New knowledge of the pathogenesis of periodontal disease

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The aim of this study was to evaluate the relationship between the accumulation of interleukins IL-1\textbeta, TNF-\alpha, IL-8, and chemokine RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted) in gingival fluid and periodontal support tissues in patients with periodontitis. A review is also provided of apoptotic processes as events of major importance, highlighting the presence of TUNEL cells and ultrastructural morphologic changes associated with cell apoptosis. There appears to be further evidence to support the important role of inflammation control. Cytokines may be considered as markers of the progression and severity of periodontitis as well as indicators of an appropriate response to treatment. However, further studies are needed to support and characterize this concept. (Quintessence Int 2004;35:706-716)

Periodontal diseases are a heterogeneous group of diseases characterized by inflammation and the subsequent destruction of the tooth-supporting tissue. Today it is quite clear that periodontal diseases are of an infectious nature and that the microorganisms present in the subgingival bacterial plaque are the primary etiologic agents.\textsuperscript{1-3} The destruction of the periodontium is associated with the presence of gram-negative anaerobic bacteria localized in the subgingival region, and include typically Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Actinobacillus actinomycetemcomitans (Aa), and Bacteroides forsythus (Bf). These bacteria are considered to play a significant role in the pathogenesis of periodontitis and the formation of the periodontal pocket, destruction of the connective tissue, and resorption of the alveolar bone. While it is the bacterial infection that triggers the destructive process, it is the host's immune response to the bacterial challenge that is responsible for the molecular processes leading to periodontal tissue destruction.\textsuperscript{4}

The bacteria colonizing the subgingival region multiply and extend in an apical direction and, in the process, bring about loss of epithelial and connective tissue attachment. The bacteria may give rise to destruction processes caused by both direct and indirect mechanisms due to the activation of the host's immunologic and inflammatory reactions.\textsuperscript{5-9} Although it is not possible to attribute the etiology of periodontal diseases to a specific bacterial agent, there are a number of studies pointing to a group of bacteria believed to play a special role in the triggering and subsequent development of the disease.\textsuperscript{10} There are over 500 bacterial species capable of colonizing the subgingival region, but the number of these commonly implicated in the disease process is around 10 or 15 gram-negative anaerobes and spirochaetes.\textsuperscript{11,12} The designation of periodontal pathogen applies to those bacteria that possess specific mechanisms to break down the host's defense systems and cause destruction of the periodontal tissues.
Gingivitis does not always progress to periodontitis although the latter is always preceded by gingivitis. Yet, the proportion of gingivitis cases progressing to periodontitis and the factors involved in this process are unknown.

The disease progression model points to a periodic or episodic phenomenon, with periods of quiescence, when neither periodontal destruction occurs nor periods of exacerbation and destruction of the periodontal structures. These are characterized histologically by acute inflammation, with a significant increase in the number of neutrophils (Fig 1).

The interaction between the pathogenic bacteria and a host's defense systems could lead to the development of a periodontal pocket, loss of connective tissue, and bone resorption. Once periodontitis is established, the inflammatory infiltration present is composed of different cell types, such as neutrophils, T and B lymphocytes, and macrophages migrating to the perivascular connective tissue, as demonstrated by immunologic studies. These cells produce various specific subtypes of cytokines that take part in the destruction of periodontal connective tissue attachment. The immune response is also regulated by the selection and death of the immunocompetent cells brought about by a programmed cell death mechanism, referred to as apoptosis, and which could play a significant role in the pathogenesis of periodontitis.

The presence of proinflammatory cytokines and chemokines, such as interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), interleukin-10 (IL-10), interleukin-8 (IL-8), and chemokine RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted) S, in the gingival fluid is more marked in sites with progressive loss of connective tissue attachment. Following treatment, there is a significant reduction in the levels of IL-1β, TNF α, IL-10, IL-8, and RANTES in the gingival crevicular fluid, thus suggesting a relationship between cytokine production and the occurrence of disease.

