherosclerosis. Once the inflammatory process is gradually amplified, macrophages start to accumulate in the endothelial layer, forming the atherosclerotic lesion. In later stages (not shown), smooth muscle cells proliferate into the endothelial lesion, leading to the formation of a fibrous cap. Cytokines, specifically IL-6, induce production of CRP by hepatocytes, which also can amplify systemic inflammation.

severity of atherosclerosis in rabbits orally challenged with live *P gingivalis* on a high-fat diet. Importantly, the severity of the periodontal lesions as depicted by bone loss was positively correlated with the extent of the lipid deposition observed in the aorta. Additional recent evidence obtained by a controlled study was provided by Lalla et al,[114] who showed that orally delivered, *P gingivalis*–induced periodontal infections accelerated the development of atherosclerotic lesions in heterozygous apolipoprotein E knockout mice (meaning that apolipoprotein production is suppressed but not completely eliminated). A number of studies have recently pinpointed this relationship through a series of clinical assessments. In these studies, strong evidence suggests that periodontal infections significantly increase the levels of CRP.[88,89] Supporting these observations, clinical parameters of periodontitis-like PD and the community periodontal index of treatment needs correlated positively with the levels of LDL and total cholesterol in male patients.[115]

Historically, systemic diseases have been considered by the dental profession in the context of their influence on the severity of and predisposition to periodontal disease.

Controversial Aspects of the Link Between Periodontal Diseases and Atherosclerosis

Some evidence to date has shown that the bacterial gene for 16s ribosomal RNA from different periodontal pathogens may be amplified from total DNA extracted from human carotid and aortic lesions.[71] This evidence, however, does not pinpoint whether bacteria had, in fact, a key role in the development of the lesion or whether they are only bystanders in the process. It is not clear whether these bacteria stay in the bloodstream long enough to elicit important inflammatory changes. Another possible explanation is the continuous expression of inflammatory mediators within the periodontal tissues, which could gain access to the bloodstream and then induce inflammatory changes on endothelial cells, monocytes, and macrophages. As mentioned previously, some of the cytokines expressed in the mouth are upstream regulators of the expression of acute-phase proteins by the liver (ie, CRP, serum amyloid A). Evidence to date seems to consistently indicate that periodontally diseased patients indeed present higher levels of CRP.[111] This evidence, however, does not establish a cause–effect relationship; most of these studies recruited patients with a history of heart disease, and they did not adjust the sample to include other important, confounding variables also capable of influencing the levels of CRP. The influence of proinflammatory mediators expressed in periodontal diseases and their influence in the levels of systemic markers of inflammation has yet to be determined. Recently, Glurich et al[90] analyzed a number of different systemic markers in patients with either periodontal disease or CVD, patients with both diseases, and healthy subjects. Patients with either periodontal disease alone or CVD alone had their levels of CRP elevated twofold above healthy individuals, whereas a threefold increase was observed in patients with both alterations; serum amyloid A and alpha (1)-antichymotrypsin also were increased in comparison to those with one or neither condition. In seeking a relationship between systemic markers of inflammation and periodontal disease, the recruitment of patients with a known history of heart disease may lead to misleading information. This is attributable to the fact that these patients are generally exposed to other, yet nonamenable, risk factors that may account for the levels of systemic markers. These include hypertension, high levels of LDL, triglycerides, reduced levels of HDL, and sedentary habits.

Conclusion

As the goal of modern health care continues to shift from an attitude of treatment to one of prevention, investigations will increasingly be directed toward elucidating predisposing factors that lead to atherosclerosis and developing appropriate early intervention. Periodontal diseases may represent one such factor. Periodontitis itself is endemic in North America and Europe, despite the fact that treatment modalities have proven successful over the long term and preventive measures are well understood. Periodontal diseases, like atherosclerosis, are an inflammatory disease. Herein may lie the most

plausible link between the two diseases, in that periodontal disease may be contributing to a heightened systemic inflammatory state that, in turn, contributes to the progression or exacerbation of atherosclerosis, although other explanations have been put forth. If, as has been suggested in this article, periodontitis constitutes an independent risk factor for atherosclerosis, it will be incumbent on all dental professionals to take appropriate measures to both counsel patients in the prevention of periodontal diseases and, where necessary, arrange treatment for affected individuals to receive appropriate care either in a general practice or specialist setting. Ongoing and future investigations in this area will focus on establishing the molecular basis for the relationship between periodontal diseases and atherosclerosis, and designing more stringently controlled interventional studies to establish the presence of a causal relationship if one indeed does exist. Likewise, development of controlled studies on the use of additional antimicrobial and anti-inflammatory strategies may provide important new tools in the control of atherosclerosis as a health problem.

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Evidence that Diabetes Mellitus Aggravates Periodontal Diseases and Modifies the Response to an Oral Pathogen in Animal Models

Dana T. Graves, DDS, DMSc
Professor

Hesham Al-Mashat, DDS
DSc Candidate

Rongkun Liu, DDS, PhD
Visiting Scholar

Department of Periodontology and Oral Biology
Goldman School of Dental Medicine
Boston University
Boston, Massachusetts

Abstract: ***Bacterial plaque has been shown to initiate periodontal diseases. Most studies indicate that the host response, rather than the direct effect of bacteria, is responsible for much of the destruction associated with periodontitis. Bacteria or their products have an indirect role by stimulating inflammation, which is associated with the excessive production of inflammatory mediators, such as prostaglandins, or cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1. These mediators, in turn, induce the production and activation of enzymes that destroy gingival connective tissue and stimulate the formation of osteoclasts to resorb bone. Based on results in animal models and studies in humans showing that similar responses occur, the initial steps in the breakdown of connective tissue attachment to the tooth surface and bone resorption involve the production of inflammatory cytokines. Moreover, the risk and severity of periodontal diseases is affected by systemic factors, such as diabetes. Diabetes in particular seems to impair the ability to produce new bone formation after bone loss by preventing the formation of new bone that normally occurs after bone is resorbed, a process called coupling. In addition, the cytokines that stimulate loss of tissue, particularly TNF-α, may kill the cells that repair damaged connective tissue or bone. In diabetes there may be more TNF-α produced, leading to an even more limited capacity to repair tissue. The diminished capacity to form new bone may make it more difficult for diabetics in particular to repair the loss of tissue that occurs in periodontal diseases.***

Learning Objectives

After reading this article, the reader should be able to:

- describe the types of host response induced by bacterial products of plaque, such as lipopolysaccharides.
- explain the role of cytokines, such as interleukin-1 and tumor necrosis factor-alpha, on the progression of periodontal disease.
- describe the relationship between periodontal disease and a systemic disorder such as diabetes.
- discuss the effect of diabetes on bone resorption and bone formation that results in a net bone loss.

A central question in the pathophysiology of periodontal diseases concerns how tissue destruction happens and how systemic conditions such as diabetes affect tissue loss. This article is divided into two parts. The first part addresses the question of how bacterial plaque leads to tissue destruction in periodontal diseases. The second part discusses how a systemic condition, such as diabetes, affects periodontitis. This connection is becoming an increasingly important health issue.

Periodontal Diseases

Periodontal diseases are a significant cause of tooth loss among adults. Periodontal diseases are defined as polymicrobial infections that stimulate an inflammatory response in periodontal tissues and result in a loss of support of the affected teeth.[1] This process is characterized by destruction of the periodontal attachment apparatus, loss of crestal alveolar bone, apical migration of the epithelial attachment, and formation of periodontal pockets.[2]

It is well understood that periodontal diseases are initiated by plaque that

accumulates on the tooth surface. Plaque exists as a complex community of up to hundreds of different bacterial species that can act cooperatively. One of dentistry's important contributions to medical science was to establish that there are very significant differences between the behavior of bacteria in a biofilm such as plaque vs the behavior of individual bacteria, which are referred to as "planktonic bacteria."[3]

There are different ways that bacteria can cause periodontal destruction, two of which will be discussed here. Bacteria can produce toxic compounds and enzymes that can cause tissue destruction. Alternatively, bacteria or their products may stimulate inflammation that leads to the activation of host enzymes that are responsible for tissue destruction.[1] An important issue regarding periodontal diseases is which of these mechanisms predominate.

The effect of bacterial plaque on periodontal tissue destruction can be evaluated by blocking bacterial growth. This was established by demonstrating that antibiotic treatment improved periodontal health by removing periodontal pathogens.[4] However, these studies did not determine whether bacteria alone or the resultant inflammatory host response to bacteria caused the periodontal damage. If bacteria alone are responsible for most of the tissue damage, then blocking inflammation would have relatively little effect. If the inflammatory host response to bacteria is the more important component, blocking it would significantly decrease periodontal disease progression. Thus, blocking the host response represents a key test in determining whether bacteria alone or the response to bacteria is more crucial in the pathogenesis of periodontal diseases.

Bacterial Plaque and the Inflammatory Host Response

Previously, the host response to a bacterial challenge was characterized as either acute or chronic inflammation. However, chronic inflammatory diseases, such as periodontitis, have simultaneous acute and chronic components. As a result, the terms *acute* or *chronic inflammation* have been replaced by *innate* or *acquired immune response*, respectively.

From an evolutionary standpoint, the innate immune response developed before the acquired immune response. The innate immune response depends on pattern recognition and is carried out by cells such as polymorphonuclear leukocytes and monocytes or macrophages. These cells have receptors called *toll-like receptors*, which can discriminate between classes of foreign molecules. For example, these receptors recognize bacterial deoxyribonucleic acid (DNA) but not mammalian DNA, or the bacterial cell wall component, lipopolysaccharide (LPS) but not mammalian cell walls.[5] Thus, any molecule that binds to these receptors is recognized as "foreign" and elicits a host response. This response is characterized by the production of inflammatory mediators, including cytokines. Cytokines such as interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF-α) stimulate a number of cellular events, including recruitment of phagocytic cells to the site of infection which, taken together, represent the innate immune response.

