Mediators of Periodontal Osseous Destruction and Remodeling: Principles and Implications for Diagnosis and Therapy

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Osteoclastic bone resorption is a prominent feature of periodontal disease. Bone resorption via osteoclasts and bone formation via osteoblasts are coupled, and their dysregulation is associated with numerous diseases of the skeletal system. Recent developments in the area of mediators of osteoclastic differentiation have expanded our knowledge of the process of resorption and set the stage for new diagnostic and therapeutic modalities to treat situations of localized bone loss as in periodontal disease. This review describes the current state of knowledge of osteoclast differentiation and activity, mediators, and biochemical markers of bone resorption and their use and potential use in clinical periodontics. Finally, therapeutic strategies based on knowledge gained in the treatment of metabolic bone diseases and in periodontal clinical trials are discussed, and the potential for future strategies is proposed relative to their biologic basis. The intent is to update the field of periodontics on the current state of pathophysiology of the osteoclastic lesion and outline diagnostic and therapeutic strategies with a rational basis in the underlying biology. J Periodontol 2002;73:1377-1391.

KEY WORDS

Bone remodeling; osteoclasts; biological markers; bone loss/diagnosis; bone loss/therapy; bone resorption/prevention and control.

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Destruction of the osseous support of the dentition is a hallmark of periodontal diseases. This localized bone resorptive process has been the target of therapeutic intervention and preventive strategies; hence, understanding the underlying mechanisms is critical for the effective treatment of periodontitis. Factors that perturb either bone formation or bone resorption will alter the overall quality and quantity of bone. Bone formation and resorption are coupled, and in a healthy adult, are in a state of balance. During development, bone formation is greater than bone resorption, with a resultant increase in bone mass. Optimal periodontal regenerative procedures recapitulate this process. Inhibiting the mediators of osteoclast activation can also result in an increase in bone mass. Consequently, it is important to consider the mechanisms of action of factors that stimulate resorption, their clinical identification, and their control. Furthermore, agents that are currently in use to diagnose and treat conditions of systemic bone loss, such as osteoporosis, may prove valuable to also target the periodontium. This review will address basic principles and current concepts in bone remodeling, mediators of bone resorption and their clinical relevance, and diagnostic and therapeutic potentials to treat bone resorption specifically associated with periodontitis.

BONE REMODELING: BASIC PRINCIPLES AND CURRENT CONCEPTS

It has long been accepted that bone formation and bone resorption are processes that are "coupled," although periodically there is evidence suggesting they can act independently.[1] This coupling process entails that osteoclasts resorb an area of bone, and osteoblasts are signaled to come in and replace bone (Fig. 1). The resorptive phase occurs over a 3- to 4-week period, whereas the formative phase occurs over a 3- to 4-month period for one unit called a basic multicellular unit (BMU) that includes osteoblasts and osteoclasts.[2] Between the resorptive and formation phases is a period termed the reversal phase. During the reversal phase, cells that appear morphologically inactive line the resorption lacunae.

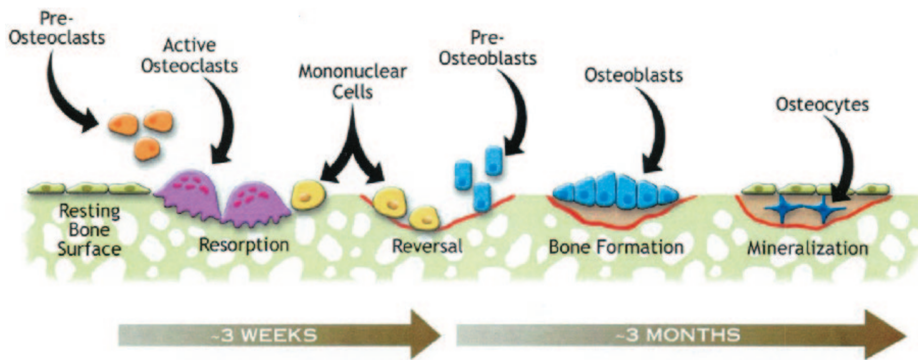

Figure 1.
The bone remodeling cycle. Preosteoclasts are recruited to sites of resorption, induced to differentiate into active osteoclasts, and form resorption pits. After their period of active resorption, they are replaced by transient mononuclear cells. Through the process of coupling, preosteoblasts are recruited, differentiate into active matrix secreting cells, and form bone. Some osteoblasts become entrapped in the matrix and become osteocytes.