The cytotoxicity of various bacterial cell components, such as short-chain carboxylic acids present in Porphyromonas gingivalis, Prevotella loescheii, and Fusobacterium nucleatum give rise to apoptosis in T cells and keratinocytes. On the other hand, lipopolysaccharides (LPS) are common components of the cell wall of the gram-negative bacteria that will stimulate butyric acid, to induce apoptosis in the mononuclear cells. In addition, Actinomyces comitans leukotoxin can cause apoptosis in the periodontal tissue B lymphocytes.

There are many bacteria able to induce tissue degeneration, but it is likely that it is the host's mechanisms that are responsible for damaging the connective tissue. In this setting, loss of connective tissue may be the result of the host's mechanism of self-defense to prevent the progression of the lesion, by promoting the proliferation of the junctional epithelium in an apical direction in order to avoid contact with a "toxic" root surface.

It has been shown that there is a significant relationship between the severity of the disease and the total amount of antibodies. Thus, antibody titres could be a more accurate indicator of disease severity than the number of microorganisms present.

In view of the complexity of the host's immunologic response phenomena, the progression from gingivitis to periodontitis and its rate of development cannot be explained merely by the presence of a microbiota. The latter, though necessary, cannot account for the connective tissue loss. Clinical studies have shown that the individual's inherent susceptibility is of great importance in determining the initiation and progression of the disease. Understanding the factors that affect this susceptibility may be crucial in the elucidation of the initiation and progression of periodontitis. These factors include the genetic makeup of the individual determining different cell responses. Environmental risk factors, bacteria, smoking, and stress, as well as diseases (eg, diabetes), may alter the balance between the host and the putative microbiota and lead to different clinical forms of periodontitis.
Accordingly, the model of periodontitis progression has changed from the concept of continuous slow progression to a pattern of discontinuous progression characterized by periods of exacerbation and remission.

**BASIC IMMUNOLOGIC MECHANISMS**

The manifestation of periodontitis depends on the interaction between the host and environmental and microbiologic factors, and it is probable that specific environmental and/or genetic factors determine the individual’s susceptibility to disease. There are, however, specific host defense mechanisms to the bacterial challenge in the adaptive response of the immune system.

Gingival crevicular fluid is a serous transudate that covers the sulcular and junctional epithelium. This fluid increases in cases of inflammation and contains most of the serum components. In periodontitis, components of the serum are enriched with other substances from the inflammatory cell infiltrate. Bacterial lipopolysaccharides (LPS) have a proinflammatory effect on most of the periodontal tissue cell types, such as macrophages, lymphocytes, fibroblasts, and osteoblasts/osteoclasts.

The lipopolysaccharide-binding protein (LPS) and the surrounding protein form a complex, which assists in their binding to the specific CD14 receptor expressed on monocytes. The activation of the CD14 receptor produces the secretion of proinflammatory molecules such as IL-1, TNFα, PGE2, and IL-6, which, in turn, will lead to the release of mediators such as platelet-activating factor (PAF), bioactive amines (bradykinin and histamine), and prostaglandins.

The complement system is important in periodontal disease, as is the role it plays in protecting against periodontal pathogenic bacteria. Its activation is one of the host’s earliest immune responses in the gingival crevicular space. Activation of C5 varies according to the severity of the periodontal inflammation and may contribute to distinguishing between different forms of periodontitis. The bacteria related to periodontal disease exhibit different mechanisms to evade the action of the complement system.

Some bacterial strains express polysaccharides on their surface that mask the molecules activating the complement system. In other words, the carbohydrate structures on the surface can determine the nature of the interaction between bacteria and the components of the complement system. Certain periodontal pathogenic bacteria exhibit cell surface proteolytic activity that is capable of degrading specific components of the complement system, such as C3 and C5, thereby preventing opsonization. Bacteria such as Pg and Aa present complex carbohydrate structures with intense antigenic and immunogenic potency capable of inducing a significant IgG2 response.