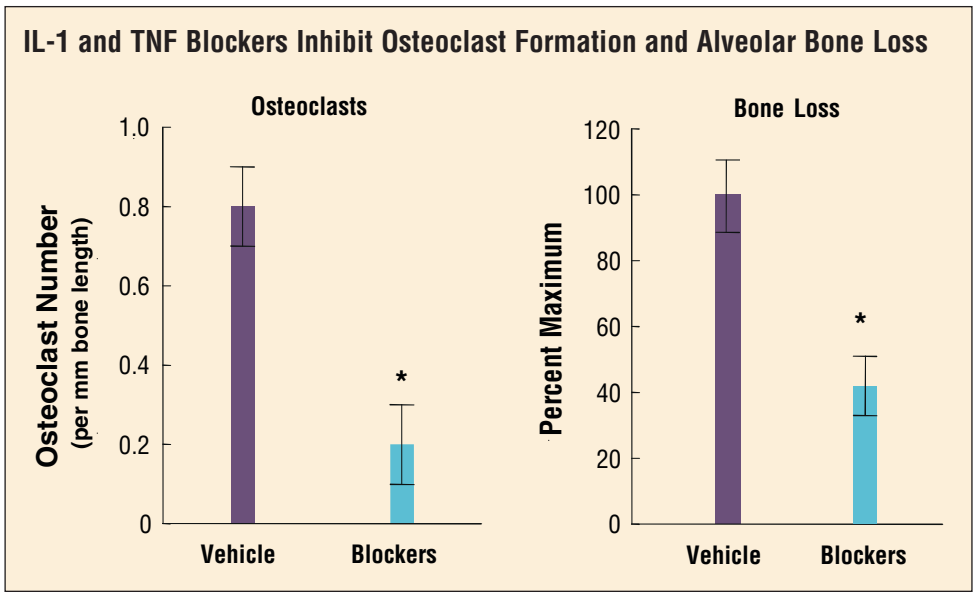

Figure 1— Periodontal disease was induced by tying a ligature around the posterior teeth of animals. After 6 weeks there was a significant loss of bone in animals injected with placebo (vehicle), but when IL-1 and TNF blockers were applied, the bone loss was substantially reduced. *indicates statistical significance. Adapted from Assuma et al.[8]

The acquired immune response is not based on pattern recognition but on the very precise binding of a T-cell receptor to a specific molecule. Rather than binding to a class of molecules, such as bacterial LPS, which varies considerably depending on the bacterium, a given T-cell receptor in the acquired immune response binds to only a single LPS molecule produced by a specific bacterium.[6] This allows precision in the response, characterized by the formation of antibodies or activation of specific lymphocytes. However, acquired immunity also is able, under certain circumstances, to recognize self-antigens, allowing development of lethal autoimmune diseases. Because the acquired immune response has the potential to be very self-destructive, there are elaborate checks and balances that control its activation.[6] As a result of these checks and balances, the acquired immune response takes much longer to become fully activated.

Periodontal diseases are difficult to study because cause-and-effect relationship studies in humans cannot be carried out for ethical reasons. Thus, animal models are used for this purpose. Our laboratory has evaluated both a nonhuman primate model and a mouse model to study host-bacteria interactions relevant to periodontal diseases. The primate model shares many similarities to human periodontal diseases, including bacterial etiology. In this model, periodontal diseases are initiated by tying silk ligatures containing *Porphyromonas gingivalis* around the mandibular posterior teeth of Macaca fascicularis monkeys. The ligatures cause plaque accumulation and the conversion of gingivitis to periodontitis.[7] More recently, in our laboratory and others, rodent models are used because the gene sequences of the animals are generally known and various reagents are readily available, which facilitates research studies.

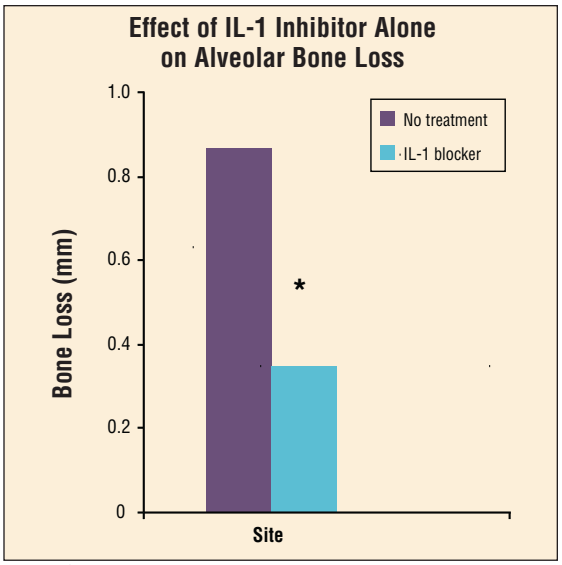

Figure 2—In this experiment the bone loss was evaluated. There was a substantial reduction in bone loss in animals treated with an IL-1 blocker. In the animals not treated with this blocker, there was a high degree of bone loss. *indicates statistical significance. Adapted from Delima et al.[10]

Innate Inflammatory Host Response in Experimental Periodontitis

As mentioned, the host response to periodontal infection involves both innate and acquired immune responses. To determine whether the innate immune response contributed to periodontal destruction, experiments were conducted in the nonhuman primate model focusing on the cytokines IL-1 and TNF-α.[8-10] These molecules are potent stimulators involved in activating the innate immune response and have been shown to be at higher levels in individuals with periodontal diseases.[11] Although IL-1 and TNF-α levels were shown to be higher in periodontitis, there was no evidence that IL-1 or TNF were involved in periodontal breakdown. To investigate this issue, a control group of nonhuman primates with periodontal disease was treated only with a placebo (vehicle), while an experimental group of animals was treated with two blocking agents (antagonists).[8,9] One of these blockers specifically inhibited IL-1 and the other inhibited TNF-α. After 6 weeks, there was a significant loss of bone with increased numbers of osteoclasts in the control group treated with placebo. When IL-1 and TNF-α were blocked, there was significantly less bone and fewer osteoclasts (Figure 1). The data indicate that the presence of IL-1 and TNF-α are important factors in bone loss and that when these molecules are inhibited, the amount of bone loss is considerably reduced.

The effect of inhibiting IL-1 alone also was determined.[10] In the animals not treated with blockers, there was a high degree of bone loss compared to the zero time point. This bone loss was significantly reduced by the inhibition of IL-1 (Figure 2). This study indicated that IL-1 was one of the principal factors in causing bone loss in experimental periodontitis because the loss was substantially reduced when IL-1 was inhibited.

The previous group of experiments demonstrated that IL-1 and TNF-α contributed to bone loss in experimental periodontitis. However, the data did not show whether IL-1 and TNF-α were responsible for the buildup of

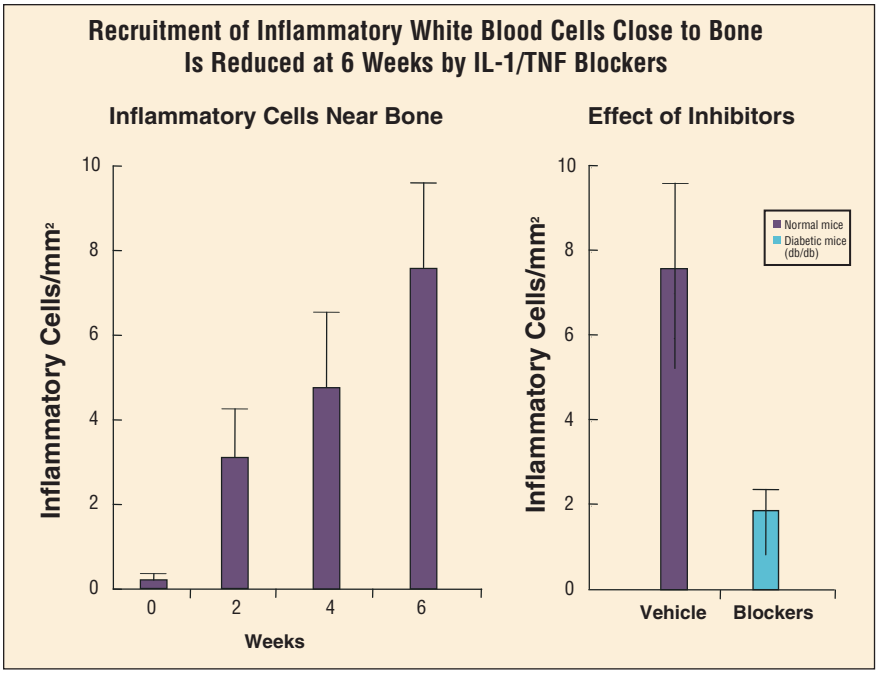

Figure 3—The number of inflammatory cells close to bone was measured after experimental periodontitis was induced by tying silk ligatures around the posterior teeth. Left panel: Over 6 weeks, the cells close to alveolar bone increased. Right panel: Treatment with blockers to IL-1/TNF-α significantly reduced the number of inflammatory white blood cells close to bone. Adapted from Graves et al.[9]

inflammation close to bone. The study results shown in Figure 3 demonstrate that a significant aspect of periodontitis was caused by the recruitment of inflammatory cells in close proximity to bone and that this depended, to a large extent, on IL-1/TNF-α activity. Under normal conditions, when IL-1 and TNF-α were not inhibited, the conversion from gingivitis to periodontitis involved the migration of inflammatory cells deep into the connective tissue close to bone. The migration of inflammatory cells was largely reduced when IL-1 and TNF-α antagonists were present. It can be concluded from this study that the recruitment of the inflammatory white blood cells close to bone involved the production of IL-1 and TNF-α, most likely in response to bacteria that invaded the gingiva. This conclusion was reached because the IL-1 and TNF-α blockers did not directly affect the bacteria but did affect the migration of inflammatory cells close to bone.