It is estimated that the adult skeleton contains more than 1 million BMUs at any time, with nearly 5-fold more occurring in trabecular bone versus cortical bone.[3] The net result is an exchange of 10% of the skeleton over a 1-year period. It follows that trabecular bone is more susceptible to conditions of turnover. This is an important concept when considering the impact of systemic skeletal diseases on bone of the oral cavity, which has a higher cortical-to-trabecular bone ratio than vertebrae, for example.

Osteoblasts, the cells responsible for bone formation in the BMU, are derived from mesenchymal precursor cells and go through a well-defined pattern of differentiation.[4] Current data suggest that osteoblasts arise from progenitors of the marrow and also pericytes, mesenchymal cells adherent to the endothelial layer of vessels.[5,6] Precursor cells differentiate into preosteoblastic cells through the action of bone morphogenetic proteins (BMPs). BMPs are the only factors that have been found capable of initiating osteoblastogenesis from uncommitted progenitors.[7] Other factors such as transforming growth factor-β, platelet-derived growth factor (PDGF), insulin-like growth factors (IGFs), and fibroblast growth factors (FGFs) promote osteoblast proliferation and hence support their use in periodontal regenerative strategies.[8] Once the osteoblast matures into an active matrix-producing cell, it expresses many of the phenotypic characteristics that are used to identify it such as osteocalcin, the PTH/PTHrP receptor, and bone sialoprotein.[4] The lifespan of an osteoblast has been estimated to be 3 months. The osteoblast subsequently becomes an osteocyte entrapped in the bone matrix, dies via apoptosis, or becomes a lining cell.[2] Interestingly, periodontal pathogens, specifically *Actinobacillus actinomycetemcomitans,* have been shown to stimulate apoptosis of osteoblastic cells.[9]

Osteoclasts are cells derived from the hematopoietic lineage and gain access to sites of resorption via the blood supply. They are identified by their expression of an osteoclastic enzyme, tartrate-resistant acid phosphatase (TRAP) and by typically having 3 or more nuclei per cell. Other characteristic morphologic features of osteoclasts are the ruffled membrane and clear zone that assure the resorptive process remains localized beneath the osteoclast, and the proton pump that regulates hydrogen ion concentration and thus pH in the bone resorptive microenvironment. It has long been known that the development of osteoclasts depends on the presence of accessory cells, most notably marrow stromal cells or osteoblasts.[10] Interestingly, more recently, lymphocytes have also been found to support osteoclast generation.[11] There are 2 molecules considered essential and sufficient to support osteoclastogenesis: macrophage colony-stimulating factor (M-CSF, or CSF-1) and receptor activator of nuclear factor kappa B ligand (RANKL) (Fig. 2). M-CSF is a secreted factor, but the cell surface form of RANKL requires a juxtacrine (cell to cell) interaction. Other transcription factors and enzymes associated with osteoclast development include PU.1, c-src, c-fms, carbonic anhydrase II, matrix metalloproteinase 9, and cathepsin K.[10] Many of these have been the focus of therapeutic strategies to target systemic bone loss.[12] Recently, a new member of the leukocyte receptor complex (LRC)-encoded family was identified and found to be expressed exclusively in osteoclasts.[13] Named OSCAR for osteoclast-associated receptor, it is expressed exclusively in osteoclasts late in differentiation and, hence, is thought to play a regulatory role in the differentiation of osteoclasts via interaction with a ligand on osteoblasts/stromal cells.

The process of bone resorption is initiated with a resorptive stimulus (Table 1).[12,14] These stimulators typically affect bone resorption through the activation of M-CSF or RANKL,[11] although in pathologic states,

