CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

D.T. Graves[1]*, J. Li[2], and D.L. Cochran[3]

[1]Department of Periodontics, School of Dental Medicine, University of Pennsylvania; [2]Department of Periodontics, New Jersey Dental School, Newark, NJ, USA; and [3]Department of Periodontics, UTHSCSA, San Antonio, TX, USA; *corresponding author: dtgraves@dental.upenn.edu

J Dent Res 90(2):143-153, 2011

Inflammation and Uncoupling as Mechanisms of Periodontal Bone Loss

ABSTRACT

Periodontal disease is characterized by both inflammation and bone loss. Advances in research in both these areas have led to a new appreciation of not only each field but also the intimate relationship between inflammation and bone loss. This relationship has resulted in a new field of science called osteoimmunology and provides a context for better understanding the pathogenesis of periodontal disease. In this review, we discuss several aspects of the immuno-inflammatory host response that ultimately results in loss of alveolar bone. A proposal is made that periodontal inflammation not only stimulates osteoclastogenesis but also interferes with the uncoupling of bone formation and bone resorption, consistent with a pathologic process. Furthermore, arguments based on experimental animal models suggest a critical role of the spatial and temporal aspects of inflammation in the periodontium. A review of these findings leads to a new paradigm to help explain more fully the impact of inflammation on alveolar bone in periodontal disease so that it includes the effects of inflammation on uncoupling of bone formation from resorption.

KEY WORDS: adaptive immunity, bone matrix, cytokine, innate immunity, IL-1, osteoblast, osteoclast, RANK ligand, TNF.

DOI: 10.1177/0022034510385236

Received March 30, 2010; Last revision May 7, 2010; Accepted May 8, 2010

INTRODUCTION

It is widely recognized that bacteria initiate periodontal disease. Early studies by Löe and others (Löe *et al*., 1965; Theilade *et al*., 1966) demonstrated a direct relationship between bacterial plaque accumulation and gingival disease. In these studies, tissues without bacterial plaque had little inflammation, while those with plaque exhibited a strong inflammatory reaction. This process was reversible, indicating a direct correlation with bacterial plaque. Other studies in animals demonstrated a relationship among microbial plaque, inflammation, and periodontal bone loss (Keyes and Jordan, 1964; Saxe *et al*., 1967; Lindhe *et al*., 1975). These early studies established the bacterial etiology of gingivitis and periodontal diseases involving bone loss.

More recent studies have not only reinforced the bacterial etiology of periodontal disease but have also emphasized the role of inflammation in the pathologic process. These studies were performed in animal models to establish a cause-and-effect relationship between bacteria and the initiation of periodontal disease. In one animal model, a ligature is tied around the teeth, causing plaque accumulation and facilitating bacterial penetration into the gingival, which leads to inflammation and alveolar bone resorption (Graves *et al*., 2008). In contrast, the placement of ligatures in gnotobiotic rats does not cause significant increases in gingival inflammation or periodontal bone loss (Rovin *et al*., 1966), again demonstrating the essential role of bacteria as an initiating trigger. Further studies with other animal models also reinforce the relationship between bacteria and periodontal disease. For example, treatment of animals with antibiotics or topical application of chlorhexidine reduces the bacterial load and significantly reduces bone resorption (Weiner *et al*., 1979; Kenworthy and Baverel, 1981). In contrast to reducing bacteria, an increase in bacterial load enhances periodontal disease (Nagahata *et al*., 1982). In other animal models, the inoculation of periodontal pathogens into the oral cavities of rodents induced bone loss. In several studies, the introduction of *Porphyromonas gingivalis* into the oral cavity by oral gavage induced alveolar bone resorption in the mouse (Baker *et al*., 1994, 1999, 2000; Lalla *et al*., 1998). Similarly, oral gavage with *Actinobacillus actinomycetemcomitans* (Garlet *et al*., 2006) or *Tannerella forsythia* (Sharma *et al*., 2005) has been reported to stimulate periodontal bone loss. Introduction of *A. actinomycetemcomitans* in rats leads to colonization and the loss of alveolar bone (Schreiner *et al*., 2003; Fine *et al*., 2005). Thus, experimental studies in animal models support the human clinical trials implicating bacteria in the initiation of inflammation and periodontal disease (Reddy *et al*., 2003; Kirkwood *et al*., 2007).

HOST RESPONSE AND PERIODONTAL BONE LOSS

It has been well established that manipulation of the host response can attenuate periodontal bone loss (Graves, 2008). When the host response is reduced

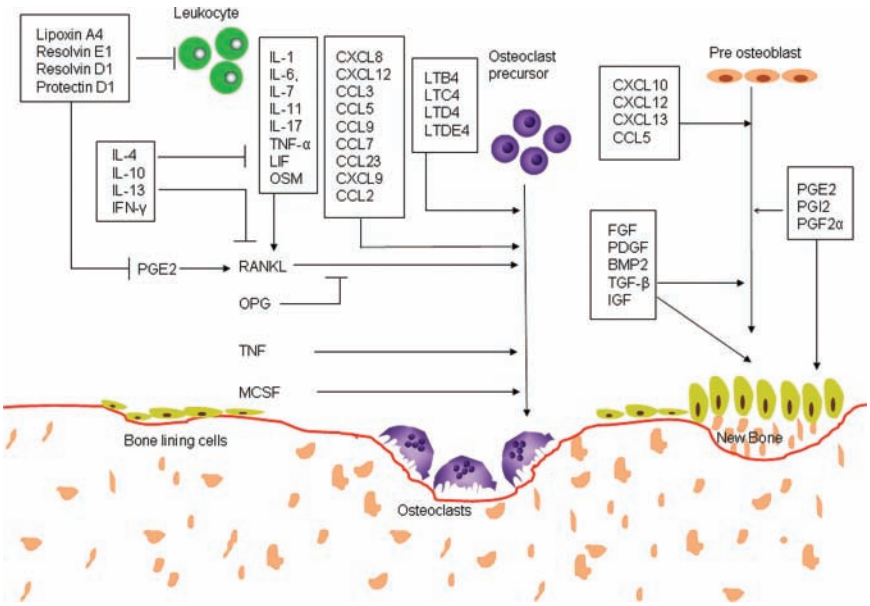

Figure 1. Stimulation of osteoclastogenesis, bone resorption, and coupled bone formation. RANKL, M-CSF, and TNF directly stimulate the formation of osteoclasts, other cytokines or lipid-based mediators such as prostaglandins or leukotrienes indirectly stimulate osteoclastogenesis by effects on RANKL, M-CSF, or TNF-α, and chemokines affect resorption by stimulating recruitment of osteoclast precursors or osteoclast activity. In periodontitis, inflammatory cytokines IL-1, IL-6, IL-7, IL-11, IL-17, TNF-α, LIF, OSM, and RANKL are thought to be primarily produced by leukocytes. Growth factors such as FGF, PDGF, BMP-2, TGF-β, and IGF are released from bone matrix or synthesized locally by various cell types after bone resorption and stimulate proliferation of osteoblast precursors, osteoblast differentiation, or synthesis of bone matrix. Some chemokines, such as CXCL10, CXCL12, CXCL13, and CCL5, may affect bone formation by effects on osteoblast precursors or osteoblasts.

either through the application of inhibitors or through genetic manipulation, the severity of bone loss stimulated by periodontal pathogens is typically reduced, even if antibacterial defenses may be weakened. This suggests that the host response plays an essential role in bacteria-induced periodontal bone resorption. There are several classes of molecules that activate a host response that can stimulate osteoclastogenesis either directly or indirectly, including lipid-based mediators such as prostaglandins or leukotrienes, cytokines, and chemokines (see Fig. 1 and Table 1). One of the first studies to demonstrate a cause-and-effect relationship examined the impact of a non-steroidal anti-inflammatory drug on periodontal bone loss in the beagle dog (Williams *et al*., 1985). This study demonstrated that flurbiprofen over a 12-month period significantly decreased naturally occurring alveolar bone loss in dogs compared with control animals treated with placebo. Thus, products of the cyclooxygenase pathway of arachidonic acid metabolism are significantly involved in tissue destruction induced by periodontal disease. Prostaglandins are elevated in gingival crevicular fluid from patients with localized aggressive periodontitis, and *P. gingivalis* stimulates leukocyte infiltration concomitant with elevated PGE-2 levels and increased cyclooxygenase-2 expression *in vivo* (Pouliot *et al*., 2000). Human clinical trials suggest that cyclooxygenase inhibitors are useful in inhibiting periodontal disease but have not been widely adopted because of side-effects (Paquette *et al*., 2000). More recent evidence indicates that omega-3 fatty acid products, lipoxins, and resolvins reduce inflammation and alveolar bone loss in experimental periodontitis (Hasturk *et al*., 2006).