Previous studies on blocking prostaglandins provided the first indication that inflammation caused by bacteria resulted in periodontal tissue destruction.[12,13] Prostaglandins are signaling molecules that are activated as part of the innate immune response and are blocked by nonsteroidal anti-inflammatory drugs. When the formation of prostaglandins was inhibited, bone resorption in dogs was reduced. If these results are combined with our results from blocking TNF-α and IL-1, a conclusion can be

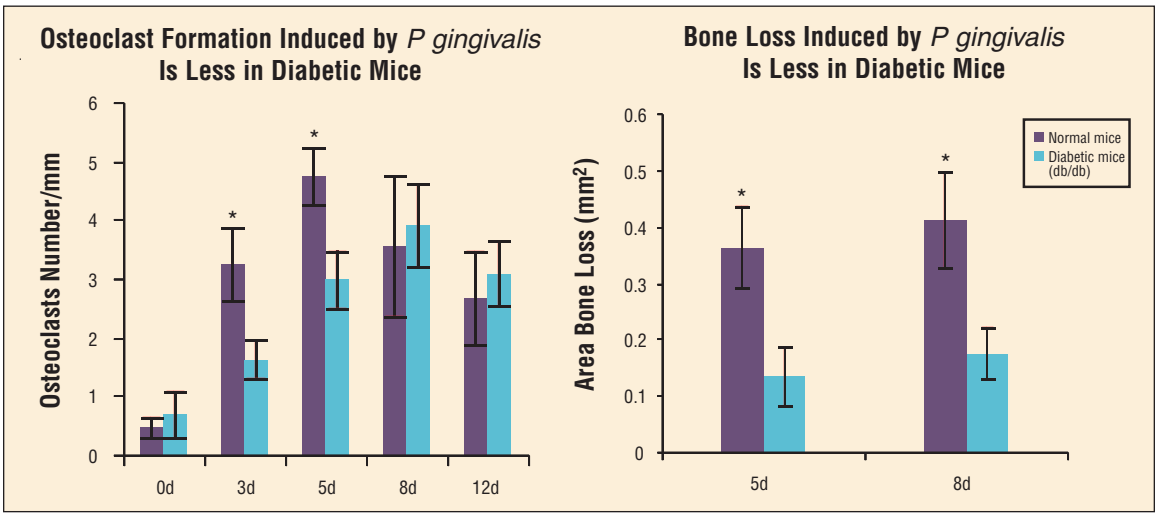

Figure 4—*P gingivalis* was inoculated into the scalp of mice. The number of osteoclasts and amount of bone loss was significantly higher in the control group than the diabetic group. Diabetic (db/db) and normoglycemic littermate control mice. * indicates significant difference between diabetic and normoglycemic mice, $P < .05$. Adapted from He et al.[24]

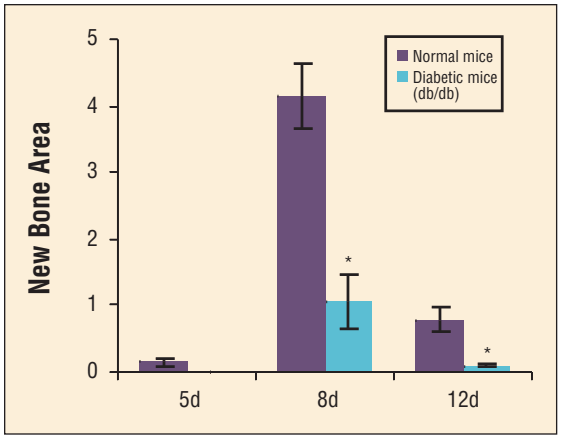

Figure 5—The amount of new bone formation was quantified by computer-assisted image analysis. The amount of new bone formation in the control group was four to eight times higher than that of diabetics. *indicates significant difference between diabetic and normoglycemic mice, $P < .05$. Adapted from He et al.[24]

reached that bacteria-induced inflammation, stimulated and amplified by cytokines and prostaglandins, was responsible for much of the bone loss that occurred with periodontal diseases. Thus, one can draw a model in which bacteria or bacterial products from the gingival sulcus penetrate the gingiva. If the invasion is deep enough, inflammation is produced close to bone that stimulates bone loss. The small degree of inflammation initially induced becomes amplified because the early signals, such as IL-1 or TNF-α, enhance the production of later proinflammatory molecules, such as prostaglandins. Both, in turn, can stimulate the formation of osteoclasts and bone resorption.

Gingival connective tissue also can be destroyed as a result of the release and activation of tissue-degrading enzymes produced during inflammation. In this case, inflammatory signals stimulate the production and activation of matrix metalloproteinases that break down collagen, which is the most important structural unit of the gingiva.[1,11]

Recent data have suggested that the other major arm of the immune system, the acquired immune response, may also contribute to periodontal bone loss.[6] Taken together, it appears that some, if not much, of the periodontal damage that occurs in periodontitis is a result of the host immune response. (Note: In the authors' studies in monkeys and other studies in dogs, it is clear that modulating the host can prevent the majority of tissue loss. There is little or no evidence that bacteria directly cause periodontal bone loss in humans or animals.) Because modulation of the host prevented most of the damage observed, one can conclude that it is the inflammation rather than the direct action of bacteria that produces this effect. This is consistent with the idea that periodontal disease is started by bacteria in plaque, and that these bacteria can penetrate the gingiva and stimulate inflammation. By effective removal of plaque through good oral hygiene and scaling, there is less invasion of the periodontal tissues by bacteria. With fewer bacteria, there is less inflammation and, as a result, reduced periodontal tissue loss. The sequence of events also raises the question as to whether it would be better to turn off the host response to preserve the periodontal tissues. It is the opinion of the authors that the answer to this question is decidedly no, because significantly compromising the inflammatory response has the potential to facilitate much greater penetration of the gingiva by bacteria with potentially serious systemic consequences. This is thought to occur when the host response is significantly reduced, for example, in patients with leukocyte adhesion deficiency.[14] Thus, one can think of periodontal diseases as localized collateral damage control which effectively prevents bacteria that colonize the teeth from penetrating into the gingiva and causing a generalized septic response. However, it may be possible in the future to fine-tune the inflammatory response so that it is not as destructive to the periodontium.

Diabetes Mellitus

It is estimated that 15 million individuals in the United States have diabetes mellitus. There are two major forms of diabetes. Type 1 diabetes, formerly called juvenile diabetes, occurs when the beta cells of the pancreas are destroyed—usually by autoimmune disease—and insufficient amounts of insulin are produced. Type 2 diabetes occurs when cells are resistant to the action of insulin. Type 2 diabetes constitutes about 90% to 95% of the diabetes cases in the United States while most of the remainder have type 1 diabetes.[15] In type 2 diabetic individuals, insulin levels may initially be normal or even slightly higher than normal. The principal failure is resistance to insulin stimulation so that glucose transport from the vasculature into cells of the liver and muscle is not adequately induced. Therefore, glucose levels remain high in circulating blood. Type 2 diabetes is commonly associated with obesity and, for reasons that are not entirely clear, obesity causes cells to not respond well to insulin (ie, to become insulin resistant).[15] Studies have

shown that both types of diabetes increase the risk of periodontal diseases and cause more severe periodontal breakdown.[16-18]

The Effect of Diabetes Mellitus on Periodontal Diseases

It has been shown repeatedly that the risk of periodontal diseases or periodontal disease progression is greatly influenced by systemic factors such as diabetes. Diabetes increases two- to fivefold the likelihood of developing periodontal diseases.[16-18] Treatment of diabetes often reduces the risk of more severe periodontal disease.[19]

Because diabetes has a significant impact on bone and periodontal diseases, people with this disease need a thorough periodontal evaluation and special consideration in treatment planning. When the diagnosis of periodontal diseases is established, active treatment is recommended and should be instituted promptly. One consideration, which is currently the subject of considerable research, is that periodontal treatment should be carried out because untreated periodontitis may negatively impact systemic diseases, including diabetes.[20] Along these lines, it has been reported that effective periodontal treatment helps in the stabilization of serum glucose levels.[20] This may be another example of how a healthy periodontium benefits the overall health of the individual by lowering the probability of systemic inflammation and its consequences.

Several mechanisms have been proposed to explain the greater incidence and severity of periodontal diseases in diabetics. Diabetes tends to increase susceptibility to bacterial infection by decreasing the effectiveness of cells that kill bacteria.[21] Because periodontal diseases are triggered by bacteria, a decreased capacity to kill bacteria may make it easier for bacteria to invade the gingiva. Another possibility is that inflammation tends to be enhanced in those with diabetes.[22] As a result, diabetes might cause the production of higher levels of proinflammatory cytokines, such as IL-1 and TNF-α, thereby leading to greater bone loss.[22,23] Thus, both of these concepts suggest that there is more inflammation in those with diabetes, either because there is a greater tendency of bacteria to invade the gingiva or more inflammation is stimulated by bacteria that do invade as a result of the diabetic condition. In both scenarios, a greater degree of inflammation would produce enhanced levels of bone loss. However, this concept has not been proven conclusively. Another possibility, which has not been investigated, is that diabetes enhances net periodontal bone loss because diabetes interferes with the capacity to form new bone after periodontal diseases have caused bone resorption.

