Inflammation and Bone Loss in Periodontal Disease

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Inflammation and bone loss are hallmarks of periodontal disease (PD). The question is how the former leads to the latter. Accumulated evidence demonstrates that PD involves bacterially derived factors and antigens that stimulate a local inflammatory reaction and activation of the innate immune system. Proinflammatory molecules and cytokine networks play essential roles in this process. Interleukin-1 and tumor necrosis factor-alpha seem to be primary molecules that, in turn, influence cells in the lesion. Antigen-stimulated lymphocytes (B and T cells) also seem to be important. Eventually, a cascade of events leads to osteoclastogenesis and subsequent bone loss via the receptor activator of nuclear factor-kappa B (RANK)–RANK ligand (RANKL)–osteoprotegerin (OPG) axis. This axis and its regulation are not unique to PD but rather are critical for pathologic lesions involving chronic inflammation. Multiple lines of evidence in models of PD clearly indicate that increases in RANKL mRNA expression and protein production increase the RANKL/OPG ratio and stimulate the differentiation of macrophage precursor cells into osteoclasts. They also stimulate the maturation and survival of the osteoclast, leading to bone loss. OPG mRNA expression and protein production do not generally seem to be increased in the periodontitis lesion. Studies of RANKL and OPG transgenic and knockout animals provide further support for the involvement of these molecules in the tissue loss observed in PD. Ironically, periodontal practitioners have focused on the bacterial etiology of PD and believed that plaque removal was aimed at eliminating specific bacteria or bacterial complexes. However, it seems that the reduction of inflammation and attenuation of the host's immune reaction to the microbial plaque, eventually leading to a decrease in the ratio of RANKL/OPG and a decrease in associated bone loss, are the actual and desired outcomes of periodontal therapy. Future therapeutic options are likely to have regulation of the RANK–RANKL–OPG axis as their goal. *J Periodontol 2008;79:1569-1576.*

KEY WORDS

Bone resorption; inflammation; osteoclasts; periodontal disease.

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Although investigations into the pathogenesis of periodontitis have traditionally centered on the role of bacterial infection, over the past 2 decades there has been increasing interest in the host response factors that drive periodontal disease (PD).[1] It is now understood that the immune and inflammatory responses are critical to the pathogenesis of periodontitis and are shaped by a number of host-related factors, both intrinsic (e.g., genetics) and induced (e.g., pollutants).[1-3]

The initial response to bacterial infection is a local inflammatory reaction that activates the innate immune system.[4,5] Amplification of this initial localized response results in the release of an array of cytokines and other mediators and propagation of inflammation through the gingival tissues.[4,5] The failure to encapsulate this "inflammatory front" within gingival tissue results in expansion of the response adjacent to alveolar bone.[4] The inflammatory process then drives the destruction of connective tissue and alveolar bone that is the cardinal sign of PD.

The recognition that periodontitis involves an inflammatory component as well as altered bone metabolism has provided a new perspective on the etiology of the disease. Investigations[6,7] into the pathogenesis of PD are now considered to fall under the umbrella of "osteoimmunology." This interdisciplinary field of study, which emerged almost a decade ago, integrates the disciplines of immunology and bone biology and has served

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as a useful framework for improving our understanding of PD.[7] The framework has catalyzed continued advances in our knowledge of specific cytokines and other mediators involved in the propagation of the inflammatory response in periodontitis and in further elucidation of the mechanisms underlying bone resorption.[7]

ROLE OF THE "INFLAMMATORY FRONT" IN PERIODONTAL BONE RESORPTION

Whether bone loss will occur in response to an inflammatory reaction is now known to depend on two critical factors.[4] First, the concentration of inflammatory mediators present in gingival tissue must be sufficient to activate pathways leading to bone resorption. Second, the inflammatory mediators must penetrate gingival tissue to reach within a critical distance to alveolar bone.

