

Cytokines That Promote Periodontal Tissue Destruction

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Although periodontal diseases are initiated by bacteria that colonize the tooth surface and gingival sulcus, the host response is believed to play an essential role in the breakdown of connective tissue and bone, key features of the disease process. An intermediate mechanism that lies between bacterial stimulation and tissue destruction is the production of cytokines, which stimulates inflammatory events that activate effector mechanisms. These cytokines can be organized as chemokines, innate immune cytokines, and acquired immune cytokines. Although they were historically identified as leukocyte products, many are also produced by a number of cell types, including keratinocytes, resident mesenchymal cells (such as fibroblasts and osteoblasts) or their precursors, dendritic cells, and endothelial cells. Chemokines are chemotactic cytokines that play an important role in leukocyte recruitment and may directly or indirectly modulate osteoclast formation. This article focuses on aspects of osteoimmunology that affect periodontal diseases by examining the role of cytokines, chemokines, and immune cell mediators. It summarizes some of the key findings that attempt to delineate the mechanisms by which immune factors can lead to the loss of connective tissue attachment and alveolar bone. In addition, a discussion is presented on the importance of clarifying the process of uncoupling, a process whereby insufficient bone formation occurs following resorption, which is likely to contribute to net bone loss in periodontal disease. *J Periodontol* 2008;79:1585-1591.

KEY WORDS

Chemokines; cytokines; lymphocytes; osteoclasts; periodontal diseases; tumor necrosis factor.

The pathogenesis of periodontal disease (PD) is an inflammatory process involving innate and adaptive immune responses. PD is characterized by the host-mediated destruction of soft tissue caused by the induced production and activation of lytic enzymes and stimulated osteoclastogenesis.¹ Although it is widely recognized that PD is chronic, the nature of the chronicity has not been established. It is uncertain whether PD is a continuous process or consists of episodes of exacerbation and remission.^{2,3} Furthermore, the duration of the periods of exacerbation, if they exist, is not known.

There is an established relationship between periodontal bone resorption and bacteria. The inflammatory process occurring in PD is characterized by the infiltration of leukocytes, which limit the level of bacterial invasion. There are a number of factors that promote leukocyte recruitment, including bacterial products, cytokines, cross-talk between innate and adaptive immune responses, chemokines, lipid mediators, and complement. Page and Schroeder⁴ showed that bone resorption ceases when a 2.5-mm zone is created between the site of bacteria and bone. They concluded that distances >2.5 mm are caused by bacterial invasion of gingival connective tissue: the closer the cells of the inflammatory infiltrate are to the bone, the greater the number of osteoclasts formed and, hence, the greater amount of bone degraded.^{5,6} This osteoclast formation is stimulated by secreted factors

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(i.e., cytokines) from inflammatory cells in the infiltrate, which stimulate bone resorption.^{1,7} In PD there is uncoupling of bone resorption with subsequent bone formation so that a net loss of bone occurs.^{8,9} This contrasts with normal conditions in which the amount of bone formation is equal to the amount of resorption that occurs and is referred to as coupling.¹⁰

Animal models have proven invaluable to study how bacteria lead to periodontal tissue destruction and uncoupling. By use of gain or loss of function, cause-and-effect relationships between cytokines and periodontal tissue loss have been established. Research over the last 2 decades elucidated the relationships among inflammation, immune cell mediators, bacterial-induced cytokines, and periodontal tissue. This article focuses on the role that immune cell mediators and their secreted products play in regulating the inflammatory process during PD and summarizes some of the key findings that attempt to delineate the mechanisms by which cytokines lead to the loss of connective tissue attachment and alveolar bone loss.

UNDERSTANDING THE ROLE OF THE HOST RESPONSE

The role of the host response in periodontal bone loss is complex. There is evidence that a deficient host response increases periodontal destruction and, at the same time, evidence that a too vigorous response leads to PDs. The first conclusive evidence that the host response played an important role was shown when treatment with a prostaglandin inhibitor reduced the amount of bone loss.¹¹ Evidence that cytokines played a critical role was shown in a non-human primate model. In this report, inhibition of interleukin (IL)-1 and tumor necrosis factor (TNF) reduced the progression of periodontal bone loss and loss of attachment,^{12,13} which was attributed to the recruitment of inflammatory cells (notably monocytes and lymphocytes) toward the bone.¹⁴ In later years, various knock-out mouse models supported the hypothesis that cytokines are integral in the disease process. Mice deficient for interferon-gamma (IFN- γ), IL-6, or TNF receptor signaling exhibited reduced alveolar bone loss in oral gavage models.^{15,16} Mitogen-activated protein (MAP) kinase inhibitors for p38 reduced lipopolysaccharide (LPS)-induced PD in a rat model, suggesting that reduced cytokine signalling during the host immune response protects against bone loss.¹⁷