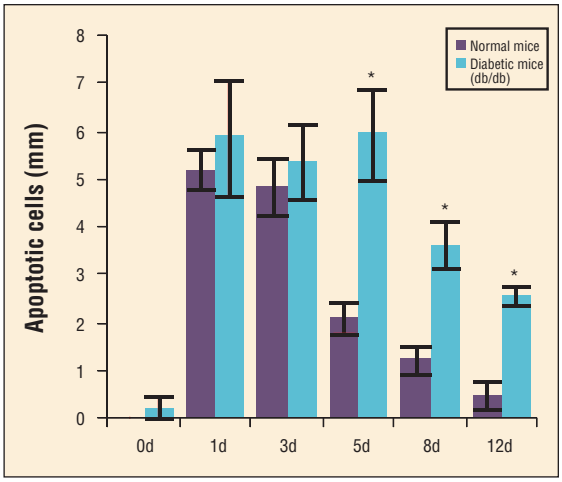

Figure 6—Apoptotic bone lining cells on histologic sections after inoculation with *P gingivalis*. The number of bone lining cells undergoing cell death (apoptosis) per mm of bone length was counted at 1000 X magnification. After day 3, bone cell death is greatly reduced in the normal mice, while it remains high for the entire time in the diabetic (db/db) mice. * indicates statistical significance, $P < .05$. Adapted from He et al.[24]

The Effect of Diabetes Mellitus on Bacteria-Stimulated Bone Loss

A dynamic tissue, bone is well adapted for repair. After bone resorption occurs, growth and remodeling are automatically triggered in a process called *coupling*. However, in periodontal diseases, there is a net loss of bone so that coupling is incomplete. We conducted experiments to determine whether the principal effect of diabetes on bacteria-stimulated bone loss was caused by an increase in bone resorption or a decrease in bone repair after bone loss. Genetically diabetic mice (db/db mice) with type 2 diabetes and their nondiabetic littermates were used as a model. The diabetic mice shared many similarities to human type 2 diabetes. A human periodontal pathogen, *P gingivalis*, was injected into the connective tissue of the scalps of both the control and experimental groups of mice—a method that is useful to study the inflammatory response to oral pathogens and the impact on bone[24,25] because it causes a host response and bone resorption. The bone resorption is then repaired by the formation of new bone. The results of this experiment are shown in Figure 4. Contrary to expectations that diabetic mice would have enhanced bone resorp-

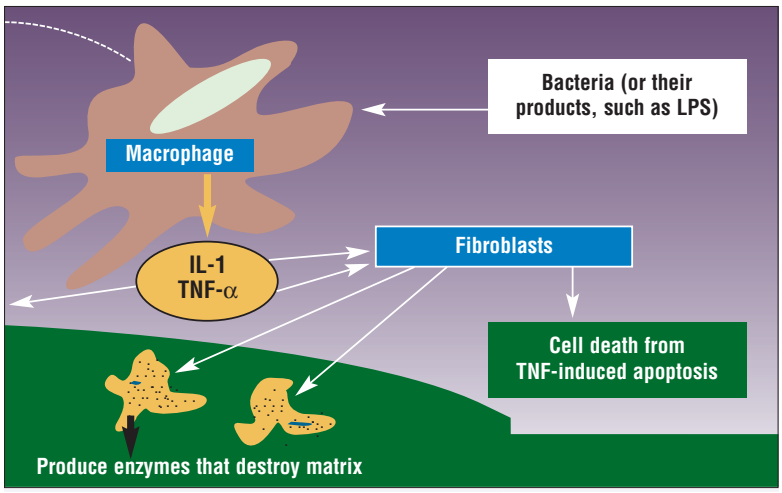

Figure 7A—IL-1 and TNF-α can be produced by a number of cells and are able to stimulate many different biologic activities. IL-1 and TNF-α can stimulate fibroblasts to produce lytic enzymes that can destroy connective tissue matrix. TNF can also cause apoptosis of fibroblasts.

tion, we found the opposite. *P gingivalis* infection caused less osteoclast formation and less bone resorption compared to normoglycemic (nondiabetic) mice. This was surprising because it has been widely held that diabetics have increased bone resorption after bacterial challenge.

The capacity to repair the resorbed bone was then measured at the histologic and molecular levels. Because bone resorption is followed by new bone formation, net bone loss occurs when there is less new bone formed. In this study, diabetes had a significant impact on the capacity to form new bone, thus contributing to net bone lost primarily by diminishing new bone formation (Figure 5).

To further understand why the diabetic mice were not producing enough new bone, the death of osteoblasts—the cells that produce new bone after resorption—was measured. The results indicated that there was a much more prolonged high rate of osteoblast cell death, also called apoptosis, in the diabetic group (Figure 6). Thus, the increased death of osteoblasts may contribute to the diminished capacity of diabetic mice to form new bone after infection. To look at this another way, when osteoblasts (the cells that produce bone) die prematurely, the capacity to repair the bone defect by producing new bone is severely limited. This means that the normal coupling of bone formation after resorption does not occur.

Discussion

Although bacteria in plaque are required to initiate the periodontal disease process, they are not necessarily responsible for the resultant actual loss of bone tissue. The indirect role of bacterial plaque products results in an excessive production of inflammatory mediators, such as prostaglandins, or cytokines, such as TNF-α and IL-1, which initiate alveolar bone loss. The severity of periodontal diseases also is affected by systemic factors such as diabetes mellitus. Diabetes mellitus in particular seems to impair the ability to produce new bone after bone loss by preventing the normal coupling of bone formation and bone resorption. These concepts are described for connective tissue in Figure 7A and for bone in Figure 7B. In connective tissue bacterial products (such as LPS) stimulate cells (such as macrophages) to produce IL-1 and TNF-α. IL-1 and TNF-α stimulate the production of enzymes that destroy gingival connective tissue and may also cause the death of fibroblasts that are capable of repairing damaged tissue. In bone, bacteria or their products stimulate nearby macrophages to produce IL-1 or TNF. This stimulates bone lining cells such as osteoblasts to produce other factors that induce formation of osteoclasts that resorb bone. TNF in particular may also cause cell death of osteoblasts that repair bone. It is interesting to note that the initial steps in both gingival connective tissue destruction and bone resorption

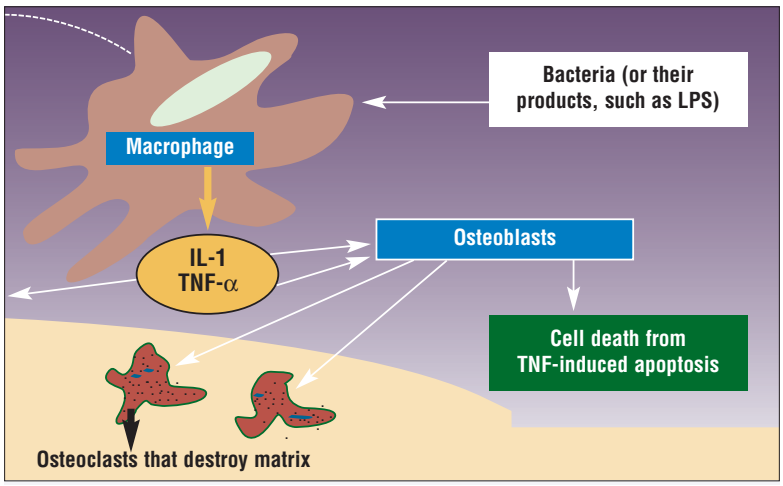

Figure 7B—IL-1 and TNF-α can stimulate osteoblasts to induce the formation of osteoclasts that resorb bone matrix. TNF-α can also directly induce fibroblast and osteoblast death.

are thought to be similar. In addition, these cytokines, particularly TNF-α, may kill the cells that repair damaged connective tissue, such as fibroblasts or bone-lining osteoblasts. In diabetics, there may be more TNF-α produced, leading to an even more limited capacity to repair tissue. Moreover, diabetes may also make it more difficult for osteoclasts to form for reasons that are unknown at this time. However, the greatest effect of diabetes in the mouse model appears to be on diminished bone formation, which is consistent with clinical observation of greater net bone loss in humans with diabetes. This hypothesis that bacteria causes enhanced net bone loss in diabetic animals because of an inability to form new bone after resorption was developed in a rodent model and needs to be corroborated in other animal models.

Conclusion

Several studies have shown that bacterial plaque initiates periodontal diseases and that treatment which addresses plaque buildup are the most reliable approach to treatment. Until recently, however, the mechanisms by which bacteria causes periodontal tissue loss were not well understood. Studies in nonhuman primates and in dogs suggest that bacterial invasion of the gingiva stimulates the formation of inflammatory mediators such as IL-1, TNF-α, and prostaglandins, and that inhibition of these mediators reduces most of the tissue loss associated with periodontal diseases. This is consistent with human studies showing that these inflammatory mediators are elevated in patients with periodontitis. On this basis, it is likely, although not absolutely proven, that the production of IL-1, TNF, and prostaglandin is a critical step in tissue destruction associated with various periodontal diseases. These mediators are also elevated in both animals and humans who have diabetes. The precise mechanism by which diabetes enhances the risk and severity of periodontal disease is not known. Studies in mice suggest that diabetes may reduce the capacity of bone to repair itself after bone resorption. If an individual had bone loss and was able to repair some of the lost bone, there would be less net bone loss than in another individual who had reduced capacity for repair. The latter may be the case in diabetes. However, many more studies are needed to refine this concept and determine whether a similar paradigm exists in other animal models and in humans.

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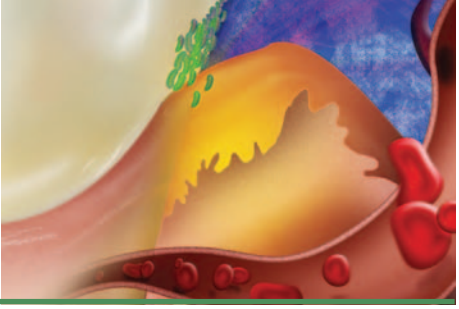

Effectiveness of a Triclosan/Copolymer Dentifrice on Microbiological and Inflammatory Parameters

Tao Xu, DMD, PhD
Associate Director

Meenal Deshmukh, PhD
Research Scientist

Virginia Monsul Barnes, DDS
Senior Technical Associate

Harsh M. Trivedi, MS
Technical Associate

Diane Cummins, PhD
Worldwide Director

The Colgate-Palmolive Company
Technology Center
Piscataway, New Jersey

Abstract: ***According to the US Surgeon General's report, "Oral Health in America," published in 2000, most adults in the United States show some degree of periodontal pathology, with severe periodontal diseases affecting about 14% of middle-aged adults. Periodontal diseases are polymicrobial-induced inflammatory diseases, and they vary from mild gingival inflammation to severe deterioration of the periodontium, ie, loss of periodontal supportive tissues and, ultimately, tooth loss. New evidence shows that periodontal diseases may impact systemic health. For this reason, the maintenance of a healthy mouth is becoming increasingly important for the overall health of the body. This article summarizes laboratory research conducted during the development of a novel, multibenefit, oral-care technology based on triclosan—a broad-spectrum antibacterial agent—and a polyvinylmethylether/maleic acid copolymer. This unique combination of agents is found in Colgate® Total®, a clinically proven efficacious dentifrice for control of dental plaque and gingivitis. Data are presented that demonstrate the unique antibacterial properties of this dentifrice: (1) a broad-spectrum antimicrobial profile; (2) the long-lasting retention of triclosan on hydroxyapatite and epithelial cells; and (3) molecular evidence of antibacterial activity against specific pathogens in clinical dental plaque. In addition, data are presented that demonstrate the anti-inflammatory effects of triclosan on specific cytokines, the interruption of inflammatory pathways, and the inhibition of bone resorption. Overall, these data support the multibenefit clinical effects of Colgate® Total® and suggest a plurality of mechanisms of action.***

Learning Objectives

After reading this article, the reader should be able to:

- specify the disease pathways and consequences of periodontal diseases.
- describe the antibacterial efficacy of a triclosan/copolymer/fluoride dentifrice, including new molecular techniques available to document the antibacterial effect.
- discuss the anti-inflammatory effects of triclosan, including the specific markers measured to detect anti-inflammatory efficacy.