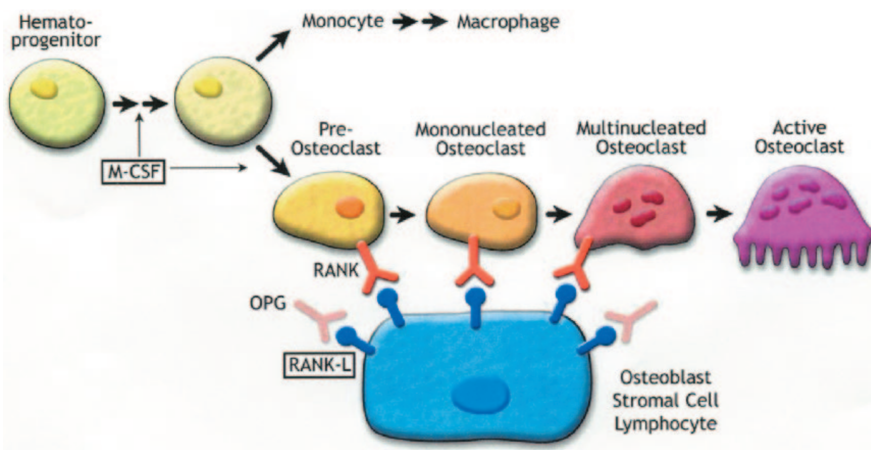

Figure 2.
Osteoclast differentiation pathway. Cells of the hematopoietic lineage are induced to become osteoclasts through the action of M-CSF and RANKL. RANKL is a cell surface protein on stromal cells (osteoblasts and lymphocytes also have RANKL) that is critical for differentiation and activation of osteoclasts. OPG can effectively block the action of RANKL by acting as a decoy receptor.

Table 1.
Mediators of Bone Resorption

Stimulators	Inhibitors
Interleukin-1 (IL-1)	Interferon gamma (IFN-γ)
Interleukin-6 (IL-6)	Osteoprotegerin (OPG)
Tumor necrosis factor (TNF)	Estrogens
Parathyroid hormone (PTH)	Androgens
PTH-related protein (PTHrP)	Calcitonin (CT)
Prostaglandin E_2 (PGE_2)	Cyclosporin
Macrophage colony-stimulating factor (M-CSF)	
Receptor activator of NFκB (RANK)	
RANK ligand (RANKL)	
1,25 dihydroxyvitamin D_3 (Vitamin D)	

mediators such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1 may act independently of RANKL.[15] A key early event in the bone resorptive process is the attachment of the osteoclast to the bone matrix.[14] This matrix attachment is mediated by integrins, primarily αVβ3, and results in the intimate contact of the osteoclast with the matrix to be resorbed and the formation of the sealing zone that enables the osteoclast to isolate a microenvironment beneath it to facilitate resorption.[16] Once activated, the osteoclast creates the acidic environment beneath it via H^+-ATPase activity of the ruffled membrane proton pump, which is responsible for dissolving the mineral component of the matrix (Fig. 3). Secretion of HCl into the resorptive microenvironment results in a pH of 4.5 necessary for mobilization of the bone mineral.[10] The organic component is degraded by matrix metalloproteinases (MMPs) and cathepsin K, a lysosomal cysteine protease, in addition to several other cathepsins that may be involved.[16] Products of the degradation process are then endocytosed by the osteoclast and transported to the opposite membrane of the cell and released. Osteoclasts likely undergo repeated cycles of resorption in an area and then move to a new site to evoke another cycle of resorption or alternatively undergo apoptosis and, hence, cease resorption. Since the lifespan of an osteoclast is, on average, about 2 weeks, it follows that these cells are intensely active during their tenure, and agents that alter osteoclast apoptosis can dramatically change the bone resorptive process.[2]

Mediators of Bone Resorption

Numerous cytokines have been identified as mediators of bone resorption, and it is not surprising that these are also found as putative mediators in periodontal disease (Table 1). The classic studies, performed by Horton et al., identified an osteoclast activating factor (OAF) that was produced in response to periodontal plaque bacteria.[17] This OAF was later found to be IL-1, one of the most potent bone resorptive agents. IL-1 is secreted in 2 molecular forms, IL-1α and IL-1β, by a variety of cells including macrophages, B cells, neutrophils, fibroblasts, and epithelial cells, and is involved in the proinflammatory process, matrix degradation, and wound healing.[18] Due to its strong relationship with bone resorption, this cytokine has received considerable attention as an inflammatory cytokine with potential as a marker for active bone loss. Interleukin-1 has also received special attention because a high percentage of the pop-