Achieving critical concentrations of inflammatory mediators that lead to bone resorption depends on the expression of proinflammatory cytokines, such as interleukin (IL)-1, -6, -11, and -17, tumor necrosis factor-alpha (TNF-α), leukemia inhibitory factor, and oncostatin M.[8] The kinins, such as bradykinin and kallidin, and thrombin and various chemokines also have a stimulatory effect on bone resorption.[8] This is the opposite of the expression of anti-inflammatory cytokines and other mediators, such as IL-4, -10, -12, -13, and -18, as well as interferon-beta (IFN-β) and -gamma (IFN-γ), which serve to inhibit bone resorption.[8]

That proinflammatory cytokines are integral to the propagation of the inflammatory response to regions proximal to bone was demonstrated in a study[9] of a *Macaca fascicularis* primate model of experimental periodontitis. In this animal model, *Porphyromonas gingivalis* (*Pg*)-soaked silk ligatures were applied to posterior mandibular teeth to induce experimental periodontitis. Primates received local injections, over a period of 6 weeks, of antagonists to TNF-α and IL-1 (soluble TNF-α and IL-1 receptors) or vehicle control. Analysis of gingival connective tissue sections in close proximity to bone demonstrated significant inflammatory cell recruitment and osteoclast formation surrounding bone in the control primates. Thus, infection with *Pg* in these animals was associated with expansion of the inflammatory front to alveolar bone (Fig. 1B). In contrast, antagonists to cytokines TNF-α and IL-1 reduced the appearance of inflammatory cells in this region and the formation of bone-resorbing osteoclasts (Fig. 1A). Injection of these antagonists reduced recruitment of inflammatory cells by 80%, osteoclast formation by 67%, and bone loss by 60% compared to control sites ($P < 0.01$).[9] These findings suggested that inhibition of the inflammatory mediators can prevent the inflammatory front from reaching alveolar bone, and it was associated with a reduction in bone loss in this animal model.

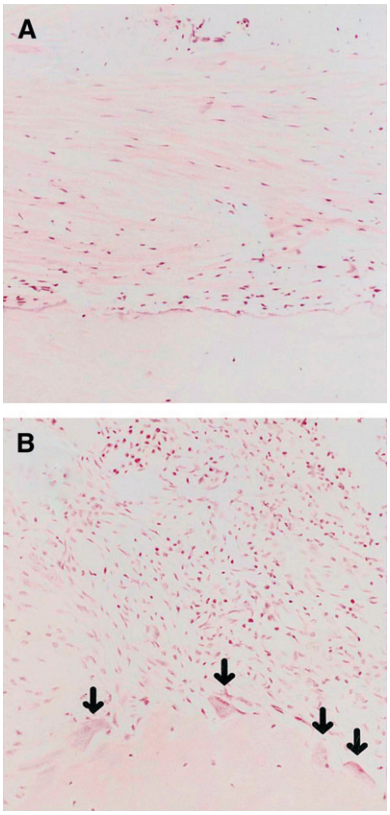

Figure 1.
*Inflammatory response in infiltrate proximal to alveolar bone in primate model of experimental periodontitis receiving soluble antagonists to TNF-α and interleukin-1 (IL-1) **(A)** or vehicle control **(B)** for 6 weeks. Although osteoclasts and inflammatory cells are evident with vehicle treatment alone, there were no osteoclasts present and few inflammatory cells in primates receiving TNF-α/IL-1 blockers. Arrows in B denote osteoclasts on bone surface. (Hematoxylin and eosin; original magnification, ×200.) Copyright 1998 The American Association of Immunologists.*[9]

RANKL IS CRITICAL FOR REGULATING BONE METABOLISM

During an inflammatory response, cytokines, chemokines, and other mediators stimulate periosteal osteoblasts (Fig. 2), altering expression levels of a protein called receptor activator of nuclear factor-kappa B ligand (RANKL) on the osteoblast surface.[8,10] RANKL is expressed by osteoblasts in a membrane-bound protein or cleaved into a soluble form.[11,12] In addition to osteoblasts, RANKL is expressed by a number of other cell types, including fibroblasts and T and B lymphocytes. RANKL is expressed at low levels in fibroblasts; however, its expression is induced in response to cytolethal distending toxin from *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*; *Aa*).[8] Activated T and B