Cytokines also play a prominent role in bacteria-induced periodontal bone resorption (summarized in Table 1). In a non-human primate model, inhibitors of two prominent pro-inflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF), reduced periodontal bone loss and loss of attachment compared with that in animals treated with vehicle alone (Assuma *et al*., 1998; Delima *et al*., 2001, 2002). Experiments were carried out in a *Macaca fascicularis* primate model in which *P. gingivalis*-soaked silk ligatures were tied around the posterior teeth. The ligature results in greater plaque accumulation coupled with the addition of *P. gingivalis* to the oral bacteria to induce periodontal disease. IL-1 and TNF were specifically inhibited by the local injection of soluble receptors to these cytokines into the gingiva. The inhibition of TNF and IL-1 with specific antagonists reduced the inflammatory cell infiltrate that forms close to bone and inhibited bone resorption. Thus, IL-1 and TNF contribute significantly to the pathologic bone loss in periodontal disease. In a follow-up study with the same model, IL-1 and TNF blockers inhibited the loss of connective tissue attachment (Delima *et al*., 2001). Moreover, inhibition of IL-1 alone had an effect, significantly reducing both the "migration" of an inflammatory infiltrate toward bone and the loss of alveolar bone.

From the above results, the authors suggest that inflammation associated with gingivitis is actively protective, since blocking further up-regulation of the host response with IL-1/TNF inhibitors reduced host destruction. This is in contrast to situations where the absence of TNF-α and IL-1 renders the tissue more susceptible to bacterial infection. For example, in endodontic infections where there is no pre-existing inflammatory response in the dental pulp (as there is in the gingiva), exposure of the naïve dental pulp to oral pathogens results in significantly greater destruction when TNF receptor signaling is absent (Chen *et al*., 1999). In contrast, periodontal disease is reduced in similar TNF-receptor-deficient mice (Garlet *et al*., 2007). Thus, in experimental periodontitis, inhibition or knockout of inflammatory mediators typically reduces the severity of the disease process, whereas blockage or deletion of the same genes in animals with endodontic lesions typically enhances the pathologic process and disease severity (Assuma *et al*., 1998; Baker *et al*., 1999; Chen *et al*., 1999; Huang *et al*., 2001; Garlet

Table 1. Summary of Regulatory Factors Likely to Participate in Osteoclastogenesis, Bone Resorption, and Coupled Bone Formation in Periodontitis

Activity	Regulatory Factors	Sources	Function
Direct regulation of osteoclast precursor (reviewed in Teng, 2006; Han *et al.*, 2007; Sims and Gooi, 2008; Bartold *et al.*, 2010)	RANKL	Primarily lymphocyte	Activates osteoclastogenesis through RANK at osteoclasts
	OPG	Lymphocyte and mesenchymal cell lineage	Inhibits RANKL, inhibits osteoclastogenesis
	M-CSF	Bone-lining cells	Activates osteoclastogenesis
Indirect regulation of osteoclast formation and activity (reviewed in Han *et al.*, 2007; Noguchi and Ishikawa, 2007; Silva *et al.*, 2007; Cochran, 2008; Graves, 2008; Hikiji *et al.*, 2008; Bartold *et al.*, 2010)	IL-1	Mesenchymal cell lineage, immune cells	Enhances RANKL
	IL-6	Mesenchymal cell lineage, immune cells	Enhances RANKL
	IL-7	Mesenchymal cell lineage, immune cells	Enhances RANKL
	IL-11	Mesenchymal cell lineage, immune cells	Enhances RANKL
	IL-17	Th17 lymphocytes	Acts on osteoblasts, stimulates synthesis of PGE_2, then enhances RANKL
	TNF-α	Mesenchymal cell lineage, immune cells	Directly acts on osteoclasts or indirectly activates osteoclasts through the stimulation of RANKL
	LIF	Mesenchymal cell lineage, immune cells	Enhances RANKL
	OSM	Mesenchymal cell lineage, immune cells	Enhances RANKL
	IL-4	Immune cells	Inhibits RANKL
	IL-10	Immune cells	Inhibits RANKL
	IL-13	Immune cells	Inhibits RANKL
	IFN-γ	Immune cells	Inhibits RANKL
	IL-8/CXCL8	Mesenchymal cell lineage, immune cells	Chemoattractant to PMNs and induces osteoclast differentiation
	SDF-1/CXCL12	Immune cells	Induces chemotaxis and differentiation of osteoclasts, increases MMP-9 activity
	MIP-1α/CCL3 RANTES/CCL5 MIP-1γ/CCL9 MCP-3/CCL7 CKβ8/CCL23	Mesenchymal cell lineage, immune cells	Stimulates chemotaxis of osteoclast precursor cells
	MIG/CXCL9	Immune cells	Induces migration and adhesion of osteoclast precursor
	MCP-1/CCL2	Immune cells	Induces osteoclast chemotaxis and differentiation
	PGEs (PGE2, PGI2, PGF2α)	Monocyte/macrophage, gingival epithelial cell, fibroblast	Stimulates osteoclast formation through RANKL and direct effect on osteoclast precursor cells to stimulate osteoclast formation. Stimulates bone formation.
	Leukotrienes (LTB4, LTC4, LTD4)	Immune cells	Stimulates osteoclast formation, most likely independent of RANKL
Osteoblast differentiation/formation (reviewed in Serhan *et al.*, 2003; Troen, 2003; Silva *et al.*, 2007; Sims and Gooi, 2008; Van Dyke, 2008)	FGFs (FGF1, FGF2)	Bone matrix	Stimulates proliferation of osteoblast precursors and angiogenesis
	PDGF	Platelets, macrophages, bone matrix, various cell types	Stimulates proliferation of osteoblast precursors and angiogenesis
	IGF1, IGF2	Bone matrix	Stimulates production of bone matrix
	BMP-2	Osteoclasts, bone matrix	Induces osteoblast differentiation
	Mim-1	Osteoclasts	Stimulates migration and differentiation of osteoblastic precursor cells
	TGF-β	Bone matrix, osteoblasts and osteoclasts	Stimulate synthesis of bone matrix, inhibits MMP synthesis
	IP-10/CXCL10	Immune cells	Induces osteoblast proliferation
	SDF-1α/CXCL12	Immune cells	Induces osteoblast proliferation and type I collagen mRNA expression
	BCA-1/CXCL13	Immune cells	Induces osteoblast proliferation and type I collagen mRNA expression
	RANTES/CCL5	Immune cells	Induces chemotaxis of osteoblasts and promotes cell survival
	Lipoxin A4	Vascular lumen during cell-cell interaction, leukocyte-epithelial cell interaction	Inhibits chemotaxis of neutrophils
	Resolvins (Resolvin E1, D1)	Metabolic mediator	Inhibits neutrophil migration
	Protectin D1	Metabolic mediator	Inhibits TNF secretion from T-cells and promotes apoptosis of T-cells

et al., 2007; De Rossi *et al*., 2008). This difference may be due to the protective presence of a low-level inflammatory response that is always present in the superficial gingiva close to teeth, whereas this pre-existing leukocyte response is not present in naïve dental pulp.