Although these studies suggested that inhibition of the host response decreases periodontal bone loss, there is evidence to suggest that inhibition of the host response also has the opposite effect, increasing the progression of PD. For example, it is widely acknowledged that neutrophil depletion contributes to periodontal tissue destruction.¹⁸ Also, mice deficient for P/E-selectin have similarities to human leukocyte ad-

hesion deficiency (LAD) and have increased bacterial colonization of the oral cavity, increased IL-1 α , and greater alveolar bone loss.¹⁹ A number of human immune deficiencies are associated with enhanced bone loss, such as LAD, Chédiak-Higashi syndrome, Papillon-Lefèvre, and acquired immune deficiency syndrome. Together, these studies demonstrate that modulation of the host response is critical in clarifying the processes leading to periodontal bone loss and that the host response plays a protective and destructive role in the periodontium.

A critical aspect of the host response is the detection of bacteria by Toll-like receptors (TLRs). Activation of the innate immune response by the binding of various bacterial components (i.e., diacyl lipopeptides, peptidoglycan, LPS, flagellin, and bacterial DNA) to TLRs results in the production of cytokines and chemokines.²⁰ Upon activation of TLRs, an intracellular signaling cascade is stimulated that leads to the activation of transcription factors (e.g., nuclear factor-kappa B, activator protein 1 (AP-1), and p38) and the production of various cytokines, many of which directly or indirectly stimulate osteoclast formation.

OSTEOIMMUNOLOGY IN PERIODONTAL BONE LOSS

Osteoimmunology is an emerging area of study focusing on the interactions between the cellular and molecular components of the skeletal and immune systems. This section serves as an overview to the cell mediators and cells governing immune-related activities that affect bone loss associated with PD.

CHEMOKINES AND CYTOKINES IN PERIODONTAL BONE LOSS

Chemokines

Chemokines are a large family of chemotactic cytokines that stimulate the recruitment of inflammatory cells. They are produced by a number of cell types in the periodontium, such as fibroblasts, endothelial cells, macrophages, osteoclasts, epithelial cells, polymorphonuclear leukocytes, monocytes, lymphocytes, and mast cells. Chemokines are divided into two major families based on the structure of the ligand; they are referred to as CC and CXC chemokines, whereas their receptors are referred to as CC chemokine receptor (CCR) and CXC chemokine receptor (CXCR). Some chemokines contribute to inflammation-induced bone resorption because they can stimulate one or more steps of bone resorption, including the recruitment, differentiation, or fusion of precursor cells to form osteoclasts or enhance osteoclast survival.²¹ Chemokines could also affect periodontal bone loss by their role in recruiting cells, such as neutrophils, which protect against bacterial invasion. A recent study²² examined the role of the chemokine

receptor CXCR2, which binds to several chemokines that are neutrophil chemoattractants. When CXCR2-deficient mice were given an oral gavage of *Porphyromonas gingivalis*, they showed an increase in periodontal bone loss compared to wild-type mice, suggesting that chemokines are important in protecting the host from bacteria-induced bone loss.

IL-1

From human and animal models, there is strong evidence of a role for IL-1 in mediating bone loss stimulated by periodontal pathogens. In humans, IL-1 β expression was elevated in gingival crevicular fluid at sites of recent bone and attachment loss in patients with PD.²³ Using a non-human primate model, Delima et al.²⁴ showed that inhibition of IL-1 using human soluble IL-1 receptor type I (IL-1RI) significantly reduced inflammation, connective tissue attachment loss, and bone resorption induced by periodontal pathogens compared to controls (Fig. 1). In another study,²⁵ IL-1RI-deficient mice had less *P. gingivalis* LPS-induced osteoclastogenesis compared to similarly treated wild-type mice. The exogenous application of recombinant human IL-1 β in a rat ligature model accelerated alveolar bone destruction and inflammation over a 2-week period.²⁶ In addition, transgenic mice overexpressing IL-1 α in gingival epithelium developed a syndrome that paralleled all of the classic features of PD, including loss of attachment and destruction of alveolar bone.²⁷ Taken together, these studies strongly support the role of IL-1 in promoting destruction of the periodontium.