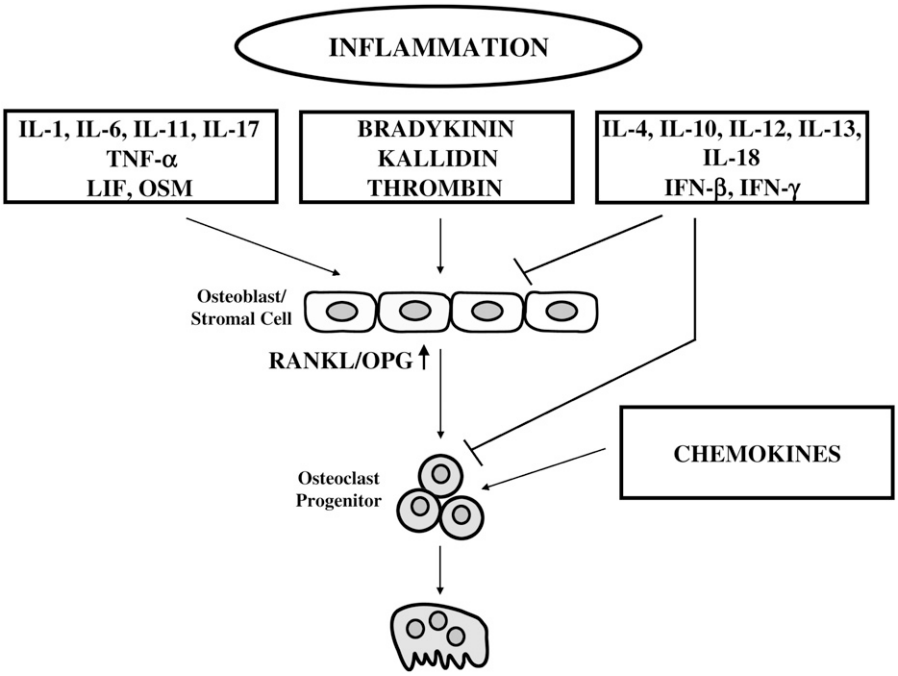

Figure 2.
Stimulation and inhibition of osteoclast formation and bone resorption involves the interplay between a number of inflammatory cytokines and other mediators acting through RANKL binding to RANK on osteoclast progenitor cells. LIF = leukemia inhibitory factor; OSM = oncostatin M. Reprinted with permission from the International and American Associations for Dental Research.[8]

lymphocytes seem to be a particularly abundant source of RANKL in gingival tissues isolated from individuals with periodontitis.[13-15] In one study,[14] $CD4^+$ T cells were the predominant cell type present in periodontitis gingival tissues, and they expressed RANKL more highly than dendritic cells or monocytes. In a similar study,[13] T and B cells were the predominant mononuclear cell types in periodontitis gingival tissues (total mononuclear cells were made up of 45% T cells, 50% B cells, and 5% monocytes) and highly expressed RANKL (>50% of T cells and 90% of B cells expressed RANKL compared to <20% of T and B cells combined in healthy gingiva).[13] However, B cells do not seem to require the presence of T cells to drive bone resorption. In a congenitally athymic rat model of experimental periodontitis injected with donor B cells, RANKL expression and the corresponding induction of osteoclast differentiation was increased in rats receiving B cells from *Aa*-immunized animals compared to non-immune B cells.[15]

Bone resorption and formation are regulated by the relative concentrations of RANKL expressed by various cells, as well as the RANKL receptor RANK on osteoclast precursor cells and the soluble decoy receptor osteoprotegerin (OPG) (Fig. 3).[10] When RANKL expression is enhanced relative to OPG, RANKL is available to bind RANK on osteoclast precursors, tipping the balance to favor activation of osteoclast formation and bone resorption (Fig. 3, left).[10] The binding of RANKL to osteoclast precursors occurs at a stage when hematopoietic stem cells have differentiated from the colony forming unit for granulocytes and macrophages to the colony forming unit for macrophages (CFU-M). Binding of RANKL to RANK on CFU-M in the presence of macrophage colony-stimulating factor induces differentiation of the preosteoclast into a multinucleated cell that becomes a mature osteoclast.[7] The mature osteoclast is a polarized cell that undergoes structural changes to allow it to form a tight junction between the bone surface and basal membrane; it also secretes lytic enzymes into a resorption pit to erode underlying bone.[10]

When OPG concentrations are high relative to RANKL expression, OPG binds RANKL, inhibiting it from binding to RANK (Fig. 3, right).[10] Preventing the binding of RANKL to RANK leads to reduced formation of osteoclasts and apoptosis of preexisting osteoclasts.[10]

THE RANKL–RANK–OPG AXIS

Under normal physiologic conditions, there is a balance between bone resorption and bone formation.[10] This balance promotes bone homeostasis, including the maintenance of structural integrity and calcium metabolism.[6]

In certain inflammatory bone conditions, the balance is altered such that bone formation is enhanced, as in osteopetrosis, or excessive bone resorption occurs, such as that observed in osteoporosis and periodontitis.[8,16] Accordingly, excessive formation of bone may be attributed to an abundance of OPG or reduced expression of RANKL, resulting in a net increase in OPG, also known as a decrease in the RANKL/OPG ratio (Fig. 4). Conversely, a relative decrease in concentrations of OPG or increase in RANKL expression may result in a net increase in RANKL and pathologic bone resorption, also known as an increase in the RANKL/OPG ratio.