IL-1 and TNF stimulate the formation of osteoclast-like cells *in vitro* (Devlin *et al*., 1998). IL-1 stimulates osteoclastogenesis and bone resorption, largely through up-regulation of receptor activator for nuclear factor-κB (RANK) ligand, while TNF can stimulate osteoclastogenesis directly or indirectly through RANK ligand (Wei *et al*., 2005). RANK ligand inhibitor, osteoprotegerin (OPG), may also be produced, so the ratio of RANK ligand to OPG is an important consideration. Lymphocytes are thought to be a particularly important source of RANK ligand in periodontal disease, while in physiologic bone remodeling, RANK ligand appears to be principally from bone-lining cells (Teng *et al*., 2000; Kawai *et al*., 2006; Rauner *et al*., 2007). Inhibition of RANK ligand caused a decrease in alveolar bone loss in several models of periodontal disease (Teng *et al*., 2000; Han *et al*., 2006; Jin *et al*., 2007). In one study, the participation of CD4+ T-cells in mediating periodontal bone loss was demonstrated (Teng *et al*., 2000). Furthermore, the administration of the RANK ligand inhibitor, osteoprotegerin, in these mice reduced osteoclastogenesis and inhibited alveolar bone destruction. Another group has determined that B-cells contribute to periodontal bone loss in the absence of T-cells through the production of RANK ligand (Han *et al*., 2006). Transfer of B-cells from rats immunized against *A. actinomycetemcomitans,* followed by injection of the periodontal pathogen, significantly increased periodontal bone loss compared with transfer of B-cells from non-immunized animals. Increased bone loss was inhibited by the injection of osteoprotegerin. The role of RANK ligand in a ligature-induced model of periodontitis has been examined (Jin *et al*., 2007). In this study, rats were administrated subcutaneously an OPG-Fc fusion protein to block RANK ligand. By μCT and histomorphometric analysis, reduced osteoclastogenesis and significant preservation of alveolar bone volume were observed among OPG-Fc–treated rats compared with the controls. In humans, there have been several clinical trials testing the RANKL-inhibitor Denosumab, a human monoclonal antibody specific for RANKL (Lewiecki, 2009). Denosumab increases bone density and reduces fracture risk in post-menopausal women and inhibits osteoclast-mediated damage caused by rheumatoid arthritis (Lewiecki, 2009).

Mitogen-activating protein (MAP) kinase inhibitors also reduce lipopolysaccharide-induced alveolar bone loss, consistent with the role of MAP kinase in mediating the effects of pro-inflammatory signals (Rogers *et al*., 2007). Application of a p38 MAPK inhibitor reduced LPS-induced osteoclast formation and periodontal bone loss, which is consistent with *in vitro* results demonstrating that upstream regulators of p38 MAP kinase, MKK3 and MKK6, are required for IL-1beta and TNF-alpha-induced RANK ligand expression in bone marrow stromal cells (Rossa *et al*., 2008).

Manipulation of the host response through genetic studies further supports the relationship between the host response and periodontal bone loss. To identify the role of adaptive immune response in periodontal bone loss, a murine model has been used with oral infection by a Gram-negative anaerobic bacterium, *P. gingivalis*. Severe combined immunodeficient mice, which lack B- and T-lymphocytes, had much less bone compared with normal control mice, suggesting that the adaptive immune response plays a critical role in periodontal bone loss. To further identify the lymphocyte subtype involved, several immune-deficient mice were examined, including MHC II CD4+ T-cell-deficient, MHCI CD8+ T-cell-deficient, and natural killer T-cell-deficient mice. Only MHC class II-responsive CD4+ T-cell-deficient mice had diminished bone loss compared with normal controls (Baker *et al*., 1999). Thus, CD4+ T-cells but not CD8+ T-cells in this study were critical for bacteria-induced alveolar bone loss in response to oral infection of *P. gingivalis*.

To investigate the role of pro-inflammatory cytokines, researchers have examined mice with genetic deletion of IL-6 and IFN-γ. Introduction of *P. gingivalis* to IFN-γ and IL-6 knockout mice stimulated less periodontal bone loss compared with that in normal control mice, indicating that these cytokines contribute to periodontal disease progression. Although IFN-γ directly inhibits osteoclastogenesis, the results suggest that the stimulatory effect of IFN-γ on monocytes and lymphocytes overrides the direct inhibitory effect on osteoclasts and contributes to *P. gingivalis*-induced bone loss (Yang *et al*., 2002; Gao *et al*., 2007). In other studies, it was shown that IFN-γ was necessary in preventing systemic dissemination of a periodontal infection, and that the absence of IFN-γ reduced the levels of inflammatory cytokines and recruitment of leukocytes to the periodontium, supporting the role of IFN-γ as contributing to bacteria-induced periodontal bone loss (Garlet *et al*., 2008). Likewise, the role of TNF receptor signaling has been investigated by study of the response of mice with genetic deletion of TNF receptors to *P. gingivalis* backspace in a calvarial model and inoculation of the oral cavity with *A. actinomycetemcomitans* (Graves *et al*., 2001; Garlet *et al*., 2006). In the calvarial model, experimental mice with targeted deletion of TNF receptor-1 and TNF receptor-2 showed significant reduction of PMN infiltration and osteoclast numbers in response to injection of *P. gingivalis* compared with the wild-type mice (Graves *et al*., 2001). In addition, this study established that *P. gingivalis* stimulated fibroblast apoptosis *in vivo* through a mechanism that involved up-regulation of TNF-α. An alternative approach to investigating the role of TNF in periodontal disease was recently studied with adeno-associated virus vector to deliver a TNF blocker consisting of the TNF receptor 2 and the Fc domain of IgG1 (Cirelli *et al*., 2009). In this model, *P. gingivalis* was injected into the gingiva of rats with and without gene therapy treatment. The gene therapy blocked LPS-stimulated alveolar bone loss and osteoclastogenesis and reduced the local levels of several pro-inflammatory cytokines. In a periodontal model, the absence of TNF receptor-1 signaling in mice inoculated with *A. actinomycetemcomitans* reduced the levels of chemokines and their receptors, and reduced matrix metalloproteinase and RANK ligand mRNA levels. Interestingly, the antibacterial defense was also reduced, which was associated with the detection of higher levels of *A. actinomycetemcomitans*. Even with greater levels of *A. actinomycetemcomitans,* there was less bone loss, since the impact on the pro-inflammatory levels and reduced inflammatory infiltrate was relatively more important.

Taken together, these studies involving manipulation of the host response through inhibitors or genetics demonstrate that the host inflammatory response to bacteria mediates periodontal bone loss.

The studies discussed above have provided insight into the role of the host response with regard to its being protective *vs*. destructive (*i.e*., contributing to tissue loss). For example, the finding that reducing the host response would lead to less bone loss was initially counter-intuitive, since it is widely known that the host response is protective. For example, a compromised host response in leukocyte adhesion deficiency, Chédiak-Higashi syndrome, Papillon-Lefèvre, and acquired immune deficiency syndrome leads to greater susceptibility to periodontal disease (Nualart Grollmus *et al*., 2007; Yin *et al*., 2007). Animal studies also support the notion that the host response is protective. For example, adoptive transfer of *A. actinomycetemcomitans*-specific T-cell clones has been reported to protect against *A. actinomycetemcomitans*-induced periodontal bone loss (Yamashita *et al*., 1991), and immunization against *P. gingivalis* significantly reduces bone loss in a non-human primate model (Persson *et al*., 1994). In addition, cytokines such as IL-17 play a protective role in limiting bone loss by *P. gingivalis* (Yu *et al*., 2007). This study demonstrated that the enhanced bone loss observed in IL-17 receptor-deficient mice is a result of a defective ability to stimulate neutrophil migration. This failure was linked to defective chemokine production in IL-17 receptor-deficient mice and demonstrated that IL-17 plays an important role in the host defense against oral pathogens.

Taken together, the studies above indicate that the host response has both protective and destructive functions in the periodontium. This apparent contradiction has led to the hypothesis that the host response has dual roles and is more complicated than simply being assigned a "protective" or "destructive" role.