Periodontal diseases are polymicrobial diseases, characterized as dental-plaque–induced gingival inflammation with loss of periodontal support tissues, bone loss, and, ultimately, tooth loss.[1-3] For the past several decades, significant clinical and basic research has established the complex pathology of periodontal diseases, and, specifically, that they involve bacterial infection, host immune reaction, and bone metabolism, as well as genetic and environmental risk factors. More recently, a potential link has been identified between periodontal health and systemic diseases, including diabetes, cardiovascular disease, respiratory disease, and low birth weight or preterm birth.

The US Surgeon General's first-ever "Oral Health in America" report indicated that most adults in the United States have some degree of periodontal pathology, with severe forms of periodontal disease measured as 6 mm of periodontal attachment loss, affecting 14% of adults aged 45 to 55.[4] One of the major themes of this report was the notion that with increased life expectancy and improved standards of living, oral health means more than

just healthy teeth. Although more research is needed to substantiate the link between oral health and systemic health, the available data have suggested the importance of oral health to the control of systemic sequelae.[5-9]

The importance of bacterial plaque to the onset and progression of periodontal diseases is well accepted.[1-3,10] While more than 400 species of bacteria can be detected in the oral cavity, only selective pathogenic species produce products harmful to gingival tissues.[11,12] The pathway to periodontal destruction is depicted in Figure 1. It shows that dental plaque bacteria initiate pathogenesis. Microbial products of specific pathogens, ie, lipopolysaccharide (LPS) and proteolytic enzymes, directly or indirectly trigger a host tissue response by inducing cytokine production and increasing the levels of inflammatory mediators, leading to inflammation and tissue destruction. Without intervention or treatment, supporting tissues will be destroyed, clinical pockets will form, bone resorption will occur, and, ultimately, the tooth will be lost. Potentially more serious than tooth loss is the possibility that local oral pathology could negatively impact the whole body's immune response, thereby impacting systemic health. These concepts and the mechanisms involved in periodontal disease etiology have been the subject of a number of recent literature reviews.[13-18]

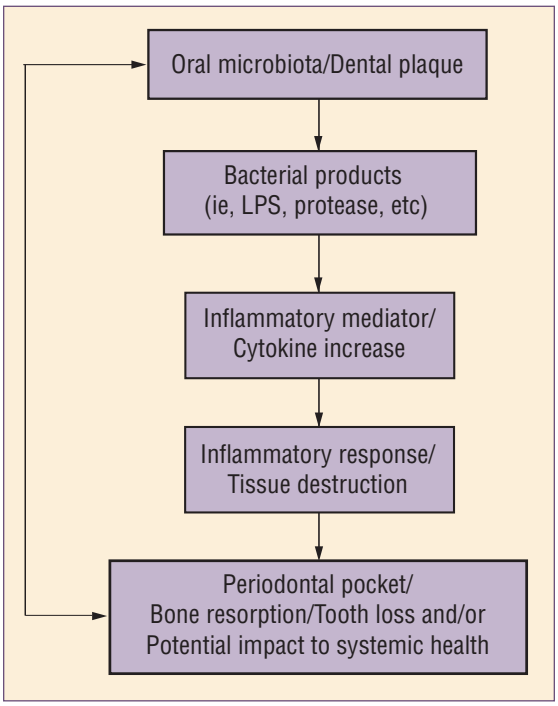

Figure 1—This illustration of periodontal pathogenesis shows where bacteria and dental plaque can initiate pathogenesis. Microbial-breakdown products, ie, lipopolysaccharide (LPS) and proteolytic enzymes, can directly or indirectly trigger tissue response and inflammatory mediator and cytokine increase, which lead to inflammation and tissue destruction. If the pathology moves forward without intervention, periodontal tissues can be destroyed, clinical pockets can form, and bone resorption can occur, leading to possible tooth loss.

Periodontal diseases are polymicrobial diseases, characterized as dental-plaque–induced gingival inflammation with loss of periodontal support tissues, bone loss, and, ultimately, tooth loss.

Given the complexity of periodontal diseases and the importance of oral health, one of the critical questions is how to best prevent and treat periodontal infection. Clinical procedures such as scaling and root planing provide immediate and universal benefits, whereas effective routine oral care can help maintain a healthy oral environment and decrease the occurrence of oral disease.[19]

It is interesting to speculate that a combination of antibacterial and anti-inflammatory efficacy may provide a unique and beneficial approach to the prevention and treatment of periodontal diseases via daily oral-care procedures, not only for high-risk individuals, but also for the general population. Thus, while the prevention and treatment of periodontal diseases can be achieved, for the most part, through the control of dental plaque, the concomitant control of inflammation may add incremental benefit.

A unique triclosan/copolymer/fluoride dentifrice technology, Colgate® Total®,[a], has been developed and clinically proven to enhance conventional oral-care procedures.[20] This technology uses a broad-spectrum antibacterial agent, triclosan, and a polyvinylmethylether/maleic acid (PVM/MA) copolymer to deliver sustained antibacterial activity in the oral cavity, thereby controlling dental plaque and preventing and treating gingival inflammation. In practice, this triclosan/copolymer/fluoride dentifrice has been proven to deliver statistically significant and clinically relevant benefits in the prevention of caries, the reduction of dental calculus buildup and oral malodor, as well as

[a]Colgate-Palmolive Company, New York, NY 10022; 800-338-8388

the control of dental plaque and treatment of gingivitis.[21] Such a multibenefit oral-care technology can significantly enhance routine oral-care procedures and help to maintain a healthy oral environment.

This article reviews data pertinent to the mechanism of action of the triclosan/copolymer/fluoride dentifrice and its use in routine oral care for the prevention of plaque and gingivitis. It is focused primarily on laboratory research into the effects of this dentifrice on oral bacterial pathogens and on triclosan's role on host response parameters associated with inflammation.

Antibacterial Efficacy and Control of Dental Plaque

As a broad-spectrum antibacterial agent, triclosan has a nearly three-decade history of safe use in soaps and deodorants.[17] More than 10 years ago, triclosan was successfully introduced into oral-care products, specifically dentifrices and mouthwashes.[20] One of the most significant challenges for incorporating the use of triclosan in oral care was to develop a dentifrice formula in which triclosan would be fully compatible with the excipient ingredients and also would ensure maximum delivery and retention of triclosan in the oral cavity. The solution was to combine triclosan with a PVM/MA copolymer, which stabilizes the triclosan in a bioavailable form within the product matrix, facilitates its release during toothbrushing, and ensures its adherence in the oral cavity for a prolonged period.

The antibacterial effects of the triclosan/copolymer/fluoride dentifrice on oral organisms were reported by Gaffar et al.[22] The authors performed the minimum inhibitory concentration (MIC) assay using fresh clinical isolates as well as standard strains from the American Type Culture Collection. The MIC values for the majority of the dental plaque pathogens tested were less than 1 µg/mL (Table 1). In a clinical study,[23] dental plaque was collected from human subjects at various time intervals after they had brushed with the triclosan/copolymer/fluoride dentifrice. The concentration of triclosan in dental plaque decreased over time, being present at 38.8 ± 18.3 µg/mL and 4.1 ± 1.7 µg/mL at 2 and 14 hours, respectively (Table 2). Importantly, the level of triclosan present in plaque 14 hours after toothbrushing was found to exceed the MIC values for most of the organisms isolated from dental plaque (as indicated in Table 1). Thus, this triclosan/copolymer/fluoride dentifrice has been found to provide an effective dose of triclosan to dental plaque during toothbrushing, where it is retained at antibacterial levels to deliver long-lasting effects for up to 14 hours.

Nabi et al[24] published data showing that the

Table 1—Determination of Minimum Inhibitory Concentration of Colgate® Total® on Oral Bacteria*

Source of Bacteria	Minimum Inhibitory Concentration (MIC in µg/mL)	
Laboratory strains		
	MIC values between < 0.38 and 1.14	
	Streptococcus mitis (two species)	*Fusobacterium nucleatum*
	Actinomyces naeslundii	*Caphocytophaga ochracea*
	Actinomyces odontolyticus	*Peptococcus asaccharolyticus*
	Prevotella intermedia	
	MIC value = 5.35	
	Veillonella parvula	
Clinically isolated strains		
	MIC values between < 0.29 and 0.78	
	Actinobacillus actinomycetemcomitans	(two species)
	Actinomyces odontolyticus	(two species)
	Capnocytophaga spp	(two species)
	Fusobacterium nucleatum	
	Actinomyces naeslundii	
	MIC value = 2.34	
	Capnocytophaga spp 290	

*Adapted from Gaffar et al.[22]