During an inflammatory response, proinflammatory cytokines, such as IL-1β, -6, -11, and -17 and TNF-α, can induce osteoclastogenesis by increasing the expression of RANKL while decreasing OPG production in osteoblasts/stromal cells.[12] In contrast,

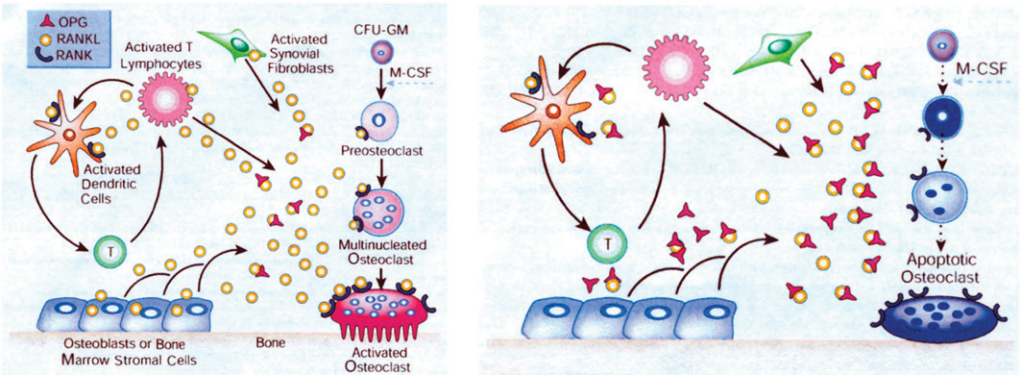

Figure 3.
*Mechanism of action of RANKL expression by various cell types in the induction of osteoclastogenesis following binding to RANK on osteoclast precursors **(left)**. An abundance of OPG relative to RANKL **(right)** inhibits binding of RANKL to RANK, resulting in reduced osteoclastogenesis and the promotion of apoptosis of existing osteoclasts. M-CSF = macrophage colony-stimulating factor; CFU-GM = colony forming unit for granulocytes and macrophages. Reprinted with permission from Macmillan Publishers.*[10]

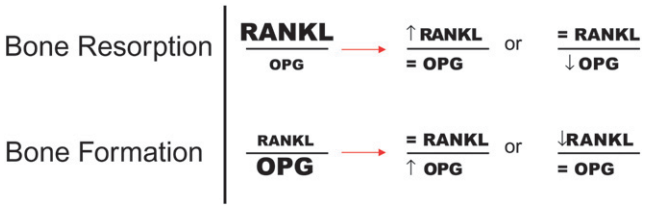

Figure 4.
Whether bone resorption or bone formation occurs depends critically on the RANKL/OPG ratio, which is a function of relative expression levels of RANKL and OPG.

anti-inflammatory mediators, such as IL-13 and IFN-γ, may lower RANKL expression and/or increase OPG expression to inhibit osteoclastogenesis.[12]

How the relative concentrations of RANKL and OPG are altered during the progression of experimental PD was investigated in detail in a study[5] of C57BL/6 mice orally inoculated with *Aa*. Following harvesting of periodontal tissues from maxillary molars, the mRNA expression of inflammatory and regulatory cytokines and other mediators were quantified over a 60-day postinfection period. Inoculation with *Aa* resulted in infiltration of leukocytes within periodontal connective tissue, as indicated by histologic analysis. A corresponding increase in leukocyte count was observed, occurring from postinjection days 0 through 60 (P <0.001). This increase in leukocyte count occurred just prior to the rapid increase in alveolar bone loss observed during the first 30 days postinfection, which began to increase at a slower rate after day 30 (P <0.01). To help explain this loss in alveolar bone that occurred most markedly during the first 30 days postinfection, an analysis of contributing cytokines, as well as levels of matrix metalloproteinases (MMPs) and RANKL (involved in the destruction of connective tissue and bone loss, respectively), was undertaken. An increase in the expression of proinflammatory cytokines occurred early, during the initial 15-day period studied, which corresponded to an increase in leukocyte count and a rapid increase in bone loss. In addition, increases in the concentrations of MMPs and RANKL were observed during this time. However, during days 30 to 60, in which a slower rate of bone loss was observed, the concentrations of proinflammatory cytokines, MMPs, and RANKL decreased. Instead, there was a dramatic increase in the concentrations of anti-inflammatory cytokines (e.g., IL-4 and -10), as well as inhibitors to MMPs and RANKL (e.g., tissue inhibitors of metalloproteinases and OPG, respectively). Thus, the bone loss observed correlated with an expression pattern in which RANKL was increased relative to OPG over the early part of the study period, coincident with a rapid increase in bone loss (days 0 to 15). During the latter part of the study period (days 30 to 60), in which the rate of bone loss slowed, there was a marked decrease in RANKL concentration, whereas OPG concentration was at its highest.