IL-6 and -11

IL-6 and -11 are related cytokines but seem to have different functions in periodontal bone loss. In response to *P. gingivalis* oral gavage, mice with genetically deleted IL-6 had decreased bone loss compared to wild-type mice, suggesting that the production of IL-6, which is proinflammatory, contributed to bone resorption.¹⁵ In another study,²⁸ the systemic injection of IL-11 in a ligature-induced beagle dog model caused a significant decrease in periodontal bone attachment loss compared to controls. This likely was due to an anti-inflammatory effect of IL-11.

TNF- α

A number of studies make the case for TNF- α having a significant role in the bone loss that is characteristic of

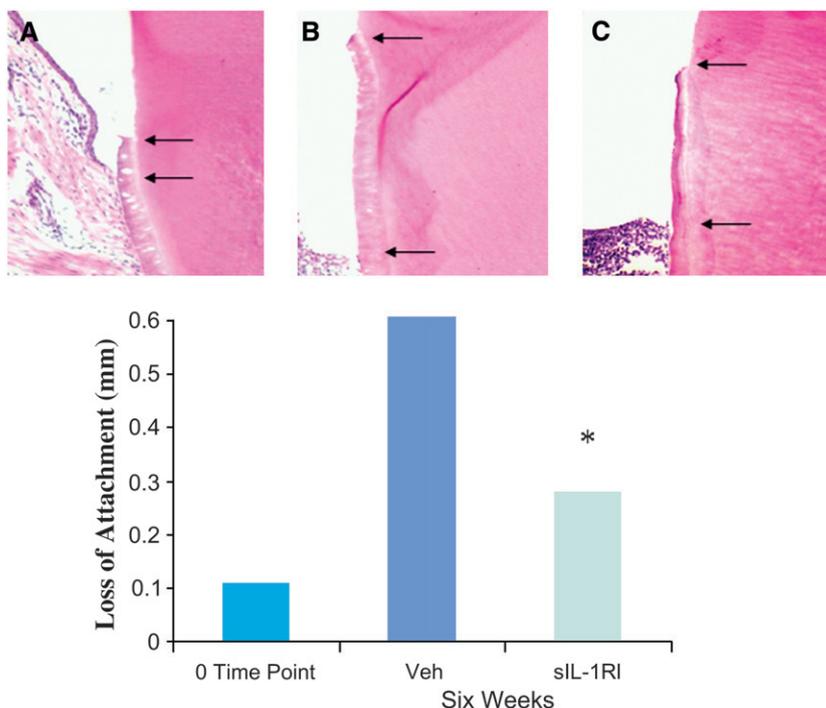


Figure 1.

Blocking IL-1 reduced the loss of connective tissue attachment in a primate model. Upper panel: Periodontal tissue was compared to that of primates in the control group (A) after PD was initiated in *Macaca fascicularis* primates (B and C). Animals that had periodontitis received treatment with vehicle (phosphate-buffered saline) (B) or sIL-1RI (C) for 6 weeks. The upper arrows indicate the original coronal extent of the connective tissue attachment, denoted by the presence of cementum, and the lower arrows indicate the coronal extent of the connective tissue attachment in hematoxylin and eosin stained sections (original magnification $\times 10$) at the time that the animal was sacrificed. Lower panel: The length of connective tissue attachment was assessed in histologic sections by image analysis. *sIL-1RI treated animals had less attachment loss than vehicle-treated control animals ($P < 0.05$). Reprinted with permission from The University of Chicago Press.²⁴ Veh = vehicle; sIL-1RI = soluble IL-1 receptor type I.

PD. *P. gingivalis*-induced osteoclastogenesis was reduced in TNF receptor-deficient mice compared to wild-type controls, indicating that osteoclast formation resulted from stimulation of the host response rather than from the direct effect of bacterial products.²⁹ Gaspersic et al.³⁰ examined the influence of recombinant human TNF- α (rhTNF- α) on the inflammatory response and periodontal breakdown in a rat ligature model. The administration of rhTNF- α accelerated the progression of periodontitis in rats. In a recent study by Garlet et al.,¹⁶ TNF- α receptor-1-knockout (TNFR-1-KO) mice developed significantly less inflammation and alveolar bone loss in response to *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) oral gavage. The apparent level of *A. actinomycetemcomitans* quantified by real-time polymerase chain reaction was significantly greater in the TNF receptor ablated mice compared to wild-type controls starting at day 7 (Fig. 2). To investigate the mechanisms