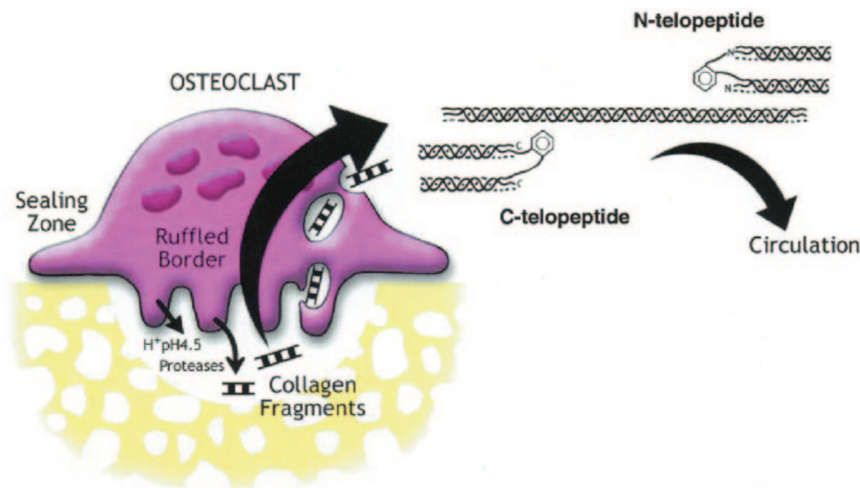

Figure 3.
The resorption process. Osteoclasts adhere to the matrix via the sealing zone and the action of integrins. This seal creates the subcellular microenvironment critical for the resorption process. The proton pump in the ruffled border acidifies the microenvironment through hydrogen ions, and proteases produced by the osteoclast are responsible for degradation of the organic matrix. Breakdown products of collagen degradation are transported through the cell to the external bone environment and can be detected in the circulation.

ulation carries a genetic polymorphism in the IL-1 gene that results in increased levels upon bacterial challenge.[19] These increased levels have been associated with increased periodontal disease severity in a population of patients. Interleukin-6 is also an inflammatory cytokine with similar activity to stimulate bone resorption and has been implicated to have a role in several disease states associated with accelerated bone remodeling.[2] Prostaglandin E_2 (PGE_2) and parathyroid hormone-related protein (PTHrP) stimulate bone resorption and have been reported to act indirectly through their activation of RANKL on cells of the osteoblast lineage,[20,21] whereas TNF-α and IL-1α are thought to act directly on cells of the osteoclast lineage to stimulate bone resorption.[15] Systemically, vitamin D_3 and parathyroid hormone (PTH) are considered the 2 main mediators of bone resorption; however, recent interest has centered on the anabolic actions of PTH, as it is a promising therapeutic agent for the treatment of osteoporosis.[22]

Recently, a family of osteoclast mediators involved in the TNF signaling pathway was identified that may have a striking impact on the treatment of clinical disorders such as periodontal disease. As indicated above, RANKL (also known as osteoprotegerin-ligand, OPG-L, or TRANCE) is a cell surface protein present on osteoblastic cells and is responsible for osteoclast differentiation and bone resorption.[11,23] RANKL interacts with its receptor, RANK, on hematopoietic cells, an interaction thought to be essential for osteoclast activation (Fig. 2). In the absence of RANKL, osteoclastic resorption is reduced, and increases in bone mass occur.[24]

There are relatively fewer inhibitors of bone resorption than stimulators of resorption (Table 1). A soluble factor, osteoprotegerin (OPG), binds to RANKL and inhibits the differentiation of osteoclasts.[25] Hence, injection of OPG in animals and humans potently inhibits bone resorption, and its use results in an increase in bone mass. OPG is being explored in human clinical trials for the treatment of osteoporosis, metastatic bone disease, and also in animal models of rheumatoid arthritis and periodontal disease.[11,26] Interferon γ is a cytokine produced by activated T lymphocytes that inhibits bone resorption by inhibiting the differentiation of committed precursors to mature cells.[27] Systemically, calcitonin is a potent inhibitor of bone resorption but, in pharmacologic use, has limited application since patients become refractory to its inhibitory action.[28] Estrogen is another systemic hormone that inhibits bone resorption as evidenced by the increase in osteoporosis that ensues with estrogen deficiency after menopause. Although its mechanisms of action are not entirely clear, it is thought to promote the programmed cell death of osteoclasts and hence reduce their period of activity.[12,28] Pharmacologic agents that inhibit bone resorption include cyclosporin, at least in part associated with its anti-inflammatory properties, and bisphosphonates, which also stimulate osteoclast apoptosis.[29,30]