RANKL/OPG RATIO IN ASSESSMENT OF THE CLINICAL SEVERITY OF PD

A number of clinical studies[13,17-21] investigated the concentrations of RANKL and OPG in gingival tissues or crevicular fluid extracted from individuals with periodontitis to determine the RANKL/OPG ratio (Table 1). Some studies[13,17] found an increase in soluble RANKL concentrations without a corresponding change in OPG levels in individuals with chronic periodontitis compared to healthy controls. However, a reciprocal relationship was also found, in which RANKL protein expression was higher and OPG levels were lower in diseased gingival tissues compared to healthy controls.[18] Although the exact concentrations of RANKL and OPG expression varied from study to study, the trend was generally the same; the RANKL/OPG ratio was higher in individuals with periodontitis than in healthy controls.[13,17-21] These findings correspond well with the critical role of RANKL in driving osteoclastogenesis and bone loss in PD.[3]

Table 1.

A Summary of Human Studies Looking at RANKL and OPG in PD

Study	Diagnosis							Total	Conclusion
	Health (subjects [n])	Gingivitis (subjects [n])	Mild Perio (subjects [n])	Moderate Perio (subjects [n])	Chronic Perio (subjects [n])	Generalized Aggressive Severe (subjects [n])	Chronic With Immuno-suppressant (subjects [n])		
Bostanci et al.[20] (crevicular fluid)	21	22			28	25	11	107	GCF RANKL and OPG were oppositely regulated in periodontitis groups.
Bostanci et al.[22] (gingival tissue)	9	8			11	12	10	50	RANKL/OPG ratio increased in all periodontitis groups.
Lu et al.[21] (gingival crevicular fluid and gingiva)	4				20			24	GCP RANKL, but not OPG, was elevated in periodontitis groups.
Mogi et al.[19] (crevicular fluid)	28		27	58		47		160	RANKL/OPG ratio was significantly elevated in periodontitis groups.
Liu et al.[23] (gingival tissue)	6			27		25		58	RANKL/OPG was elevated in periodontitis groups.
Kawai et al.[13] (gingiva and blood)	12				32			44	sRANKL, but not OPG, was significantly higher in periodontitis groups.
Vernal et al.[14] (gingival tissue)	20	7			33			60	RANKL levels were higher in periodontitis groups.
Wara-aswapati et al.[17] (gingiva and plaque)	15				15			30	RANKL/OPG ratio was significantly greater in periodontitis groups.
Garlet et al.[30] (gingival tissue)	10	7			20	16		53	RANKL/OPG and MMP/TIMP expression determined disease progression/severity.
Nagasawa et al.[31] (gingival tissue)	2				30			32	OPG is induced by LPS-stimulated gingival fibroblasts.
TOTAL	127	44	27	85	189	125	21	618	

sRANKL = soluble receptor activator of nuclear factor-kappa B ligand; TIMP = tissue inhibitors of metalloproteinases; LPS = lipopolysaccharides; perio = periodontitis.

Studies[17,18] of RANKL versus OPG concentrations in gingival tissue extracts clearly demonstrated a trend toward a higher RANKL/OPG ratio in individuals with periodontitis than in healthy controls. A semi-quantitative analysis of RANKL and OPG in immunohistochemical preparations found a RANKL/OPG ratio of 3.33:1.89 in severe chronic localized periodontitis compared to 1.8:4.0 in healthy gingiva.[18] Such trends toward a net increase in the RANKL/OPG ratio in PD are observed in gingival tissue as well as in gingival crevicular fluid (GCF). Some studies[19,20] demonstrated that levels of RANKL and OPG in GCF were reciprocally regulated in PD; i.e., an elevation in RANKL protein and a decrease in OPG were observed in GCF of individuals with periodontitis compared to healthy controls. In another study,[21] RANKL concentrations in GCF of individuals with periodontitis were increased compared to controls, whereas the OPG concentration was unchanged. However, these findings still showed a net increase in the RANKL/OPG ratio with periodontitis compared to control samples.