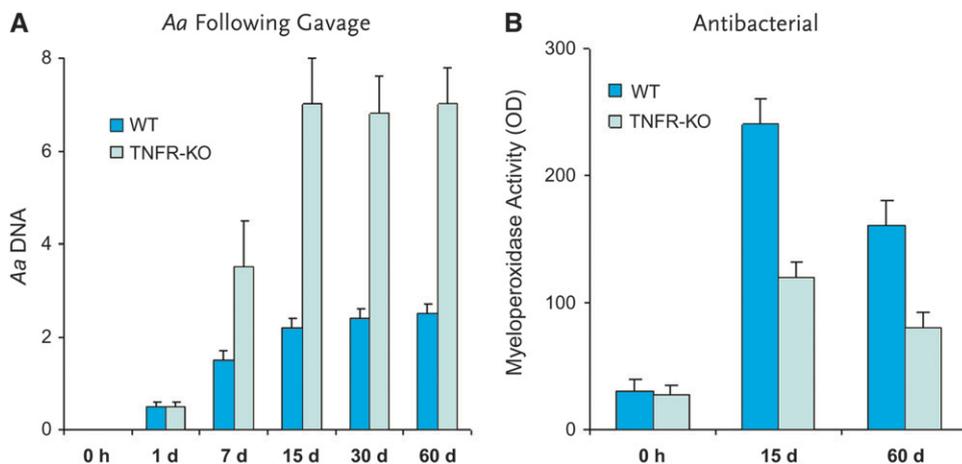


Figure 2.

Mouse oral gavage model A. *actinomycetemcomitans*. C57BL/6 and TNF- α receptor p55-deficient mice were infected orally with *A. actinomycetemcomitans* and were evaluated for *A. actinomycetemcomitans* (Aa) load in periodontal tissues, quantified by real-time polymerase chain reaction and normalized by tissue weight (A), and levels of myeloperoxidase in periodontal tissues, presented as optical density units (OD) (B). d = days; h = hours; WT = wild-type. Reprinted with permission from Blackwell Publishing.¹⁶

involved in the enhanced susceptibility of TNFR-1-deficient mice to *A. actinomycetemcomitans* infection, levels of the neutrophilic antimicrobial myeloperoxidase were measured and found to be lower in the periodontal tissues of the experimental mice. Furthermore, the quantitative analysis of mRNA expression from inflammatory cytokines IL-1 β , IFN- γ , and receptor activator of nuclear factor-kappa B ligand (RANKL) in gingival tissues revealed that it was significantly lower in infected TNFR-1-KO mice compared to wild-type infected mice. Thus, the absence of TNFR-1 resulted in a lower production of cytokines in response to *A. actinomycetemcomitans* infection. Taken together, the decrease in TNF- α seemed to reduce the host response, thereby leading to higher levels of bacteria; however, because the host response was less, there was a reduced expression of the cytokines that stimulate bone resorption, which resulted in less net bone loss.

IMMUNE CELLS

Lymphocytes

Lymphocytes are important immune cells that can produce IL-1, -6, and -17; RANKL; and TNF- α cytokines.³¹ Although osteoclastogenesis can be induced by TNF- α in a RANKL-dependent and -independent manner, IL-1 and -6 play roles in bone resorption via the induction of RANKL.³¹ In the inflammatory milieu, lymphocytes also secrete a number of inhibitory molecules that directly inhibit osteoclast formation, including osteoprotegerin (OPG); IL-4, -10, and -13; and IFN- γ .³¹

RANKL, which binds to RANK, is one of the most potent inducers of osteoclast formation and activity.⁹ Stimulation of RANK can be decreased by OPG, which binds to RANKL and inhibits the interaction between RANKL and RANK. The ratio of RANKL and OPG expression is important in inflammation-induced bone resorption, including periodontitis.⁹ The role of RANKL/OPG in periodontitis was demonstrated in rodent models in which treatment with OPG reduced periodontal bone loss following adoptive transfer of lymphocytes and infection with *A. actinomycetemcomitans* oral gavage³² and in a rat ligature model.³³