SPATIAL DISTRIBUTION OF INFLAMMATION

From the above results, it is clear that the immune response is critical in protecting the host from oral pathogens, yet at the same time is intimately involved in the destructive process. One way to resolve this apparent discrepancy in opposing roles is to consider a potentially important aspect of the pathological process, the spatial orientation of the host response. A characteristic of human gingiva is the ubiquitous presence of an inflammatory infiltrate in the gingiva adjacent to a tooth surface (Page and Schroeder, 1976; Yu and Graves, 1995). This infiltrate is present even in the absence of clinically obvious inflammation and is associated with gingivitis but not necessarily periodontitis. Bacteria or their products continually interact with gingival epithelium to stimulate a host response (Handfield *et al*., 2008). In experimental animal models, bone loss is initiated when the inflammatory infiltrate moves closer to the bone surface. For example, in a non-human primate model of experimental periodontitis, the temporal movement of an inflammatory front toward the crest of alveolar bone is associated with increased osteoclast formation and is substantially reduced by specifically blocked-in IL-1 and TNF (Graves *et al*., 1998). Thus, when inflammation is contained to the sub-epithelial connective tissue, the result is gingivitis, and when the inflammatory infiltrate moves closer to bone, osteoclastogenesis is induced. Thus, the central issue may not be the degree of inflammation or the qualitative nature of the inflammation, but may well be a consequence of where the inflammation is located in relationship to the bone. If the immune response is able to keep bacteria or their products from penetrating deeply into connective tissue, the inflammation and the host inflammatory response will be confined to the sub-epithelial space, and gingivitis is the clinical outcome. However, the progression of the inflammatory infiltrate toward bone so that it is within a critical distance to the bone surface will result in the activation and formation of osteoclasts and, subsequently, bone loss (Figs. 2A, 2B). One very significant consequence of this concept is that it provides a mechanistic basis for the universal dimensions of biologic width (Gargiulo *et al*., 1961). Building on the findings of Gargiulo and co-workers (Gargiulo *et al*., 1961), Page and Schroeder described biologic width as a 2.5-mm zone between the cemento-enamel junction and bone. They concluded that distances > 2.5 mm are caused by bacterial invasion of gingival connective tissue (Page and Schroeder, 1981), and that the closer the inflammatory infiltrate was to the bone, the greater was the amount of bone degraded (Schroeder and Lindhe, 1980; Rowe and Bradley, 1981). Thus, the proximity of the inflammatory infiltrate to bone may be an important consideration. It is likely that the spatial location of the inflammatory infiltrate will be dependent upon the depth to which bacteria or their products have penetrated the connective tissue. Thus, the spatial aspect of the host response to the bacterial challenge may contribute to the clinical manifestation of the disease (gingivitis *vs*. periodontitis) and the extent of tissue loss.

TEMPORAL ASPECTS OF INFLAMMATION

In a healthy adult with physiologic tissue turnover, an episode of bone resorption is followed by an equivalent amount of new bone formation, a well-accepted process referred to as coupling (Parfitt, 1982). In other words, bone is masterfully programmed to repair itself through the coupling of bone formation to bone resorption. This balanced bone formation and bone resorption require adequate availability of osteoblast precursor cells, osteoblast differentiation, and the formation of bone matrix in response to coupling signals. Under pathological conditions, however, an equivalent amount of bone formation does not occur, so that resorption and formation are uncoupled (Parfitt, 1982). Thus, an episode of alveolar bone resorption in a healthy adult should, under normal circumstances, be followed by an equivalent amount of bone formation. However, in periodontitis there is a failure to form bone, resulting in net bone loss. Therefore, the pathologic process that leads to net periodontal bone loss logically resides in the failure to form bone, either the inability to form compensatory bone in the quantity of bone resorbed (incomplete coupling) (Fig. 1C) or the inability to form bone at all subsequent to the bone resorption episode (total uncoupling). Thus, growth and differentiation factors that are produced during bone resorption (see Table 1) may not be sufficient to stimulate complete coupling. Alternatively, inflammatory mediators may interfere with the bone-forming process, leading to incomplete coupling. A list of molecules that could mediate the effects of inflammation on uncoupling is provided in

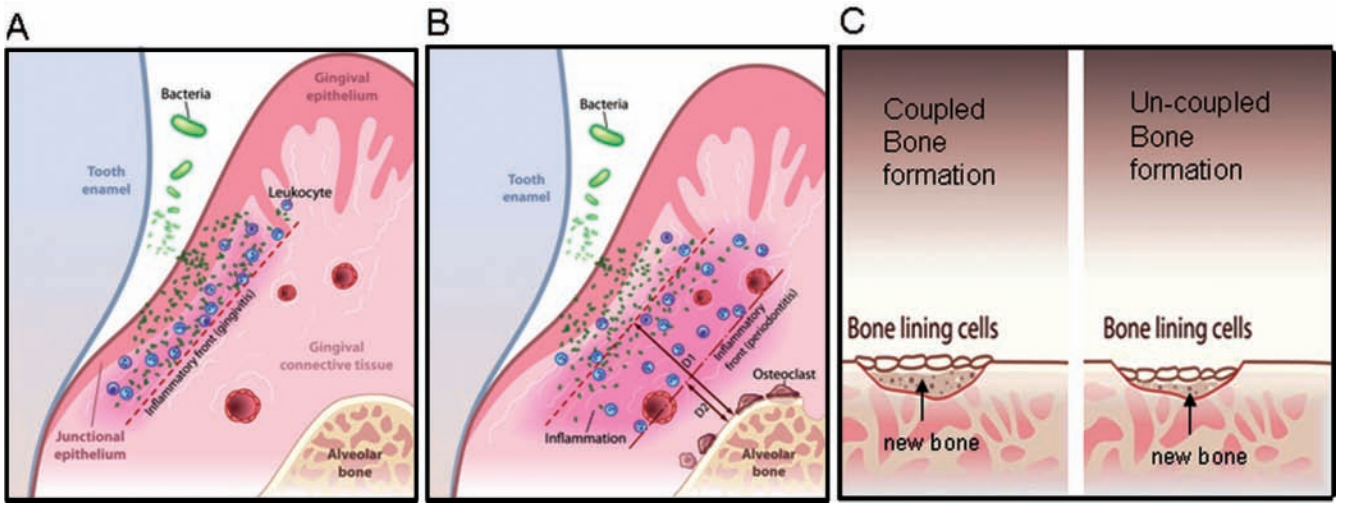

Figure 2. Migration of an inflammatory front toward alveolar bone stimulates osteoclastogenesis and may contribute to uncoupled bone formation and resorption. **(A)** In gingivitis, leukocytes in connective tissue are primarily found close to epithelium. **(B)** If bacteria or their products penetrate further into connective tissue, the inflammatory front moves closer to bone, where mediators produced by inflammatory cells can stimulate osteoclastogenesis and bone resorption. D1, the distance from the inflammatory front to bone in gingivitis. D2, the distance from the inflammatory front to bone in periodontitis. **(C)** In bone coupling, the amount of new bone produced equals the amount of resorbed bone, so that there is no net bone loss. A deficit in the number of osteoblasts or reduced bone matrix formation *per* osteoblast leads to a situation where the full amount of bone resorbed is not replaced, and there is incomplete coupling (uncoupling).

Table 2. Although this review focuses on bone, it is recognized that, for periodontal regeneration to occur, the formation of new cementum and periodontal ligament must also take place (Foster *et al*., 2007).

The inflammatory process that leads to osteoclastogenesis and bone resorption may also be responsible for the failure to form an adequate amount of new bone, *i.e*., inflammation causes uncoupling of bone formation following bone resorption. We propose that if inflammation is prolonged (temporally) so that it is present during the transition from the bone resorption to formation phase, inflammation will interfere with the elaboration of new bone. Again, evidence for this proposal can be found in animal studies. For example, injection of *P. gingivalis* into the connective tissue of a normal host leads to a brief episode of inflammation. This inflammatory process results in bone resorption followed by bone formation. When the inflammation is prolonged by a condition such as diabetes, so that the inflammatory process is extended into the phase of bone formation, the capacity to form new bone is significantly reduced (Al-Mashat *et al*., 2006; Liu *et al*., 2006a). Furthermore, if TNF production is inhibited in these same animals, the level of inflammation during this period is reduced, and, consequently, the ability to form new bone is improved in the diabetic animals (Liu *et al*., 2006a). This is strong evidence suggesting that the prolonged inflammation in diabetic animals interferes with bone formation in the periodontium following an episode of bone resorption (Liu *et al*., 2006b). This interpretation is additionally supported by evidence that the application of cytokines *in vivo* not only stimulates bone resorption but also limits bone formation. For example, injection of either TNF-α or IL-1β causes bone resorption and inhibits the coupling process by reducing new bone formation (Bertolini *et al*., 1986; Nguyen *et al*., 1991). Therefore, several lines of animal experimentation support the concept that inflammation uncouples bone formation from bone resorption. Thus, inflammation not only stimulates the formation of osteoclasts and bone resorption, but also affects bone by altering the function of osteoblasts and limiting reparative bone formation. In addition, it is possible that osteocytes are affected by inflammation and could affect the coupling process (Sims and Gooi, 2008). For example, the death of osteocytes is linked to the production of factors that stimulate bone remodeling. In addition, osteocytes produce sclerostin, a potent inhibitor of bone formation (Sims and Gooi, 2008).