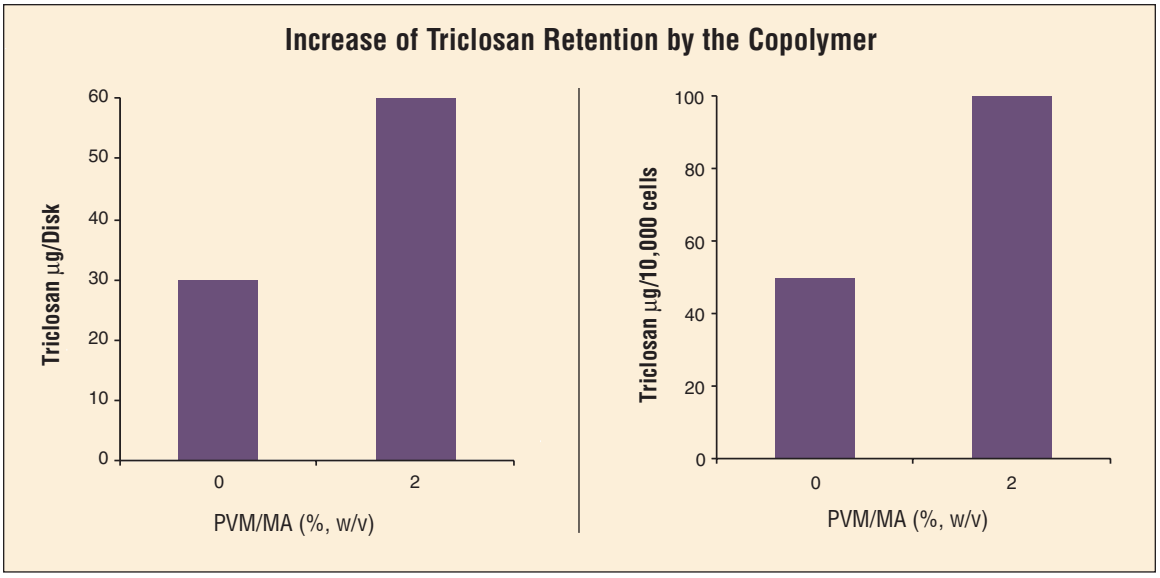

Figure 2—Triclosan uptake is increased in the presence of 2% copolymer. Left: the increase of triclosan retention on hydroxyapatite disks, which mimic the retention to the enamel surfaces. Right: the increase of triclosan retention to the buccal epithelial cells in a cell culture experiment, which simulates triclosan retention to the mucosal surfaces in the mouth. Adapted from Nabi et al.[24]

presence of 2% PVM/MA copolymer doubled the amount of triclosan deposited on saliva-coated hydroxyapatite disks (Figure 2). This method mimics the delivery and retention of triclosan to hard tissues (ie, teeth). In a second study designed to mimic the retention of triclosan on soft tissues, 2% PVM/MA copolymer was shown to increase the uptake and retention of triclosan to exfoliated epithelial cells.[25] These results support the clinical observation that the combination of triclosan and a PVM/MA copolymer delivers a unique, long-lasting antibacterial effect. Furthermore, this unique effect supports the clinically proven and well-documented long-term benefits of the triclosan/copolymer/fluoride dentifrice in regard to dental plaque control, prevention and treatment of gingivitis, reduction of dental calculus buildup and oral malodor, and the prevention of caries.

Molecular Evidence of Antibacterial Action and Long-lasting Benefit

To develop further understanding of the antibacterial effects of this dentifrice, a series of new clinical research studies has been conducted using real-time polymerase chain reaction (PCR), a state-of-the-art technique for the analysis of dental plaque samples. Clinically, the antibacterial effects of oral-care products are assessed as "gross" effects on plaque accumulation by scoring plaque indexes. These measures do not give any indication of whether this antimicrobial activity is directed against specific microorganisms. Microbiologically, antibacterial effects are measured as reductions in total bacteria and/or specific species by conventional plating and counting-numbers methods. However, it has been increasingly recognized that these cultivation techniques are inaccurate, particularly for the study of the etiology of periodontal diseases, as many oral bacteria have very complex physiological requirements and, thus, cannot be grown and counted in vitro. Furthermore, periodontal pathogens often constitute only a small fraction of the total plaque microbiota, making it difficult to specifically detect and quantify individual pathogens. On the other hand, PCR-based assays are capable of detecting the presence or absence of specific bacteria with much greater sensitivity than cultivable methods. During PCR, a single copy of a DNA template is amplified by a factor of a million or more, thus, theoretically, even a single bacterial cell can be detected. A second advantage of PCR is that it is significantly less labor-intensive than microbiological culture methods. Real-time PCR

Table 2—Determination of Triclosan Concentration in Clinical Dental Plaque After Brushing With Colgate® Total® Toothpaste*

Hours	Triclosan (µg/mL)
0	0
2	38.83 ± 18.28
14	4.14 ± 1.72

*Adapted from Gaffar et al.[23]

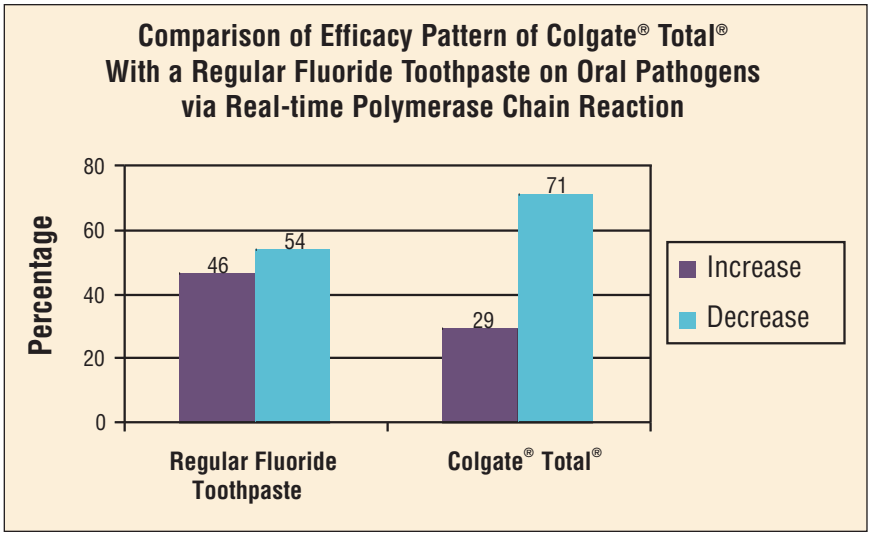

Figure 3—Comparison of reduction of oral pathogens by a unique triclosan/copolymer/fluoride dentifrice and a regular fluoride toothpaste. Bars represent percentages of data points in which bacterial DNA amounts increased or decreased 24 hours after use of the dentifrice relative to baseline levels. After 24 hours, the triclosan/copolymer/fluoride dentifrice showed a greater inhibitory effect on oral pathogens (71% of data points showed decreased amounts of pathogens) compared to regular fluoride toothpaste (54% of data points showed decreased amounts of pathogens).

overcomes the limitation of conventional PCR, which is not quantitative, by allowing quantification of species-specific DNA in a given sample.[26] As there is a direct correlation between the number of cells of a specific bacterial species and the amount of DNA of that bacterial species, quantification of DNA is a reliable way to estimate the amount of specific bacteria in a mixed microbial population.[27] Real-time PCR has been used to detect periodontal pathogens in several clinical trials and epidemiologic studies.[28,29]

For the past several decades, significant clinical and basic research has established the complex pathology of periodontal diseases, and, specifically, that they involve bacterial infection, host immune reaction, and bone metabolism, as well as genetic and environmental risk factors.

In a crossover clinical study, healthy subjects were instructed to brush with a standard commercial fluoride toothpaste and the triclosan/copolymer/fluoride dentifrice at different time intervals, with a washout period in between. Plaque indexes were recorded and available dental plaque was collected from the lingual surfaces before brushing (baseline) and 24 hours after brushing (during this time, subjects were asked to refrain from any other oral hygiene).[30,31] The wet weight of plaque was recorded and bacterial DNA was isolated from plaque. A universal primer designed to detect all bacterial DNA and species-specific primers were made for real-time PCR analysis (Table 3). Micrograms of species-specific DNA per milligram of plaque were calculated with the use of real-time PCR using species-specific primers to amplify the DNA region encoding 16S rRNA. As a part of the bacterial ribosome, the 16S rRNA interacts with specific proteins and other RNA molecules in the translation of RNA to protein. The gene coding for 16S rRNA contains highly conserved regions, suitable for the design of broadly reactive primers, flanking variable and hypervariable regions that are used for species identification.[28]

The effects of the triclosan/copolymer/fluoride dentifrice on specific bacterial species were assessed as a percent reduction or percent increase by comparing the amounts of species-specific DNA in baseline and 24-hour samples.

Table 3—Oral Bacteria in Clinical Dental Plaque Quantified by Real-time Polymerase Chain Reaction

Total Bacterial Biomass (dental plaque)
• *Porphyromonas gingivalis*
• *Actinobacillus actinomycetemcomitans*
• *Fusobacterium nucleatum*
• *Bacteroides forsythus*
• *Streptococcus mutans*
• *Actinomyces naeslundii*
• *Lactobacillus casei*

The list represents dental plaque and seven representative known oral pathogens selected for DNA quantification by real-time PCR. A universal primer designed to detect all bacterial DNA and species-specific primers were made from the above bacteria to be used for real-time PCR.

Figure 3 shows the results for five representative subjects for this triclosan/copolymer/fluoride dentifrice and the standard fluoride toothpaste at the 24-hour time point. These data indicate that the use of the triclosan/copolymer/fluoride dentifrice inhibited the regrowth of a higher percentage of oral pathogens than did the standard fluoride toothpaste.