An increased RANKL/OPG ratio also may be associated with the clinical severity of PD. The RANKL/OPG ratio was elevated in GCF from individuals with chronic periodontitis (with or without the coadministration of immunosuppressant therapy) or generalized aggressive periodontitis compared to gingivitis or healthy controls.[20] This trend toward an increased RANKL/OPG ratio with more advanced PD was also found in mRNA extracted from gingival tissue in the same patient population.[22] Similarly, based on mRNA extracted from gingival tissue, the RANKL/OPG ratio was elevated in individuals with moderate periodontitis and advanced periodontitis compared to healthy subjects (RANKL/OPG ratios of 1.01, 1.04, and 0.79, respectively).[23] Nevertheless, although PD is associated with an increased RANKL/OPG ratio compared to healthy controls, the ratio may not necessarily distinguish between mild, moderate, and severe forms. One such study[19] of GCF tissue samples demonstrated an overall increase in ratio with PD compared to healthy controls; however, there was no difference in the ratio between patients with mild, moderate, or severe periodontitis.

With a net increase in the ratio of RANKL/OPG in gingival and crevicular fluids associated with bone loss and maybe with the increasing severity of PD, the possibility that interference with the RANK–RANKL–OPG axis may lead to novel treatments is intriguing. The desired outcome would be an increase in OPG or a decrease in RANKL that brings the RANKL/OPG ratio to a balance where bone formation is equal to bone resorption. Although research into regulation of the RANK–RANKL–OPG axis is still in the early stages, studies[24-29] of the osteoprotegerin fusion protein (OPG-Fc) and other inhibitors of RANK-mediated osteoclastogenesis investigated the effects of interference with the RANK-RANKL-OPG axis on PD bone loss (Table 2). Interference with the RANK–RANKL–OPG axis had a protective effect on osteoclastogenesis and PD bone loss in animal studies.[24,25] Such interference may form the basis for rational drug therapy in PD in the future.

Table 2.

A Summary of Animal Interventional Trials of PD

Study	Method
Jin et al.[25]	Systemic delivery of rhOPG-Fc
Teng et al.[26]	Intraperitoneal injection of srOPG-Fc
Valverde et al.[27]	Subcutaneous kaliotoxin, K^+-channel blocker T cells
Kawai et al.[28]	Intraperitoneal injection of OPG-Fc
Mahamed et al.[29]	Intraperitoneal injection of hu-OPG-Fc
Rogers et al.[32]	Oral gavage of SD282, a p38 MAPK inhibitor
Assuma et al.[9]	Intrapapillary injection of TNF-α and IL-1 antagonists
Li and Amar[33]	Gingival injection of SFRP1 antibody
Vaziri et al.[34]	Local subperiosteal injection of simvastatin
Han et al.[15]	Intrapapillary injection of hOPG-Fc

rhOPG-Fc = human recombinant osteoprotegerin fusion protein; MAPK = mitogen-activated protein kinase; SFRP1 = secreted frizzled-related protein 1; hOPG-Fc = human osteoprotegerin fusion protein; srOPG-Fc = soluble recombinant osteoprotegerin fusion protein; huOPG-Fc = human osteoprotegerin fusion protein; SD282 = an indole 5-carboxamide selective p38* MAPK inhibitor (Scois, Fremont, CA).

CONCLUSIONS

There is increased recognition of the importance of the inflammatory and immune responses in the pathogenesis of PD. An appreciation of the relationship between immune processes and the bone metabolism in various inflammatory bone diseases has given rise to the field of "osteoimmunology." This emerging area has provided welcome perspective and a framework for studying the mechanisms underlying PD. The amplification and propagation of the inflammatory response through gingival tissue is critical to the pathogenesis of periodontitis. However, it is the spread of the response to areas adjacent to alveolar bone that drives the cellular machinery involved in bone loss. The RANKL–RANK–OPG axis clearly is involved in the regulation of bone metabolism in periodontitis,

in which an increase in relative expression of RANKL or a decrease in OPG can tip the balance in favor of osteoclastogenesis and the resorption of alveolar bone that is the hallmark of PD. Interference with the RANKL–RANK–OPG axis may have a protective effect on PD bone loss. Such interference may form the basis for rational drug therapy in PD in the future.

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