Several other disease processes involve both inflammation and bone uncoupling. For example, similar to periodontal disease, osteoporosis is also characterized by uncoupling due to deficient bone formation following bone resorption. It is widely recognized that ovariectomy in the absence of estrogen replacement leads to bone resorption and loss of bone mass through the action of cytokines such as IL-1 or TNF (Weitzmann and Pacifici, 2006). In a recent study, Chang and colleagues investigated the impact of inflammation on the maintenance of bone mass in osteoporosis (Chang *et al*., 2009). The goal of these studies was to determine what would happen if the capacity of osteoblasts to respond to inflammatory signals was blocked, but other cell types could still respond. This was accomplished by deleting NFκB, a transcription factor that mediates gene expression stimulated by inflammatory molecules in osteoblasts, but not other cell types, through genetic manipulation. When NFκB was specifically blocked in osteoblasts, the capacity to form bone was enhanced without change in inflammation and without affecting osteoclast activities. This study also revealed NFκB

affected bone formation through its effect on Fos-related antigen-1 (Fra-1), an essential transcription factor involved in bone matrix formation. These results suggest that inflammatory mediators stimulate NFκB activity, which reduces Fra-1 expression and limits the production of bone matrix by osteoblasts. Thus, inflammation affects osteoblasts by limiting the capacity to form bone and, as a result, plays an important role in the uncoupling process of post-menopausal osteoporosis.

As in osteoporosis and periodontal disease, the loss of bone mass occurs in inflammatory arthritis. In arthritis, the inflammatory aspect of the disease is thought to cause destruction of joints and to induce lytic lesions in the peri-atricular bone which is not adequately repaired by bone coupling (Walsh *et al*., 2009). This may be partly caused by TNF-α, based on studies in which over-expression of TNF-α in transgenic mice caused the formation of lesions typical of rheumatoid arthritis (Keffer *et al*., 1991). *In vivo* administration of monoclonal antibody specific for TNF-α prevented the disease process, suggesting a critical role for TNF in the pathogenesis of arthritis. The failure to form an adequate amount of new bone, *i.e*., uncoupling, has been linked to reduced osteoblast differentiation caused by inflammatory cytokines (Diarra *et al*., 2007). Diarra and colleagues reported that TNF-α stimulates production of Dickkopf-1 (DKK-1), which suppresses bone formation by inhibiting the WNT pathway. Their findings showed that DKK-1, a negative regulator of WNT pathway, is up-regulated by TNF stimulation through TNF receptor-1 and P38 MAPK signaling. This up-regulated DKK-1 not only promotes bone resorption but also effectively blocks bone formation and repair in the diseased joint. This provides a mechanistic basis for understanding how TNF-α can limit the ability to form bone by inhibiting osteoblast differentiation. It is consistent with previous reports that inflammatory cytokines prevent bone formation *in vitro* (Stashenko *et al*., 1987).

Pro-inflammatory cytokines, such as IL-1β and TNF-α, contribute to uncoupling in bone by reducing bone formation. To clarify the mechanisms through which inflammatory cytokines can affect bone formation, several aspects of bone formation have been studied. These may include the impact of inflammatory mediators on osteoblast precursors, number of osteoblasts, or osteoblast matrix-producing activity. Decreased osteoblast numbers can be achieved from reduced proliferation or enhanced apoptosis of osteoblasts or their precursor cells, both of which may be affected by inflammation. The effects of pro-inflammatory cytokines on cultured human osteoblast proliferation have been examined *in vitro* (Frost *et al*., 1997). TNF-α stimulated proliferation of osteoblasts at low doses, while it significantly inhibited proliferation of osteoblasts at high doses or prolonged treatment. This result suggests that excessive and prolonged TNF-α may have a destructive effect on bone by inhibiting its anabolic activity, and reinforces the concept that there may be an important spatial and temporal relationship between inflammation and bone coupling. In addition to affecting proliferation, TNF-α also inhibits the differentiation of osteoblast precursor cells (Gilbert *et al*., 2000). Treatment of TNF-α has been shown to reduce the differentiation of fetal calvaria precursor cells and MC3T3-E1 clonal pre-osteoblasts to the osteoblast phenotype, and reduces formation of mineralizing nodules *in vitro*. One mechanism through which this may occur is inhibition of a critical osteoblast differentiation factor, RUNX2, by TNF-α (Gilbert *et al*., 2002). This appears to be due to signaling through TNF receptor-1 (Gilbert *et al*., 2005). Thus, TNF-α inhibits RUNX2 activity, which in turn inhibits osteoblast differentiation.

Table 2. Molecules that May Interfere with Coupling in Periodontitis

Molecule	Class	Effect on Osteoblasts or Their Precursors
TNF	Cell signaling	Induced by inflammation. Could affect osteoblasts or their precursors by reducing their numbers (decreased proliferation, increased apoptosis), inhibiting osteoblast differentiation or reducing matrix production.
p38 or jnk MAP kinases	Cell signaling	Induced by inflammation. May affect coupling by activating transcription factors DKK-1, FOXO1, and NFκB.
DKK-1	Cell signaling	Induced by inflammation. Inhibits the Wnt signaling pathway to reduce bone formation.
FOXO1	Transcription factor	Induced by inflammation. May reduce numbers of osteoblasts or their precursors by stimulating apoptosis.
NFκB	Transcription factor	Induced by inflammation. When activated, NFκB may reduce matrix production by decreasing Fra-1.
Fra-1	Transcription factor	Inhibited by NFκB. Fra-1 needed to promote expression of matrix genes.

Osteoblast survival is thought to be an important aspect of bone coupling. One of the causes of bone uncoupling could be diminished osteoblast or osteoblast precursor survival through induced apoptosis. Pro-inflammatory cytokines may directly stimulate osteoblast or osteoblast precursor apoptosis or indirectly affect it by stimulating expression of Fas, a potent pro-apoptotic mediator (Tsuboi *et al*., 1999). In periodontal bone remodeling, periodontal ligament cells are an important source of osteoblast precursors (Lin *et al*., 1994). TNF-α-induced apoptosis of periodontal ligament cells may affect the pool of osteoblast precursors (Thammasitboon *et al*., 2006). Apoptosis of mouse pre-osteoblastic MC3T3-E1 cells is induced by combined treatment of TNF-α, IL-1β, and IFN-γ and can be reduced by specific inhibitors of p38 MAP kinase (Kuzushima *et al*., 2006). More recently, we have found that MC3T3 apoptosis stimulated by pro-inflammatory cytokines is mediated by the pro-apoptotic transcription factor, forkhead box-O1 (FOXO1) (Behl *et al*., 2008). FOXO1 regulates expression of pro-apoptotic genes, including Fas-associated, *via* death domain (FADD) and caspases-3, -8, and -9. Moreover, increased apoptosis of MC3T3 cells is significantly reduced by knockdown of FOXO1 by small interfering RNA (Behl *et al*., 2008). FOXO1 belongs to the forkhead-O family, consisting of FOXO1, FOXO3, and FOXO4, which regulate cell death and cell cycle, and modulates the

response to oxidative stress. Interestingly, FOXO1 has been linked to the regulation of serum glucose levels by modulating osteocalcin (Rached *et al*., 2010).