Neutrophils, the main cell type present in the gingival sulcus, which make up the first line of defense against periodontal pathogenic bacteria, constitute another level of the immune response. Monocytes are phagocytic cells that represent the second line of defense and become activated when infection is present but only if neutrophils are ineffective. The accretion of bacterial plaque in healthy individuals produces a significant increase in the number of polymorphonuclear cells (PMN), leading to the triggering of a systemic inflammatory response. Active neutrophils in the gingival sulcus clear microorganisms through phagocytosis. However, some periodontal pathogenic bacteria such as Pg and Aa, succeed in escaping the neutrophils, giving rise to a continuous flow of these phagocytes toward the gingival crevicular space, causing continuous cell accumulation and decay as a result of ineffective phagocytosis. Neutrophil degranulation entails the release of endogenous proteases that, along with bacterial proteases, give rise to the destruction of the extracellular matrix. Thus, the normal activity of neutrophils represents an essential component of the host’s contribution to periodontal destruction (Fig 2).

Systemic exposure to oral cavity pathogens seems to occur in most patients, resulting in a specific antibody response. As a rule, the highest antibody titres are associated with the most severe forms of the disease, as has been demonstrated for Pg in chronic periodontitis.

Elevated levels of antibodies against Aa and Pg have been described in localized aggressive and in generalized aggressive periodontitis. It has been suggested that the antibody against their particular antigen is regulated by certain genes. Thus, it seems reasonable to assume that the antibody response might be more protective for one individual than for another. Therefore, some groups of patients might be more prone to periodontitis because of their inability to elicit a response of high-affinity antibodies against the dominant bacterial antigens.

Antibody response to the specific characteristics of bacterial virulence is apparently critical for the resolution or halting the progression of the disease. There are many studies showing a correlation between PMN phagocytic activity and antibody affinity and titres.

**CHEMOKINES, CYTOKINES, AND PERIODONTITIS**

Inflammatory processes give rise to macrophage activation as well as leukocyte infiltration. The activated immunocompetent cells produce and secrete cytokines. These activated host cells include monocytes,
Macrophages, lymphocytes, and fibroblasts.\textsuperscript{33,58} Cytokines are signaling proteins produced by cell activation, acting in the local milieu as autocrine, paracrine, and sometimes, endocrine substances in order to regulate cell function,\textsuperscript{68} and thereby inducing and maintaining the periodontal inflammatory response.\textsuperscript{37,69} Several cytokines, including IL-1β, TNF-α, and IL-6, take part in this tissue destruction process as a response to the bacterial plaque.

IL-1 is a proinflammatory cytokine that contributes to bone resorption by stimulating release of PGE2 and promoting metalloproteinase release that denatures extracellular matrix proteins.\textsuperscript{70,71} The predominant form of IL-1 in periodontal tissues is IL-1β produced mainly by the macrophages.\textsuperscript{72,73} IL-1 is increased in inflamed gingival tissue in patients with periodontitis.\textsuperscript{74,75} IL-1 is also detected in the gingival crevicular fluid in areas affected by periodontitis.\textsuperscript{72,76} Periodontal destruction and disease progression are associated with elevated cytokine levels.\textsuperscript{77}

TNF-α is another cytokine produced mainly by macrophages in response to such antigens as LPS. It exhibits a wide variety of biologic effects related to the destruction of periodontal tissue.\textsuperscript{33,78}

The biologic effects of IL-1 are associated with recruiting inflammatory cells, facilitating neutrophil degranulation, increasing PGE2 and MMP production, inhibiting collagen synthesis, and activating T and B cells.\textsuperscript{79} In addition, TNF-α is involved in apoptotic programmed cell death, bone resorption, MMP secretion, and intercellular adhesion molecule (ICAM) expression, while promoting IL-6 production.\textsuperscript{53} IL-6 has been identified in patients with periodontitis\textsuperscript{80} and a number of actions, proliferation of B cells and T cells as well as bone resorption, are attributed to this cytokine in periodontitis.\textsuperscript{81}