Further data analysis indicates that specific oral pathogens showed varying levels of susceptibility to the use of the standard fluoride toothpaste. Plaque samples showed significantly reduced levels of *Lactobacillus casei*, a cariogenic microorganism.[32] The results suggest that daily use of fluoride toothpaste can reduce, as well as inhibit regrowth of, cariogenic bacteria. Importantly, more significant changes were observed 24 hours after the use of the triclosan/copolymer/fluoride dentifrice, with reductions in bacterial numbers and inhibition of bacterial regrowth observed for several of the oral pathogens, particularly the periodontal pathogens *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Bacteroides forsythus*, as well as *Streptococcus mutans* and *Actinomyces naeslundii*. This study provides the first molecular evidence of the antibacterial effects of the triclosan/copolymer/fluoride dentifrice (Figure 3). The results support the clinically proven benefits of this triclosan/copolymer/fluoride dentifrice, namely, the inhibition of dental plaque and treatment of gingivitis. The data also suggest that daily use of this triclosan/copolymer/fluoride dentifrice can provide up to 24 hours of antibacterial protection and, as a result, up to 24 hours of dental-plaque control,

Table 4—Inhibition of PGE_2 Production by Triclosan

Inhibition of IL-1β–induced PGE_2 production		
IL-1β (µg/mL)	**Control (No triclosan)**	**Triclosan (1 µg/mL)**
50	~10*	<1
100	~15	<3
200	~25	~5
Inhibition of TNF-α–induced PGE_2 production		
TNF-α (µg/mL)	**Control (No triclosan)**	**Triclosan (1 µg/mL)**
1	~10	<5
10	~35	~10

PGE_2 = prostaglandin E_2; IL-1β = interleukin-1 beta; TNF-α = tumor necrosis factor-alpha.
*Values in the columns of control and triclosan represent production of PGE_2 picograms per 10^3 of human gingival fibroblasts. Adapted from Modeer et al.[33]

Table 5—Comparison of Anti-inflammatory Effect Between Ibuprofen and Triclosan

	PGE_2	COX-2
Ibuprofen	4.12	1.86
Triclosan	13.8	0.59
	Stimulation of HEPM cells by IL-1β 10 µg/mL	Stimulation of RAW cells by *Escherichia coli* LPS at 1 µg/mL

PGE_2 = prostaglandin E_2; COX-2 = cyclooxygenase-2; HEPM = human embryonic palate mesenchymal;LPS = lipopolysaccharide;IL-1β = interleukin-1 beta.

which may provide an important contribution to the prevention of oral disease, including caries and periodontal inflammation.

Anti-inflammatory Effects of Triclosan

Inflammation is the body's self-defense mechanism to deal with injury and infection. However, excessive or prolonged inflammation can lead to tissue damage. Prevention and control of inflammation may help to maintain healthy tissue. With respect to inflammation in the oral cavity, the prevention and treatment of gingivitis and periodontitis is beneficial for a healthy mouth and this, in turn, may be important for a healthy body. Clinically, the triclosan/copolymer/fluoride dentifrice treats gingivitis. Laboratory research suggests that the antigingivitis effect of this triclosan/copolymer/fluoride dentifrice results from the combined antimicrobial and anti-inflammatory properties of triclosan. The antimicrobial activity of triclosan results in better plaque control, while the anti-inflammatory action directly ameliorates the inflammation.[22-25,33] Modeer et al[33] conducted a series of laboratory studies to assess the anti-inflammatory action of triclosan. Interleukin-1 beta (IL-1β) is an important cytokine that can play multiple roles in the stimulation of the inflammatory response. Specifically, IL-1β can induce prostaglandin E_2 (PGE_2) production during the process of inflammation. PGE_2 is the most potent stimulator of bone resorption and exhibits a broad range of inflammatory effects. In one study, Modeer et al reported that as IL-1β was increased from 50 pg/mL to 200 pg/mL, the presence of triclosan at 1 µg/mL prevented a significant increase in PGE_2 (Table 4). Tumor necrosis factor-alpha (TNF-α) is also an important mediator of inflammatory reaction and may cause tissue damage. In a second study, triclosan was shown to inhibit TNF-α–induced PGE_2 production. At both 1 and 10 µg/mL

TNF-α, PGE_2 production was significantly inhibited by the presence of triclosan (Table 4). It was also found that TNF-α–induced PGE_2 production can be inhibited for up to 24 hours in a human gingival fibroblast cell culture. This was probably because of the inhibition of PGE_2 biosynthesis. In addition to its direct effects on cytokines, triclosan can reduce the activity of the enzyme cyclooxygenase-2 (COX-2), which may also lead to a decrease in the production of PGE_2. Table 5 shows the results of studies that compared triclosan in two different cell culture systems to ibuprofen, a known anti-inflammatory drug used as a positive control. Ibuprofen and triclosan both inhibited PGE_2 production. However, triclosan was more potent than ibuprofen in inhibiting COX-2. Collectively, the results show that triclosan can inhibit important cytokines, PGE_2 biosynthesis, and the COX-2 pathway. Together, these results suggest that the anti-inflammatory effects of triclosan may contribute to the end clinical benefit delivered by the triclosan/copolymer/fluoride dentifrice, namely an antigingivitis effect. Finally, in a preliminary laboratory study, triclosan was found to have the potential to directly affect bone resorption, a severe downstream consequence of periodontal inflammation. Using a parathyroid-hormone–induced release of calcium from bone cultures as a model for bone resorption, triclosan was shown to inhibit PGE_2 and calcium release.[34]

Recently, a potential link has been identified between periodontal health and systemic diseases, including diabetes, cardiovascular disease, respiratory disease, and low birth weight or preterm birth.

Summary

Colgate® Total® is a dentifrice containing a unique active system of triclosan, PVM/MA copolymer, and sodium fluoride. This combination delivers long-lasting antibacterial and antigingivitis effects for the prevention and control of periodontal inflammation. The laboratory data discussed here provides molecular evidence for the antibacterial effect of this dentifrice and shows that this effect is efficacious for up to 24 hours postbrushing. The data further confirm and support the long-term antiplaque and antigingivitis benefits of the triclosan/copolymer/fluoride dentifrice that have been documented in numerous clinical trials. In addition to reducing and inhibiting plaque and gingivitis, this triclosan/copolymer/fluoride dentifrice delivers complete oral care by providing significant reductions in caries, oral malodor, and calculus buildup, and improves whitening (as measured by the tooth color shade changes).[35] Thus, brushing with the triclosan/copolymer/fluoride dentifrice contributes to the maintenance of a complete healthy oral cavity, and this, in turn, may aid in promoting the overall health of the body. As the US Surgeon General's report states, oral health means much more than healthy teeth; oral health is linked to total health and well-being throughout life. Ignoring oral health can lead to pain and suffering. Therefore, the prevention of oral disease via daily oral care may add significant positive benefits for long-term total health.

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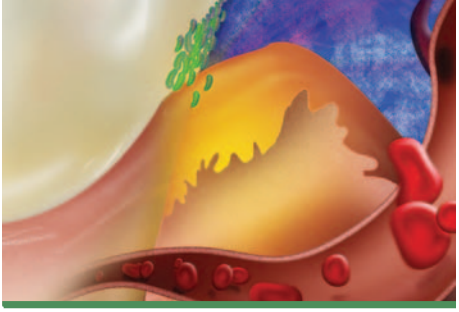

Rationale for the Daily Use of a Dentifrice Containing Triclosan in the Maintenance of Oral Health

William DeVizio, DMD
Worldwide Director
Clinical Dental Research
The Colgate-Palmolive Company
Technology Center
Piscataway, New Jersey

Robin Davies, BDS, PhD
Director
Colgate-Palmolive Dental
Health Unit
Manchester, United Kingdom

Abstract: ***This paper provides an overview and summary of the data that demonstrate the effectiveness of a dentifrice containing triclosan/copolymer/fluoride in significantly reducing plaque, calculus, gingivitis, and the onset and progression of periodontitis. The caries-preventive benefit of the triclosan/copolymer/fluoride dentifrice was at least as good as that of a fluoride dentifrice. No evidence of bacterial resistance or the development of pathogenic or opportunistic bacteria was observed.***

Learning Objectives

After reading this article, the reader should be able to:

- discuss why many patients require a multi-benefit dentifrice as part of their daily oral hygiene regimen.
- list the desirable features of a chemotherapeutic antiplaque agent.
- examine the benefits provided by a dentifrice containing triclosan, copolymer, and fluoride.
- describe the role of the copolymer in this unique dentifrice.

Few areas in the human body contain a higher concentration of, or more diverse, microbiota than the oral cavity. There are more than 300 different species of bacteria in the oral cavity, with approximately 100 billion bacteria per gram of plaque in the gingival sulcus.[1,2] It is not surprising, therefore, that the presence of bacteria in supragingival plaque can be associated with a variety of oral diseases, including gingivitis. Personal removal of supragingival bacterial plaque (ie, self-performed plaque control) on a daily basis is the most widely accepted method of oral disease prevention.[3] Long-term studies clearly demonstrate that regular, self-performed plaque control, in conjunction with professional, preventive measures, is an effective means of maintaining oral health.[4,5]

The most common method of supragingival plaque control is the mechanical removal of plaque through the use of a device such as a toothbrush.[6] A fluoridated dentifrice is often used during the teeth-cleaning process, and dental professionals often recommend flossing or some other means to improve interdental cleaning. However, such measures are used less widely and frequently.[7,8] Most patients do not brush or floss in the manner instructed, or for the amount of time necessary to achieve optimal oral health. In one clinical study, the average brushing time for subjects at home was 37 seconds; and a quarter of the subjects brushed for less than 20 seconds.[9] While many patients demonstrate improved self-performed plaque control immediately after professional care, in many instances it is only sustained for a relatively short period. More evidence to support the theory that current self-performed oral hygiene practices are not sufficient to maintain an effective level of plaque control and oral health has been reported from studies in India, Israel, Scotland, Sweden, and Switzerland.[10] For example, although 94% of the subjects reported daily brushing and flossing, all subjects had visible plaque on more than 90% of their tooth surfaces.[11] These studies and others clearly demonstrate that personal oral hygiene practices using mechanical methods of supragingival plaque control are insufficient to maintain a level of plaque control commensurate with periodontal health.[12]

Although the success of an individual's home-care regimen depends on motivation, dexterity, and compliance, it is unrealistic to assume that most

patients adhere to strict recall schedules and meticulous daily plaque control.[13] Therefore, a chemotherapeutic agent can be used to aid the patient in the mechanical removal of dental plaque. The desirable characteristics of a chemotherapeutic agent that affects dental plaque are that it should be: nontoxic, nonirritating, nonallergenic, safe, effective, pleasant tasting, economical, and easy to use. Ideally, the best delivery mechanism for this effective plaque control agent would be a toothpaste.[14]

Few areas in the human body contain a higher concentration of, or more diverse, microbiota than the oral cavity.

Toothpaste is an important mass-consumer product that provides a very convenient delivery system. It is widely used and enjoys the support of the dental profession. The dramatic reduction in dental caries in most industrialized countries since the introduction and widespread use of fluoride toothpaste is a testament to the impact that incorporation of a chemotherapeutic agent, in this case fluoride, may have on oral health.[15] The addition of an effective antimicrobial agent to a fluoride-containing dentifrice is appealing because it meets many of the criteria of a desirable antiplaque product.