Another mechanism for uncoupling is reduced function of osteoblasts mediated by diminished production of bone matrix proteins. Bone matrix has organic and inorganic components, with the majority of the former consisting of type I collagen as well as other proteins and including osteocalcin and alkaline phosphatase. TNF-α reduces collagen production and alkaline phosphatase activity in cells obtained from fetal rat parietal bone (Centrella *et al*., 1988). In addition, production of non-collagen bone matrix proteins is inhibited by pro-inflammatory cytokines. TNF-α and IL-1β induce a two- to three-fold reduction in osteocalcin synthesis in osteoblastic cells (Taichman and Hauschka, 1992). Since TNF appears to interfere with bone formation through multiple mechanisms, the TNF specific inhibitor, etanercept, has been tested for its effect on improving the coupling process. Etanercept promotes BMP-2-induced ectopic bone formation when applied either systemically or locally *in vivo* (Eguchi *et al*., 2009). Similarly, inhibition of TNF with etanercept reduces apoptosis of bone-lining cells and significantly improves bone formation following *P. gingivalis*-induced bone resorption (Liu *et al*., 2006a). Thus, it is clear that pro-inflammatory cytokines such as IL-1β and TNF-α can affect bone formation and have a negative impact on bone coupling.

There is evidence that inflammation may affect coupling associated with bone resorption induced by periodontal pathogens. When a periodontal pathogen enters connective tissue, an inflammatory response is induced. It has been shown that activation of the acquired immune response by a periodontal pathogen inhibits bone coupling by limiting the formation of new bone following resorption (Leone *et al*., 2006; Behl *et al*., 2008). In these experiments, one group of mice was immunized against *P. gingivalis*. The host response to subsequent challenge of immunized and non-immunized mice with *P. gingivalis* was significantly greater in the immunized group. Interestingly, the immunized mice had significantly higher numbers of osteoclasts and higher levels of bone resorption. However, they also had greater apoptosis of bone-lining cells, fewer bone-lining cells, and reduced capacity to form new bone compared with non-immunized mice. One mechanism through which this may occur is enhanced cell death of osteoblastic cells or their precursors by the activation of pro-apoptotic transcription factors such as FOXO1 (Behl *et al*., 2008). Similarly, prolonged inflammation caused by diabetes appears to have a negative effect on bone coupling by stimulating death of bone-lining cells (Al-Mashat *et al*., 2006; Liu *et al*., 2006a), and this mechanism has been proposed in limiting new bone formation in other disease processes (Jilka, 2007).

Thus, several diseases that involve inflammation and bone metabolism exhibit uncoupling of bone resorption and bone formation. In fact, inflammation and bone metabolism have received so much attention that a new area of science has developed, called osteoimmunology (Arron and Choi, 2000). This interdisciplinary field integrates the disciplines of immunology and bone biology and provides a new context for the understanding of chronic diseases involving inflammation and bone, such as periodontal disease. The studies reviewed above have allowed us to propose a general model for periodontal disease, whereby inflammation stimulated by bacteria leads not only to osteoclast formation and subsequent bone resorption, but also to diminished bone formation. We propose that the spatial location of inflammation in relationship to bone and its duration are both critical features of the disease. With regard to the temporal component, if the inflammation is brief and does not extend into the phase of bone formation, the impact on periodontal bone loss may be relatively small, since there should be a considerable amount of coupling and new bone formation. However, if the inflammation lasts to the period of bone formation, then uncoupling is likely to occur, and the capacity to repair the resorbed bone is likely to be compromised. There also appear to be several mechanisms through which the inflammatory response may interfere with bone formation. One mechanism may be stimulated cell death of bone-lining cells, reducing the number of available cells or precursors that can form new bone. Alternatively, cytokines associated with the inflammation may inhibit the differentiation of osteoblasts from their precursors and thus limit bone formation. A third mechanism is reduced production of bone matrix due to the impact of inflammatory mediators. There is evidence that each of these mechanisms may play a role in various bone pathologies characterized by uncoupling of bone resorption and formation (Al-Mashat *et al*., 2006; Liu *et al*., 2006a; Diarra *et al*., 2007; Behl *et al*., 2008; Wang *et al*., 2009). All three mechanisms would reduce the numbers of osteoblastic cells available to form bone to equal and balance the amount of bone resorbed. This, in turn, would lead to uncoupling and greater periodontal bone loss.

The second consideration is spatial. If the inflammation is localized to the sub-epithelial space, it is unlikely to damage the underlying bone. However, if the inflammatory infiltrate progresses toward bone at some critical distance, the proximity of the inflammatory mediators to the bone surface will stimulate the recruitment of osteoclast precursors, osteoclastogenesis, and bone resorption. If the infiltrate persists near bone, we would predict that uncoupling will occur and result in net bone loss as observed clinically. Alternatively, if the inflammation near bone is transitory, we would expect that coupling would occur and new bone formation would occur, leading to little or no net bone loss.

For years, clinicians have identified periodontal disease on radiographs by examining the amount of bone loss that occurs around the dentition. Through a better understanding of inflammation and bone metabolism, a new paradigm exists to help explain periodontal bone loss.

ACKNOWLEDGMENTS

We thank Juanita Hobson for help in preparing this manuscript. Support from NIDCR awards 5R01 DE017732 and 7R01 DE18307 is acknowledged. D. Graves was not involved in the review process of this manuscript.

REFERENCES

Al-Mashat HA, Kandru S, Liu R, Behl Y, Desta T, Graves DT (2006). Diabetes enhances mRNA levels of proapoptotic genes and caspase activity, which contribute to impaired healing. *Diabetes* 55:487-495.

Arron JR, Choi Y (2000). Bone versus immune system. *Nature* 408:535-536.

Assuma R, Oates T, Cochran D, Amar S, Graves D (1998). IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *J. Immunol* 160:403-409.

Baker P, Evans R, Roopenian D (1994). Oral infection with *Porphyromonas gingivalis* and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. *Arch Oral Biol* 39:1035-1040.

Baker P, Dixon M, Evans R, Dufour L, Johnson E, Roopenian D (1999). CD4(+) T cells and the proinflammatory cytokines gamma interferon and interleukin-6 contribute to alveolar bone loss in mice. *Infect Immun* 67:2804-2809.

Baker PJ, Dixon M, Roopenian DC (2000). Genetic control of susceptibility to *Porphyromonas gingivalis*-induced alveolar bone loss in mice. *Infect Immun* 68:5864-5868.

Bartold PM, Cantley MD, Haynes DR (2010). Mechanisms and control of pathologic bone loss in periodontitis. *Periodontol 2000* 53:55-69.

Behl Y, Siqueira M, Ortiz J, Li J, Desta T, Faibish D*, et al*. (2008). Activation of the acquired immune response reduces coupled bone formation in response to a periodontal pathogen. *J Immunol* 181:8711-8718.

Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR (1986). Stimulation of bone resorption and inhibition of bone formation *in vitro* by human tumour necrosis factors. *Nature* 319:516-518.

Centrella M, McCarthy TL, Canalis E (1988). Tumor necrosis factor-alpha inhibits collagen synthesis and alkaline phosphatase activity independently of its effect on deoxyribonucleic acid synthesis in osteoblast-enriched bone cell cultures. *Endocrinology* 123:1442-1448.

Chang J, Wang Z, Tang E, Fan Z, McCauley L, Franceschi R*, et al*. (2009). Inhibition of osteoblastic bone formation by nuclear factor-kappaB. *Nat Med* 15:682-689.

Chen C-P, Hertzberg M, Jiang Y, Graves DT (1999). IL-1 and TNF receptor signaling is not required for bacteria-induced osteoclastogenesis and bone loss but is essential for protecting the host from a mixed anaerobic infection. *Am J Pathol* 155:2145-2152.

Cirelli JA, Park CH, MacKool K, Taba M Jr, Lustig KH, Burstein H*, et al*. (2009). AAV2/1-TNFR:Fc gene delivery prevents periodontal disease progression. *Gene Ther* 16:426-436.

Cochran DL (2008). Inflammation and bone loss in periodontal disease. *J Periodontol* 79(8 Suppl):S1569-S1576.

De Rossi A, Rocha LB, Rossi MA (2008). Interferon-gamma, interleukin-10, intercellular adhesion molecule-1, and chemokine receptor 5, but not interleukin-4, attenuate the development of periapical lesions. *J Endod* 34:31-38.

Delima A, Oates T, Assuma R, Schwartz Z, Cochran D, Amar S*, et al*. (2001). Soluble antagonists to interleukin-1 (IL-1) and tumor necrosis factor (TNF) inhibits *(sic)* loss of tissue attachment in experimental periodontitis. *J Clin Periodontol* 28:233-240.