IL-10 modulates the expression of cytokines of myeloid origin resulting in major functional outcomes in the triggering and maintenance of the immune response. These could consist of an anti-inflammatory and immunosuppressive action in experimental models\textsuperscript{82,83} similar to certain regulatory effects on the immune response of periodontitis.\textsuperscript{84,85} IL-10 might be produced by type 2 T helper (Th2) lymphocytes and be responsible for the inhibition of the cytokine production of the type 1 T helper (Th1) cells.\textsuperscript{86} This action of interfering with cytokine synthesis is due to its inhibitory effects on monocytes and macrophages.\textsuperscript{87}

The presence of IL-1β (95%) and IL-8 (100%) is detected in patients with periodontitis, in comparison with healthy subjects.\textsuperscript{88} The total amount of IL-1β and IL-8 decreases noticeably after completion of periodontal treatment and after the inflammatory process is eradicated.\textsuperscript{89}

Chemokines are cytokines with low molecular weights that attract and activate different subpopulations of lymphocytes, acting as mediators in various pathologic situations, including inflammation.\textsuperscript{89–91} Chemokines represent a large family of proteins grouped in four subfamilies. One of the chemokine subtypes is "CXC" and another "CC," being differentiated by the initial two cysteine amino acids, which may be either adjoining (CC) or separated by another amino acid (CXC). On the other hand, the C family has a single N-terminal cysteine residue and in the CX3 C family, these cysteines are separated by three amino acids.\textsuperscript{92,93}
Cytokine receptors are expressed selectively in lymphocytes and have such activities as the destruction of the gingival connective tissue, loss of periodontal ligament insertion, and bone resorption. Acretion of bacterial plaque is followed by leukocyte infiltration of the gingival connective tissue, in a perivascular localization below the dentogingival junction in its initial stages. Leukocyte extravasation takes place due to an activation of the endothelial cells. Recruitment of leukocytes into the gingival connective tissue follows thereafter. There is a constant flow of neutrophils from the blood vessels of the gingival plexus toward the junctional epithelium and finally to reach the gingival crevical or periodontal pocket region. Accordingly, amongst the host's defense mechanisms, two immunologic axes are described. One is localized in the epithelial tissue and formed of complement-antibody-neutrophils, and the other is sited in the connective tissue and composed of macrophages-lymphocyte-chemokines. It is therefore of special interest to identify the different cytokines that take part selectively in the attraction of the cells forming the infiltrate present in the host's response to bacterial accumulation.

RANTES is a member of the family of CC chemokines that activates the monocytes, eosinophils, and basophils, induces chemotaxis, and releases other mediators. High levels of RANTES are found in the gingival crevicular fluid in patients with periodontitis in comparison with healthy subjects. It could be suggested that in periodontal disease, migration and accumulation of monocytes and macrophages at sites of inflammation could be associated with the release of chemokines such as RANTES.

**INFLAMMATORY INFILTRATION**

The inflammatory infiltration of periodontitis is composed of mononuclear cells, mainly mononuclear phagocytes and lymphocytes. T lymphocytes predominate in the nonprogressive stable periodontal lesion in which CD4+ cells predominate over the CD8+ type. The proportion of B lymphocytes and plasma cells increases as periodontitis progresses. It has been shown that macrophages, lymphocytes, and local cells, such as fibroblasts and vascular endothelial cells, synthesize and secrete various cytokines in response to the stimulus of bacterial products. There are marked differences in the degree of inflammation and cytokine secretion in patients with periodontitis when compared with healthy individuals. Thus, an increase in T and B cells and macrophages has been observed in the biopsy leukocyte infiltration seen in patients with periodontitis in comparison with healthy individuals (Fig 3).