Systemic Biochemical Markers of Bone Resorption

Considerable progress has been made in the identification and development of systemic biochemical markers of bone resorption; however, their utility is still not widely accepted. The 5 most intensively investigated factors are hydroxyproline; hydroxylysine glycosides; pyridinium cross-links and related collagen fragments (telopeptides); tartrate-resistant acid phosphatase (TRAP); and bone sialoprotein (BSP).[31]

The bone matrix is composed of 70% mineral and 30% organic components, the greatest of which is

type I collagen. Type I collagen comprises 90% of the organic matrix of bone; hence, during bone resorption, breakdown products of collagen are released into the circulation and may be detected in the serum and/or urine.

Hydroxyproline (OHPr) is an amino acid that occurs mainly in fibrillar collagens and accounts for 13% to 14% of the total amino acid content.[31] Because it occurs almost exclusively in collagen, its urinary levels have long been considered to reflect collagen turnover in bone and other tissues. Its drawbacks include the findings that 10% of the OHPr found in the urine represents new collagen synthesis and not collagen breakdown, and that it is found in many collagens which are not found in bone; therefore, its detection may not be specific for bone. Hydroxylysine is also unique to collagen, and its glycosylation to galactosyl hydroxylysine (GH) is associated more frequently with type I collagen of bone versus type I collagen, for example, of skin.[31] Hence, measurements of GH are thought to reflect mainly breakdown of bone collagen. Collagen pyridinium cross-links, pyridinoline or hydroxylysylpyridinoline (PYD), and deoxypyridinoline (DPD) are currently considered the most promising markers of systemic bone resorption.[31,32] PYD and DPD are derived from hydroxylysine and lysine residues in the collagen molecule. These cross-links function to covalently link collagen molecules between 2 telopeptides and a triple helical sequence and provide stability to the collagen fibrils (Fig. 3). The main sites of collagen cross-linking are the short non-helical peptides at both ends of the collagen molecule (N- and C-terminal telopeptides). These telopeptides are linked via pyridinium or pyrrole compounds to the helical portion of neighboring collagen molecules and can be detected during collagen breakdown. DPD is more concentrated in bone than PYD, and both are absent from the collagen in normal skin. During the bone resorptive process, pyridinoline cross-links are released and can be detected in the urine as free amino acid derivatives and oligopeptide-bound fractions. Several immunoassays utilizing antibodies against the free amino acids or the peptides containing the cross-link components (N- and C-terminal telopeptides) in the serum or urine are being used to monitor systemic therapy in osteoporosis.[33]

Tartrate-resistant acid phosphatase is an enzyme that is generally specific to osteoclasts, although not exclusively.[14] Immunoassays have been developed to measure TRAP in serum as a reflection of osteoclastic activity, but only limited clinical applications are available.[31] Another resorptive marker, bone sialoprotein (BSP), is an extracellular matrix, glycosylated non-collagenous protein found in high levels at areas of active mineralization. Interestingly, although BSP is expressed in highest levels by active osteoblasts, its presence in serum reflects bone resorption rather than osteoblast activity.[31]

DIAGNOSTIC POTENTIALS FOR PERIODONTAL BONE RESORPTION

Traditional periodontal diagnosis involves measures of probing depth, gingival recession, and calculated probing attachment level using a graduated periodontal probe. These are indirect measures of bone loss. A number of factors such as the presence of inflammatory changes or long junctional epithelium or the pressure applied to the probe can affect periodontal probing, leading to inaccuracies in recording the true pocket depth.[34] The height of the alveolar bone margin and the shape and form of its outline are also examined with radiographs. Unfortunately, the sensitivity of radiographs in detecting an early osseous lesion is poor.[35] Many months to years may be needed to detect measurable changes in bone density, whereas biochemical markers can detect changes in a short time. More sophisticated techniques such as subtraction radiography and computer-assisted image analysis have been used as research tools to detect small changes in bone mass, but at present, these have not found a place in routine clinical practice.[36]