Delima AJ, Karatzas S, Amar S, Graves DT (2002). Inflammation and tissue loss caused by periodontal pathogens is *(sic)* reduced by IL-1 antagonists. *J Infect Dis* 186:511-516.

Devlin RD, Reddy SV, Savino R, Ciliberto G, Roodman GD (1998). IL-6 mediates the effects of IL-1 or TNF, but not PTHrP or 1,25(OH)2D3, on osteoclast-like cell formation in normal human bone marrow cultures. *J Bone Miner Res* 13:393-399.

Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D*, et al*. (2007). Dickkopf-1 is a master regulator of joint remodeling. *Nat Med* 13:156-163.

Eguchi Y, Wakitani S, Imai Y, Naka Y, Hashimoto Y, Nakamura H*, et al*. (2009). Antitumor necrotic factor agent promotes BMP-2-induced ectopic bone formation. *J Bone Miner Metab* 28:157-164.

Fine DH, Velliyagounder K, Furgang D, Kaplan JB (2005). The *Actinobacillus actinomycetemcomitans* autotransporter adhesin Aae exhibits specificity for buccal epithelial cells from humans and old world primates. *Infect Immun* 73:1947-1953.

Foster BL, Popowics TE, Fong HK, Somerman MJ (2007). Advances in defining regulators of cementum development and periodontal regeneration. *Curr Top Dev Biol* 78:47-126.

Frost A, Jonsson KB, Nilsson O, Ljunggren O (1997). Inflammatory cytokines regulate proliferation of cultured human osteoblasts. *Acta Orthop Scand* 68:91-96.

Gao Y, Grassi F, Ryan MR, Terauchi M, Page K, Yang X*, et al*. (2007). IFN-gamma stimulates osteoclast formation and bone loss *in vivo* via antigen-driven T cell activation. *J Clin Invest* 117:122-132.

Gargiulo AW, Wentz FM, Orban B (1961). Dimensions and relations of the dentogingival junction in humans. *J Periodontol* 32:261-267.

Garlet GP, Cardoso CR, Silva TA, Ferreira BR, Avila-Campos MJ, Cunha FQ*, et al*. (2006). Cytokine pattern determines the progression of experimental periodontal disease induced by *Actinobacillus actinomycetemcomitans* through the modulation of MMPs, RANKL, and their physiological inhibitors. *Oral Microbiol Immunol* 21:12-20.

Garlet GP, Cardoso CR, Campanelli AP, Ferreira BR, Avila-Campos MJ, Cunha FQ*, et al*. (2007). The dual role of p55 tumour necrosis factor-alpha receptor in *Actinobacillus actinomycetemcomitans*-induced experimental periodontitis: host protection and tissue destruction. *Clin Exp Immunol* 147:128-138.

Garlet GP, Cardoso CR, Campanelli AP, Garlet TP, Avila-Campos MJ, Cunha FQ*, et al*. (2008). The essential role of IFN-gamma in the control of lethal *Aggregatibacter actinomycetemcomitans* infection in mice. *Microbes Infect* 10:489-496.

Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J*, et al*. (2000). Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. *Endocrinology* 141:3956-3964.

Gilbert L, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ*, et al*. (2002). Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem* 277:2695-2701.

Gilbert LC, Rubin J, Nanes MS (2005). The p55 TNF receptor mediates TNF inhibition of osteoblast differentiation independently of apoptosis. *Am J Physiol Endocrinol Metab* 288:E1011-E1018.

Graves D (2008). Cytokines that promote periodontal tissue destruction. *J Periodontol* 79(8 Suppl):S1585-S1591.

Graves D, Delima A, Assuma R, Amar S, Oates T, Cochran D (1998). Interleukin-1 and tumor necrosis factor antagonists inhibit the progression of inflammatory cell infiltration toward alveolar bone in experimental periodontitis. *J Periodontol* 69:1419-1425.

Graves D, Oskoui M, Volejnikova S, Naguib G, Cai S, Desta T*, et al*. (2001). Tumor necrosis factor modulates fibroblast apoptosis, PMN recruitment, and osteoclast formation in response to *P. gingivalis* infection. *J Dent Res* 80:1875-1879.

Graves DT, Fine D, Teng YT, Van Dyke TE, Hajishengallis G (2008). The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. *J Clin Periodontol* 35:89-105.

Han X, Kawai T, Eastcott JW, Taubman MA (2006). Bacterial-responsive B lymphocytes induce periodontal bone resorption. *J Immunol* 176:625-631.

Han X, Kawai T, Taubman MA (2007). Interference with immune-cell-mediated bone resorption in periodontal disease. *Periodontol 2000* 45:76-94.

Handfield M, Baker HV, Lamont RJ (2008). Beyond good and evil in the oral cavity: insights into host-microbe relationships derived from transcriptional profiling of gingival cells. *J Dent Res* 87:203-223.

Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N*, et al*. (2006). RvE1 protects from local inflammation and osteoclast-mediated bone destruction in periodontitis. *FASEB J* 20:401-403.

Hikiji H, Takato T, Shimizu T, Ishii S (2008). The roles of prostanoids, leukotrienes, and platelet-activating factor in bone metabolism and disease. *Prog Lipid Res* 47:107-126.

Huang GT, Do M, Wingard M, Park JS, Chugal N (2001). Effect of interleukin-6 deficiency on the formation of periapical lesions after pulp exposure in mice. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 92:83-88.

Jilka RL (2007). Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. *Bone* 40:1434-1446.

Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M Jr, Kostenuik PJ*, et al*. (2007). RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. *J Periodontol* 78:1300-1308.

Kawai T, Matsuyama T, Hosokawa Y, Makihira S, Seki M, Karimbux NY*, et al*. (2006). B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *Am J Pathol* 169:987-998.

Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D*, et al*. (1991). Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 10:4025-4031.

Kenworthy R, Baverel M (1981). Studies of a periodontal tissue lesion in the rat, untreated or treated with chlorhexidine digluconate. *J Clin Periodontol* 8:349-358.

Keyes PH, Jordan HV (1964). Periodontal lesions in the Syrian hamster. III. Findings related to an infectious and transmissible component. *Arch Oral Biol* 32:377-400.

Kirkwood KL, Cirelli JA, Rogers JE, Giannobile WV (2007). Novel host response therapeutic approaches to treat periodontal diseases. *Periodontol 2000* 43:294-315.

Kuzushima M, Mogi M, Togari A (2006). Cytokine-induced nitric-oxide-dependent apoptosis in mouse osteoblastic cells: involvement of p38MAP kinase. *Arch Oral Biol* 51:1048-1053.

Lalla E, Lamster I, Feit M, Huang L, Schmidt A (1998). A murine model of accelerated periodontal disease in diabetes. *J Periodontal Res* 33:387-399.

Leone CW, Bokhadhoor H, Kuo D, Desta T, Yang J, Siqueira MF*, et al*. (2006). Immunization enhances inflammation and tissue destruction in response to *Porphyromonas gingivalis*. *Infect Immun* 74:2286-2292.

Lewiecki EM (2009). Denosumab update. *Curr Opin Rheumatol* 21: 369-373.

Lin WL, McCulloch CA, Cho MI (1994). Differentiation of periodontal ligament fibroblasts into osteoblasts during socket healing after tooth extraction in the rat. *Anat Rec* 240:492-506.

Lindhe J, Hamp SE, Löe H (1975). Plaque induced periodontal disease in beagle dogs. A 4-year clinical, roentgenographical and histometrical study. *J Periodontal Res* 10:243-255.

Liu R, Bal HS, Desta T, Behl Y, Graves DT (2006a). Tumor necrosis factor-alpha mediates diabetes-enhanced apoptosis of matrix-producing cells and impairs diabetic healing. *Am J Pathol* 168:757-764.

Liu R, Bal HS, Desta T, Krothapalli N, Alyassi M, Luan Q*, et al*. (2006b). Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *J Dent Res* 85:510-514.

Löe H, Theilade E, Jensen SB (1965). Experimental gingivitis in man. *J Periodontol* 36:177-187.

Nagahata T, Kiyoshige T, Tomono S, Abe R, Sasaki S, Takazoe I (1982). Oral implantation of *Bacteroides asaccharolyticus* and *Eikenella corrodens* in conventional hamsters. *Infect Immun* 36:304-309.