Although the above studies indicated that lymphocytes promote periodontal bone loss, other studies reported that lymphocytes are key in preventing the loss of bone in PD. For example, antibody depletion of B lymphocytes in normal rats increased alveolar bone loss in an *Actinomyces viscosus* and *Bacteroides gingivalis* gavage model, suggesting that B lymphocytes played a protective role.³⁴ In another study,³⁵ the adoptive transfer of *A. actinomycetemcomitans*-specific T cell clones protected against *A. actinomycetemcomitans*-induced periodontal bone loss. Yu et al.²² examined the role of IL-17 in PD bone loss. IL-17 is a key cytokine produced by a newly identified subset of T helper (Th) cells, called "Th17" lymphocytes, and it is important in stimulating chemokine production to recruit neutrophils. When IL-17 signaling was ablated in mice, there was greater periodontal bone loss, suggesting that this lymphocyte product is needed to recruit cells of the innate immune response to protect against *P. gingivalis* oral gavage. The role of lymphocytes in PD bone loss also was examined in a non-human primate model. *Macaca fascicularis* immunized against *P. gingivalis* experienced significantly less bone loss for up to 36 weeks compared to non-immunized controls, demonstrating that lymphocytes played an important role in protecting the host.³⁶

In contrast to the above, there is evidence to suggest that lymphocytes are involved in enhancing PD bone loss. When severe combined immunodeficient (SCID) mice, those mice that lack B and T lymphocytes, were challenged with *P. gingivalis*, they exhibited considerably less bone loss than immunocompetent

mice. This result suggested that B and T lymphocytes are not critical for protecting the host against *P. gingivalis* gavage but do contribute to bone loss when present.¹⁵ In another study,³² greater periodontal bone loss occurred in non-obese diabetic (NOD)/SCID mice engrafted with human peripheral blood lymphocytes (CD4⁺ T cells) from patients with localized juvenile periodontitis and challenged with *A. actinomycetemcomitans* compared to controls groups not reconstituted with lymphocytes. Finally, the adoptive transfer of *A. actinomycetemcomitans*-responsive B lymphocytes to athymic rats challenged with *A. actinomycetemcomitans* showed greater periodontal bone loss compared to rats immunized with non-antigen-specific cells.³⁷ Based on these studies, it is clear that lymphocytes play destructive and protective roles and that the specific conditions and specific lymphocyte subtypes need to be considered when considering the role of lymphocytes in periodontal tissue loss.

Th cells

CD4⁺ T cells are important in determining the effect of the T-cell immune responses against pathogens. Effector CD4⁺ T cells are classified into Th1 and Th2 subsets.³⁸ Cytokines produced by Th1 lymphocytes, referred to as Th1 cytokines, include IFN- γ and TNF- α and - β . They are critical for the eradication of intracellular pathogens and are generally proresorptive through direct or indirect effects, whereas Th2 cytokines are not. The adoptive transfer of antigen-specific Th1 cells resulted in the enhancement of periodontal bone loss in rats and mice stimulated by bacteria.^{32,39} In contrast, recipient rats that received the adoptive transfer of Th2 cells exhibited less bone loss.⁴⁰ Overall, these data suggested that the adaptive immune response and, in particular, CD4⁺ T cells and the proinflammatory cytokines that they secrete, are important effectors of bone loss as a result of bacterial infection. Although many cross-sectional studies have explored the production of Th1 and Th2 cytokines in human PD, few longitudinal studies linked the expression of these cytokines with the loss of periodontal bone or attachment, i.e., there is an absence of data that temporally link the expression of cytokines to clinical periodontal destruction in humans. Thus, it is difficult to draw firm conclusions in human studies

as to the association of Th1 or Th2 cytokines with periodontal destruction.

CYTOKINES IN BONE COUPLING

Bone resorption occurs in the context of a bone metabolic unit. The process starts with osteoclasts resorbing bone, followed by new bone formation by osteoblasts in the resorption lacunae. Under physiological conditions, the two activities are coupled, i.e., the amount of bone formed by osteoblasts is equal to that resorbed by osteoclasts. However, in pathologic processes, the two processes are uncoupled.¹⁰ For example, in PD, the amount of bone resorbed exceeds that formed, resulting in net bone loss.⁹

The author's laboratory is focused on understanding the process of uncoupling in PD, particularly in diabetes. To investigate this issue, we used rat ligature models in diabetic mice to establish how diabetes affects bone resorption and subsequent alveolar bone formation. Bone resorption was induced following the placement of ligatures around rat molars for 7 days.⁴¹ Following removal of the ligatures, bone resorption ceased, and new bone formed. At 4 and 9 days following removal of the ligatures, new bone formation occurred in normal mice but was significantly less in diabetic mice (Fig. 3A). Bone coupling (calculated by dividing new bone formation by a measurement of bone resorption) was significantly reduced in diabetic mice compared to controls, indicating a coupling defect in diabetic mice. As a potential mechanism, TNF- α was increased (data not shown) in the