While toothpaste is an ideal vehicle to deliver an antiplaque agent, it is very difficult in a dentifrice formulation to successfully maintain the activity and release of the agent while remaining acceptable to the user.[16] One agent that recently has been successfully incorporated into a toothpaste formulation is triclosan. Triclosan is a phenolic agent comprised of bisphenol and a nonionic germicide. It has low toxicity and a broad spectrum of activity. Triclosan is effective against both gram-positive and gram-negative bacteria. It has been used successfully in numerous consumer products for more than 30 years. If triclosan is to be used as an antiplaque agent, substantivity—or retention—of the antimicrobial agent to the teeth and gingiva is a critical component of efficacy. One toothpaste formulation, Colgate® Total®,[a], has successfully improved the substantivity of triclosan by incorporating a copolymer, polyvinylmethylether/maleic acid (PVM/MA). Studies have shown that this combination of triclosan and PVM/MA copolymer provides long-lasting (up to 12 hours) clinical efficacy.[17-19]

The clinical efficacy of this triclosan/copolymer/fluoride dentifrice on plaque, and its positive effects on oral health, has been demonstrated in approximately 2,000 subjects who participated in 13 independent, double-blind clinical studies.[20] These studies were designed in compliance with most regulatory agencies and professional associations worldwide, including the US Food and Drug Administration and the American Dental Association. From these clinical studies, subjects using the triclosan/copolymer/fluoride dentifrice had on average 27% less plaque than subjects using a fluoride dentifrice. This improved level of plaque control was accompanied by a 57% reduction in gingival bleeding.[20] It is well known that using dentifrices containing fluoride has resulted in significant reduction in caries.[21] It is reasonable to assume that a dentifrice containing triclosan and copolymer in addition to fluoride would provide benefits that are at least similar. Indeed, 4 studies with approximately 10,000 participants demonstrated that the triclosan/copolymer dentifrice containing fluoride provided a significant anticaries benefit that is at least as effective as that provided by other dentifrices containing fluoride alone.[22-25] Several studies also have shown that the PVM/MA copolymer, in the presence of triclosan, inhibited crystal growth of hydroxyapatite,[26] which suggested that the dentifrice might provide an anticalculus benefit. Four clinical studies were conducted on more than 400 subjects with a history of supragingival calculus formation.[27-30] It was demonstrated that the use of a dentifrice containing triclosan and PVM/MA copolymer resulted in a 23% to 57% reduction in supragingival calculus when

There are more than 300 different species of bacteria in the oral cavity, with approximately 100 billion bacteria per gram of plaque in the gingival sulcus.

[a]The Colgate-Palmolive Company, New York, NY 10022; 800-338-8388

compared with a fluoride dentifrice, with an average reduction of 37%.

In addition to a reduction in dental caries and calculus formation, the triclosan/copolymer/fluoride dentifrice has been shown to be effective in maintaining gingival health and also may be beneficial for controlling the onset and rate of progression of attachment loss in high-risk individuals. For example, randomized, controlled studies in adolescents and adults demonstrated that the unsupervised use of the triclosan/copolymer/fluoride dentifrice significantly reduced the onset and progression of periodontitis when compared with a fluoride dentifrice.[31-33]

While it is highly desirable to use a dentifrice to deliver an effective antimicrobial agent, the benefit to oral health must be balanced against potential deleterious shifts in oral ecology. More specifically, because antibiotic resistance may have the potential to become a problem worldwide, the indiscriminate use of antimicrobials must be avoided. Therefore, the benefit-to-risk ratio with regard to use of triclosan must clearly be favorable for society.

Most patients do not brush or floss in the manner instructed, or for the amount of time necessary to achieve optimal oral health.

To ascertain the effects of microbial resistance to triclosan, clinical studies were conducted with a triclosan/copolymer/fluoride dentifrice on more than 1,000 patients over prolonged periods (up to 5 years).[34-38] The overall conclusion from the microbiology studies was that the use of this triclosan/copolymer/fluoride dentifrice does not cause the development of pathogenic, opportunistic, or resistant oral microorganisms. These findings corroborate an earlier study conducted on a triclosan/zinc dentifrice.[39] Furthermore, these microbiology studies were reviewed by an expert panel,[40] which concluded that:

- there is substantial evidence that the use of a dentifrice containing triclosan and a copolymer provides a significant clinical oral health benefit in the general population.
- the ultimate indicator of the ability of an antimicrobial agent to induce bacterial resistance in humans can only be determined from clinical studies.
- data from clinical studies of a triclosan/copolymer dentifrice up to 1 year in duration clearly indicate that there is no evidence to support the acquisition of bacterial resistance in the supragingival oral microflora.
- no further studies of the effect on the oral microflora of a triclosan/copolymer dentifrice are required to support microbiologic safety.

Conclusion

Clinical studies clearly indicate that the use of Colgate® Total® may provide oral health benefits beyond those associated with "traditional" toothpaste use, in a manner that is safe and effective. Dental professionals can confidently recommend this triclosan/copolymer dentifrice to their patients for use as part of their normal oral hygiene regimen.

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Quiz

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CE 1—Dr. Mariotti

1. **Immediately after cellular injury, which cells release preformed biochemical mediators?**
   a. stem
   b. mast
   c. histamine
   d. B helper cells

2. **The complement, clotting, and kinin systems are all tied together through a mixture of interactions. Which of the following from one system can activate one or both of the other plasma protein systems?**
   a. mRNA
   b. protein
   c. ribosome
   d. mitochondria

3. **The process in which a cytokine alters the function of adjacent cells is:**
   a. autocrine.
   b. paracrine.
   c. found only in dorsal root ganglia.
   d. found routinely in osteocytes.

4. **What are biologically active molecules that are released into the bloodstream and stimulate functional activity of cells distant from the site of secretion?**
   a. autosomal recessive neutrophils
   b. autosomal dominant basophils
   c. prokaryotic
   d. hormones

5. **What is the dividing line between acute and chronic inflammation?**
   a. 3 days
   b. 7 days
   c. 3 months
   d. There is no clear dividing line.

6. **The most common systemic effects of inflammation include fever and:**
   a. leukocytosis.
   b. pinocytosis.
   c. phagocytosis.
   d. bacterial endocarditis.

CE 2—Dr. Scannapieco

7. **Recent work has elucidated complex signaling pathways between bacteria referred to as:**
   a. PCR.
   b. quorum sensing.
   c. histopathological balancing.
   d. ion balance.

8. **Periodontitis is associated with extensive formation of biofilm that is dominated by spirochetes and:**
   a. anaerobic gram-negative bacteria.
   b. aerobic gram-positive bacteria.
   c. facultative aerobic bacteria.
   d. aerobic gram-negative bacteria.

9. **The surface area of the periodontal ligament has been calculated to cover about:**
   a. 250 square millimeters.
   b. 250 square centimeters.
   c. 75 square centimeters.
   d. 180 square micrometers.

10. **Low-grade but persistent bacteremia may allow oral bacteria to aggregate platelets through:**
    a. protein synthesis.
    b. receptor-ligand interactions.
    c. biofilm exudates.
    d. histamine activation.

11. **Recently, it has been reported that DNA of oral bacteria could be amplified directly from:**
    a. gingival fluid.
    b. oral biofilm.
    c. parathyroid hormone.
    d. atherosclerotic plaques.

12. **During parturition, the uterus is influenced**

by the hypothalamus through the production of:
a. prostaglandins.
b. oxytocin.
c. TNF-α.
d. acute-phase proteins.

CE 3—Dr. Dave et al

13. About how much of all coronary artery disease can be explained by conventional risk factors?
a. one quarter
b. half
c. three quarters
d. almost all

14. Atherosclerotic plaque, in addition to being an accumulation of lipids, is a:
a. bacterial infection only.
b. viral infection only.
c. inflammatory lesion.
d. completely encapsulated entity.

15. One of the hallmarks of the early atherosclerotic lesion is the recruitment and adhesion of which of the following to the site of endothelial damage?
a. neutrophils
b. monocytes
c. lymphocytes
d. all of the above

16. One of the most consistent markers of systemic inflammation and unfavorable cardiovascular prognosis is:
a. cytomegalovirus.
b. acute-phase protein CRP.
c. low-density lipoprotein uptake.
d. IL-1β.

17. Separating the "inner" and "outer" environments at the base of the gingival sulcus is the:
a. junctional epithelium.
b. periodontal ligament.
c. remnants of Hertwig's root sheath.
d. epithelial rests of Mallassez.

18. What is a part of the outer membranes released after cell death?
a. microtoxin
b. macrotoxin
c. endotoxin
d. exotoxin

CE 4—Dr. Graves et al

19. Which of the following represents a key test in determining whether bacteria alone or the response to bacteria is more crucial in the pathogenesis of periodontal disease?
a. blocking the host response
b. testing of different antibiotic regimens on agar diffusion plates
c. Cochran-Mantel-Haenszel statistics
d. determination of intraluminal vs extraluminal bacterial position

20. The term chronic inflammation has been replaced by:
a. acute inflammation.
b. innate immune response.
c. acquired immune response.
d. cytokine activation phase.

21. The innate immune response depends on:
a. T-cell receptor to a specific molecule.
b. T-cell receptor to a specific bacteria.
c. pattern recognition.
d. an autoimmune cascading agent.

22. Previous studies on which of the following provided the first indication that inflammation caused by bacteria resulted in periodontal tissue destruction?
a. migration of IL-1 antagonists
b. blocking prostaglandins
c. buildup of TNF-α
d. inhibition of gram-negative cocci

23. After bone resorption occurs, growth and remodeling are automatically triggered in a process called:
a. osteogenesis.
b. phagocytosis.
c. diapedesis.
d. coupling.

24. A prolonged high rate of osteoblast cell death is called:
a. phagocytosis.
b. osteogenesis.
c. apoptosis.
d. kinincytosis.

Gingivitis: An Inflammatory Periodontal Disease

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