Nguyen L, Dewhirst FE, Hauschka PV, Stashenko P (1991). Interleukin-1 beta stimulates bone resorption and inhibits bone formation *in vivo*. *Lymphokine Cytokine Res* 10:15-21.

Noguchi K, Ishikawa I (2007). The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease. *Periodontol 2000* 43:85-101.

Nualart Grollmus ZC, Morales Chavez MC, Silvestre Donat FJ (2007). Periodontal disease associated to systemic genetic disorders. *Med Oral Patol Oral Cir Bucal* 12(3):E211-E215.

Page R, Schroeder H (1976). Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 34:235-249.

Page RC, Schroeder HE (1981). Current status of the host response in chronic marginal periodontitis. *J Periodontol* 52:477-491.

Paquette DW, Lawrence HP, McCombs GB, Wilder R, Binder TA, Troullos E*, et al*. (2000). Pharmacodynamic effects of ketoprofen on crevicular fluid prostanoids in adult periodontitis. *J Clin Periodontol* 27:558-566.

Parfitt A (1982). The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. *Metab Bone Dis Relat Res* 4:1-6.

Persson GR, Engel D, Whitney C, Darveau R, Weinberg A, Brunsvold M*, et al*. (1994). Immunization against *Porphyromonas gingivalis* inhibits progression of experimental periodontitis in nonhuman primates. *Infect Immun* 62:1026-1031.

Pouliot M, Clish CB, Petasis NA, Van Dyke TE, Serhan CN (2000). Lipoxin A(4) analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry* 39:4761-4768.

Rached MT, Kode A, Silva BC, Jung DY, Gray S, Ong H*, et al*. (2010). FoxO1 expression in osteoblasts regulates glucose homeostasis through regulation of osteocalcin in mice. *J Clin Invest* 120:357-368.

Rauner M, Sipos W, Pietschmann P (2007). Osteoimmunology. *Int Arch Allergy Immunol* 143:31-48.

Reddy M, Geurs N, Gunsolley J (2003). Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. *Ann Periodontol* 8:12-37.

Rogers JE, Li F, Coatney DD, Otremba J, Kriegl JM, Protter TA*, et al*. (2007). A p38 mitogen-activated protein kinase inhibitor arrests active alveolar bone loss in a rat periodontitis model. *J Periodontol* 78:1992-1998.

Rossa C Jr, Liu M, Kirkwood KL (2008). A dominant function of p38 mitogen-activated protein kinase signaling in receptor activator of nuclear factor-kappaB ligand expression and osteoclastogenesis induction by *Aggregatibacter actinomycetemcomitans* and *Escherichia coli* lipopolysaccharide. *J Periodontal Res* 43:201-211.

Rovin S, Costich ER, Gordon HA (1966). The influence of bacteria and irritation in the initiation of periodontal disease in germfree and conventional rats. *J Periodontal Res* 1:193-204.

Rowe DJ, Bradley LS (1981). Quantitative analyses of osteoclasts, bone loss and inflammation in human periodontal disease. *J Periodontal Res* 16:13-19.

Saxe SR, Greene JC, Bohannan HM, Vermillion JR (1967). Oral debris, calculus, and periodontal disease in the beagle dog. *Periodontics* 5:217-225.

Schreiner HC, Sinatra K, Kaplan JB, Furgang D, Kachlany SC, Planet PJ*, et al*. (2003). Tight-adherence genes of *Actinobacillus actinomycetemcomitans* are required for virulence in a rat model. *Proc Natl Acad Sci USA* 100:7295-7300.

Schroeder H, Lindhe J (1980). Conditions and pathological features of rapidly destructive, experimental periodontitis in dogs. *J Periodontol* 51:6-19.

Serhan CN, Jain A, Marleau S, Clish C, Kantarci A, Behbehani B*, et al*. (2003). Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J Immunol* 171:6856-6865.

Sharma A, Inagaki S, Honma K, Sfintescu C, Baker PJ, Evans RT (2005). *Tannerella forsythia*-induced alveolar bone loss in mice involves leucine-rich-repeat BspA protein. *J Dent Res* 84:462-467.

Silva TA, Garlet GP, Fukada SY, Silva JS, Cunha FQ (2007). Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease. *J Dent Res* 86:306-319.

Sims NA, Gooi JH (2008). Bone remodeling: multiple cellular interactions required for coupling of bone formation and resorption. *Semin Cell Dev Biol* 19:444-451.

Stashenko P, Dewhirst FE, Rooney M, Desjardins L, Heeley J (1987). Interleukin-1B is potent inhibitor of bone formation *in vitro*. *J Bone Min Res* 2:559-565.

Taichman RS, Hauschka PV (1992). Effects of interleukin-1 beta and tumor necrosis factor-alpha on osteoblastic expression of osteocalcin and mineralized extracellular matrix *in vitro*. *Inflammation* 16:587-601.

Teng Y (2006). Protective and destructive immunity in the periodontium: Part 2—T-cell-mediated immunity in the periodontium. *J Dent Res* 85:209-219.

Teng Y, Nguyen H, Gao X, Kong Y, Gorczynski R, Singh B*, et al*. (2000). Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. *J Clin Invest* 106:R59-R67.

Thammasitboon K, Goldring SR, Boch JA (2006). Role of macrophages in LPS-induced osteoblast and PDL cell apoptosis. *Bone* 38:845-852.

Theilade E, Wright WH, Jensen SB, Löe H (1966). Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *J Periodontal Res* 1:1-13.

Troen BR (2003). Molecular mechanisms underlying osteoclast formation and activation. *Exp Gerontol* 38:605-614.

Tsuboi M, Kawakami A, Nakashima T, Matsuoka N, Urayama S, Kawabe Y*, et al*. (1999). Tumor necrosis factor-alpha and interleukin-1beta increase the Fas-mediated apoptosis of human osteoblasts. *J Lab Clin Med* 134:222-231.

Van Dyke TE (2008). The management of inflammation in periodontal disease. *J Periodontol* 79(8 Suppl):S1601-S1608.

Walsh NC, Reinwald S, Manning CA, Condon KW, Iwata K, Burr DB*, et al*. (2009). Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *J Bone Miner Res* 24:1572-1585.

Wang GL, Fu YC, Xu WC, Feng YQ, Fang SR, Zhou XH (2009). Resveratrol inhibits the expression of SREBP1 in cell model of steatosis via Sirt1-FOXO1 signaling pathway. *Biochem Biophys Res Commun* 380:644-649.

Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL (2005). IL-1 mediates TNF-induced osteoclastogenesis. *J Clin Invest* 115:282-290.

Weiner GS, DeMarco TJ, Bissada NF (1979). Long term effect of systemic tetracycline administration on the severity of induced periodontitis in the rat. *J Periodontol* 50:619-623.

Weitzmann MN, Pacifici R (2006). Estrogen regulation of immune cell bone interactions. *Ann NY Acad Sci* 1068:256-274.

Williams R, Jeffcoat M, Kaplan M, Goldhaber P, Johnson H, Wechter W (1985). Flurbiprofen: a potent inhibitor of alveolar bone resorption in beagles. *Science* 227:640-642.

Yamashita K, Eastcott JW, Taubman MA, Smith DJ, Cox DS (1991). Effect of adoptive transfer of cloned *Actinobacillus actinomycetemcomitans*-specific T helper cells on periodontal disease. *Infect Immun* 59:1529-1534.

Yang S, Madyastha P, Ries W, Key LL (2002). Characterization of interferon gamma receptors on osteoclasts: effect of interferon gamma on osteoclastic superoxide generation. *J Cell Biochem* 84:645-654.

Yin MT, Dobkin JF, Grbic JT (2007). Epidemiology, pathogenesis, and management of human immunodeficiency virus infection in patients with periodontal disease. *Periodontol 2000* 44:55-81.

Yu JJ, Ruddy MJ, Wong GC, Sfintescu C, Baker PJ, Smith JB*, et al*. (2007). An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals. *Blood* 109:3794-3802.

Yu X, Graves D (1995). Fibroblasts, mononuclear phagocytes and endothelial cells express MCP-1 in inflamed human gingiva. *J Periodontol* 66:80-88.