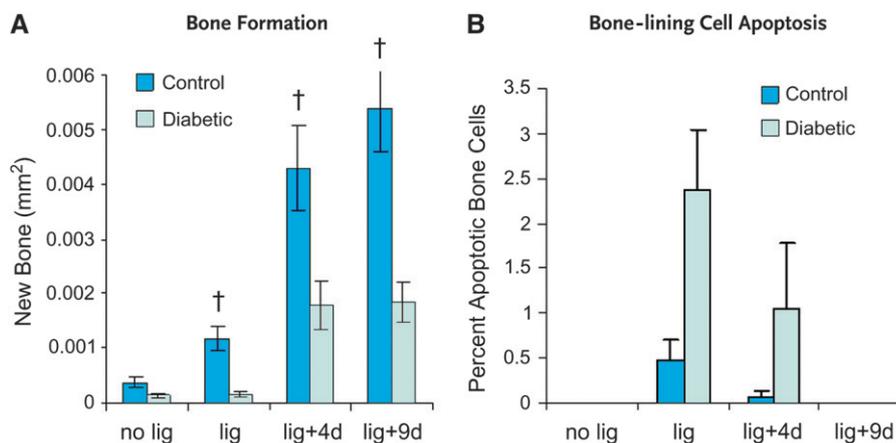


Figure 3.

Diabetes impaired new bone formation and increased osteoblastic apoptosis. **A)** The area of new bone formation was measured in tartrate-resistant acid phosphatase-stained sections. **B)** Rats were examined for the percentage of apoptotic bone-lining cells by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay. Each value is the mean of five to seven rats \pm SE. Analyses were carried out in four groups of rats: no ligation, at ligation removal, and at days 4 and 9 following ligation removal. lig = ligation; d = days. †Significant difference between diabetic and normoglycemic control rats ($P < 0.05$). Reprinted with permission from the International and American Associations for Dental Research.⁴¹

gingiva of diabetic mice compared to wild-type controls. The potential impact of TNF- α was examined in another model in which *P. gingivalis* was inoculated into the scalp of diabetic and normal mice. In this model, an injection of bacteria caused bone resorption, which was followed by bone formation, both of which could be quantified. To test the role of TNF, a specific inhibitor, pegsunercept, was injected 2 days after bacterial inoculation so that the initial inflammatory events were not affected. New bone formation was enhanced in diabetic mice treated with the TNF- α inhibitor compared to sham-treated diabetic controls. Diabetic mice also exhibited increased quantity and duration of apoptosis of bone-lining cells (osteoblasts and their precursors) compared to controls (Fig. 3B). Because elevated death of osteoblastic cells has been linked to reduced capacity to form new bone,⁴² high levels of TNF may cause uncoupling by inducing the death of osteoblasts or their precursors. These studies allowed us to propose a general model, whereby inflammation is induced by bacteria, leading to osteoclast formation and subsequent bone resorption as well as an increased rate of osteoblast apoptosis. The increased apoptosis of these cells may be linked to the immune response generated by periodontal pathogens in connective tissue. This, in turn, may cause impaired bone formation and, together, leads to uncoupling and greater periodontal bone loss.

CONCLUSIONS

Animal models showed that bacteria stimulate periodontal tissue destruction but that this effect is mediated by the host response induced by bacteria. By using inhibitors, it was shown that cytokines play an important role in this process. The effects of cytokines that promote osteoclast formation and bone resorption seem to be counteracted by other cytokines that are anti-inflammatory. It is probable that the balance between stimulatory and inhibitory cytokines, together with the regulation of their receptors and signaling cascades, determines the level of periodontal tissue loss. Another complicating factor is the deficient coupling that occurs in periodontal bone loss so that the expected replacement of resorbed bone is inadequate, leading to net bone loss. The host response induced by bacteria may also play a role in uncoupling. Consequently, the question of whether inflammatory mediators are a critical element in periodontal bone loss or attachment continues to be an important one to pursue. Seeking an answer remains challenging, however, because there is a high level of background inflammatory events caused by the ubiquitous presence of bacteria in the gingival sulcus. In addition, it remains difficult to associate inflammatory events with loss of attachment or loss of bone because of a general lack of longitudinal studies in humans.

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