Gingival crevicular fluid (GCF), an exudate that can be harvested non-invasively from the gingival sulcus or periodontal pocket, contains a rich array of cellular and biochemical mediators that reflect the metabolic status of periodontal tissues.[37,38] As GCF transverses the inflamed tissue, it carries molecules involved in the destructive process, and therefore offers great potential as a source for factors that may be associated with osteoclastic activity with the potential of being detected in advance of irreversible bone loss. Further, in contrast to biochemical markers identified in metabolic bone diseases as discussed above, GCF has the benefit of being closely approximated to the site of destruction and thus may provide more information than markers in the serum or urine. Several materials have been analyzed in crevicular fluid including plasma proteins, enzymes with collagenolytic activity, other microbial and host cell enzymes, and inflammatory mediators in attempts to identify factors to facilitate the diagnosis of active periodontal disease.[38,39] Furthermore, similarities in components of GCF from implant sites versus natural

teeth suggest that components reflecting tissue breakdown are likely derived from the alveolar bone, since a periodontal ligament source is not present in implant sites. For convenience, GCF products can be grouped into 3 general categories: 1) inflammatory mediators and products; 2) host-derived enzymes; and 3) extracellular matrix components.

Inflammatory Mediators and Products

Cytokines. Cytokines, specifically interleukin-1α (IL-1α), IL-1β, IL-6, and TNF-α have been found in GCF in relation to the progression of periodontal destruction.[40-42] IL-1 has been the focus of numerous studies because it has such potent bone resorbing activity and is an inflammatory mediator with an obvious role in periodontal tissue destruction. Both the IL-1α and IL-1β forms have been detected in gingival crevicular fluid, and IL-1β concentrations increase significantly during episodes of periodontal inflammation.[40] In a cross-sectional study, levels of IL-1β were increased at periodontitis sites compared to gingivitis and healthy sites.[43] IL-1β also has synergistic activity with other potent bone resorbing molecules such as TNF-α or lymphotoxin in stimulating bone resorption.[44] After periodontal therapy, IL-1β levels decrease significantly.[45] Alveolar bone loss in sites of active periodontal disease is associated with increased levels of IL-1β in GCF.[46,47] Furthermore, elevated levels of IL-1β and IL-6 are associated with periodontal pathogens such as *Eikenella corrodens* and *Prevotella intermedia* and continuous loss of attachment and alveolar bone loss in active sites. This suggests that these cytokines might predict and be associated with progressive attachment loss.

There is preliminary evidence that GCF from sites with progressing periodontitis contains elevated amounts of IL-6.[48,49] IL-6 appears to be elevated in GCF and biopsy specimens from sites that do not favorably respond to phase I therapy.[49] Furthermore, increased levels of bone resorbing cytokines in GCF are related to low systemic levels of estrogen.[50]

Prostaglandin E_2. Several cross-sectional studies have shown that GCF from sites with periodontitis contains significantly increased levels of PGE_2 compared to healthy sites and those with gingivitis.[51-54] Offenbacher et al. indicated that the diagnostic potential of PGE_2 increased 2- to 3-fold in inflammation and 5- to 6-fold during periods of active attachment loss and bone resorption.[51] A ligature-induced experimental periodontitis study in animals indicated that GCF levels of PGE_2 are markedly elevated during the development of attachment loss and bone resorption.[52] Total amounts and concentrations of PGE_2 are significantly higher in periodontitis as compared to healthy and gingivitis sites and are positively correlated with probing depth and gingival index.[53] Studies suggest that this marker has considerable potential as a diagnostic marker of active disease and is predictive of future bone loss.[51,52,54] Unfortunately, the currently available test required to detect PGE_2 in gingival crevicular fluid is complex and not easy to perform as a chairside diagnostic test.

Host-Derived Enzymes

Alkaline phosphatase. Alkaline phosphatase (ALP) plays a role in bone metabolism and has been used as a marker of the osteoblast-differentiated cell phenotype.[31] ALP is found in many cells of the periodontium including osteoblasts, fibroblasts, and neutrophils. Some forms of the enzyme are also produced by plaque bacteria.[55] Alkaline phosphatase levels in GCF are higher than in serum.[56] In serum, the enzyme is associated with systemic bone disease, and its elevation in GCF could well reflect changes of alveolar bone in localized areas. Increased levels of alkaline phosphatase have been noted in experimental gingivitis studies at periodontitis sites and may serve as a predictor of future or current disease activity.[56-58] Cross-sectional data indicate that GCF from sites with gingivitis or periodontitis has significantly higher concentrations of ALP than healthy sites and that it positively and significantly correlates with probing depth, but not bone loss.[56,59] GCF ALP is also elevated in patients with progressing lesions. However, with the current data regarding ALP levels in GCF relative to periodontal destruction, it would seem that its predictive value is low.