RANTES is a chemoattractant for the Th 1 (helper) but not the Th 2 cells (suppressor). For these reasons, RANTES may play a major role in the regulation of the local immune reactions by controlling the balance between the proinflammatory cells and anti-inflammatory T cells. The presence of T cells and macrophages in connective tissue biopsies in patients with periodontitis would imply a possible involvement of RANTES in the tissue destruction mechanisms associated with periodontitis.

**APOTOPSIS AND PERIODONTITIS**

Programmed cell death is a normal physiologic process that contributes to maintaining tissue homeostasis. Cells die due to apoptosis during morphogenesis or synaptogenesis, during tissue replacement or as part of the immune response.

The term apoptosis describes a cell-death process in which the cell actively participates. It is a cell death beneficial for the survival of the organism. When the cell undergoes apoptosis, it exhibits a series of morphologic changes in various structures. There is loss of asymmetry and adhesion in the cell membrane, cytoplasm, and nucleus condensation and breaking up of the DNA into internucleosomal fragments. The final state is cell fragmentation into the so-called “apoptotic bodies,” which are eliminated by the phagocytic cells without producing inflammation, thus avoiding damage to neighboring cells and surrounding tissue. Conversely, during necrosis, a cellular inflammatory reaction is triggered as a result of the release of the cytoplasmatic contents of the cells, including large amounts of proteolytic enzymes.

Apoptosis is a highly controlled process that affects various components at the onset, amplification, and completion of the destructive process. During apoptosis, caspases (cytotoxic proteases) become activated and chromatin condensation as well as the activation of specific endonucleases breaking up the DNA into internucleosomal fragments occur. In addition, the apoptotic stimulus may activate different mechanisms, which include death cell receptor ligands, such as the Fas/Fas ligand complexes, and these stimuli induce molecules directly or indirectly involved in apoptosis, such as p53. Members of the family of Bcl-2 proteins have also been shown to play a regulating role in the apoptotic process (Fig 5). This type of protein, belonging to the antiapoptotic proteins group, can prevent or limit cell death induced by different stimuli. Therefore, detection of apoptosis in inflamed gingival tissues in patients with periodontitis with pockets more than 5 mm deep and attachment tissue loss of over 5 mm could partly explain the...
mechanisms associated with the destruction of the tooth support tissues.

The most appropriate method for identifying apoptosis is dependent on the type of cells and tissue to be studied. Thus, light and electron microscopy provide the tools for detecting the changes occurring during apoptosis in the cell membrane, cytoplasm, and nucleus.

Apoptosis is accompanied by the activation of a series of cysteine-type proteases, named caspases, because of their catabolic properties. Detecting some members of this protease family may be indicative of the occurrence of apoptosis. Caspases are synthesized as inactive proenzymes, which are then processed in the cells undergoing apoptosis. Caspase 3 has been involved as a key protease activated during the development of apoptosis. Active caspase 5 is present in those cells that are undergoing apoptosis; the caspase is split by proteolysis and activates other caspases.

The induction of apoptosis in the host's cells provoked by certain pathogens, or by their products, is a phenomenon involved in the pathogenesis of infectious diseases. On the other hand, bacterial phagocytosis or exposure to different bacterial components such as LPS, may delay apoptosis of the PMNs.

DNA fragmentation is a process that takes place due to the activation of the endonucleases during the apoptotic process. These nucleases break chromatin into small fragments. The plasma membrane is the site where redistribution of the phosphatidylinerine phospholipid residues takes place, which move from the inner cell layer to the outside of the membrane. In the plasma membrane, there are receptors involved in the regulation of apoptosis, such as Fas (CD95). Fas ligand (Fas L) can induce apoptosis in cells expressing the Fas receptor; this mechanism is important in T lymphocytes and NK cells apoptosis.

Apoptosis may therefore be an important phenomenon in the regulation of the inflammatory response against chronic bacterial accumulation, affecting both the increase in cellularity and the extent of the inflammatory infiltration. The number of active caspase 3 positive cells in the biopsies of subjects with periodontitis is comparable with that obtained using the TUNEL technique (Fig 4). The presence of morphologic changes in the cell associated with apoptosis indicate that apoptosis mechanisms may be involved in the pathogenesis of periodontitis. However, the small number of positive cells for these apoptosis markers suffer a significant loss of affinity to tissue, suggesting that the tissue destruction taking place in this condition, may occur after a long time of evolution.

Neutrophils are the cells initially responding to the pathogenic action of the bacteria, which comprise the plaque; they play an especially important role in the pathogenesis of periodontal disease. These cells predominate in the junctional epithelium and the gin-
gival sulcus, representing a leukocyte wall between the bacterial plaque and the periodontal connective tissue. This interaction is crucial for understanding tissue destruction in periodontitis.

Circulating neutrophils have a short life span, and the loss of their functions, such as adhesion and phagocytosis, is closely related to the occurrence of apoptosis in these cells. Inhibition or delay of neutrophil apoptosis in the inflammatory tissues is regulated by different cytokines or by factors secreted during cell immune response such as LPS.

One must consider the presence of apoptotic cells in periodontal tissue again, as their presence poses the question of whether the induction mechanisms involve the Fas L apoptosis receptor, a member of the family of TNF-α cytokines that, on binding to the Fas receptor, produce apoptosis. It may be considered, therefore, that the Fas/Fas L complex is involved in gingival tissue apoptosis. Fas and Fas L positive cells are observed in periodontal tissue biopsies while they are not detected in biopsies from healthy subjects, thus, this finding confirms the involvement of apoptotic mechanisms in periodontitis. Since certain apoptosis activators require the presence of p53 protein, its involvement in the apoptosis present in periodontitis can be assumed. This p53 protein is a tumor-suppressant, and when activated, it induces genes related to the cell regulation cycle, DNA repair mechanism, and apoptosis induction. p53 protein is present in normal tissues and cells; due to its short half-life, it may not be detectable in healthy tissues.

The scientific studies described above support the concept of apoptosis playing a role in the pathogenesis of periodontitis. Nevertheless, further studies are required to improve our knowledge of the control of inflammation in the pathogenesis of periodontal disease.
CONCLUSION

The local immune response in periodontal tissue against bacterial antigens is characterized by an inflammatory infiltration exhibiting the clustering of leukocytes and the subsequent release and accumulation of IL-1β, TNF-α, and IL-8.

Chemokines such as RANTES have been detected in the gingival fluid of patients with periodontitis, in comparison with healthy subjects. The pattern of the released cytokines matches that of a proinflammatory immune response, with higher levels of these mediators being measured in pockets more than 5 mm deep and at sites of attachment tissue loss larger than 2 to 3 mm. On the other hand, periodontal treatment brings about a considerable reduction in the levels of IL-1β, IL-8, and RANTES.

Another important aspect in ascertaining the cellular and molecular bases of periodontal infection is the study of the inflammatory infiltration, composed of T and B lymphocytes and macrophages, at sites with periodontitis. T cells are found at places with stable disease, whereas B cells are shown at sites exhibiting progression of tissular destruction, in comparison with areas with no attachment tissue loss.

Both the cytokine levels and the characteristics of the inflammatory infiltration change 2 months after periodontal treatment, the situation returning to that observed in healthy subjects. This finding shows that the infiltrate and the cytokine levels bear a close relation to the local inflammatory features. A fundamental aspect of this series of phenomena is the presence of apoptosis, which is eventually a crucial event in periodontitis. The presence of positive TUNEL cells and the ultrastructural morphologic changes are associated with cell apoptosis. Bcl-2, p53, Fas, Fas L, and active caspase 3 have been detected in the apoptotic infiltrate.

In summary, cytokines may be considered as markers of the progression and severity of periodontitis, as well as indicators of an appropriate response to treatment, although further studies are needed to support this concept.

REFERENCES