Collagenase and related metalloproteinases. Matrix metalloproteinases, such as the collagenases stromelysin and elastase, are found in the tissue or inflammatory exudate in periodontal lesions. These enzymes are attractive candidates as markers of periodontal destruction since they can specifically cleave and degrade collagen and connective tissue matrix macromolecules. Several reports have revealed a positive correlation between collagenase or related metalloproteinases in GCF and the severity of periodontal disease.[60,61] Golub et al. recognized the diagnostic merit of collagenase, since they found the enzyme more positively correlated with probing depth than inflammation.[62] In human periodontitis, GCF collagenase activity has been shown to increase with increasing severity of gingival inflammation and increasing probing depth and alveolar bone loss.[63,64]

Cross-sectional studies clearly indicate that GCF from sites with adult or juvenile forms of periodontitis exhibits significantly elevated collagenolytic activities compared to GCF from healthy or gingivitis sites.[64-66]

Collagenase also has been considered as a diagnostic marker of periodontal bone destruction around endosseous dental implants. Levels of collagenase, gelatinase, and elastase around dental implants are similar to natural teeth.[67] Collagenase-2 and collagenase-3 in GCF have been associated with implant sites with progressive bone loss and hence may reflect peri-implant osteolysis.[68] Future studies are needed to assess disease activity around dental implants with these markers, but the diagnostic sensitivity and specificity values for active collagenase as a predictor of attachment loss are still considered low.

Extracellular Matrix Components

Osteonectin and osteopontin. Osteonectin (ON, also known as SPARC) is a non-collagenous calcium-binding protein associated with the extracellular matrix of many tissues, especially bone, and is thought to play a role in remodeling and repair.[69] Osteonectin has been reported to be elevated in GCF at sites with severe periodontitis.[70] However, ON is liberated from many different cell types as a heat shock protein, and studies have shown that heat, a major clinical sign of inflammation, induces their synthesis. Thus, ON in GCF may also relate to inflammation in general and, therefore, it may discriminate poorly between gingival inflammation and bone resorptive disease. Osteopontin (OPN) is a highly glycosylated extracellular matrix protein that is produced by osteoblasts, osteoclasts, and macophages with increased levels in active sites of bone metabolism.[71] Recently, OPN levels were detected in GCF and found to correlate with probing depth,[72] suggesting that it may be a predictor of periodontal attachment loss, but this needs to be confirmed.

Osteocalcin (OCN). Osteocalcin is another extracellular matrix protein that is associated with bone formation.[4] OCN has been identified in gingival crevicular fluid and studied in relation to clinical parameters. No detectable levels of OCN were found in crevicular fluid in a small group of patients with gingivitis, while patients with untreated periodontitis had OCN levels 200 to 500 times higher than in serum.[73] Another cross-sectional study found OCN present in higher levels in diseased sites versus healthy sites, and mean concentrations in GCF were more than 10 times higher than normal serum levels and correlated with clinical parameters such as gingival index, but not probing depth.[53] Giannobile et al. investigated the relation of GCF osteocalcin levels to the progression of ligature-induced experimental periodontitis in beagle dogs and suggested that OCN levels in GCF may serve as a predictor of bone turnover in experimental periodontitis.[74] However, in human studies, no difference in osteocalcin content has been found between active and inactive sites.[75,76]

Cross-linked carboxyterminal telopeptide of type I collagen (ICTP). There is a strong correlation between ICTP levels and deoxypyridinoline levels and clinical parameters of tissue destruction such as radiographic bone level and probing depth.[77,78] Significantly higher GCF ICTP concentrations were found in patients with periodontitis versus healthy patients, with GCF levels 100 times higher than serum reference levels.[77] Furthermore, periodontal treatment reduces the GCF ICTP concentrations to levels found in healthy subjects as early as 2 days after treatment. In contrast, although deep periodontal sites demonstrated higher ICTP levels in humans, a single episode of non-surgical therapy did not significantly reduce ICTP levels.[79] Palys et al. suggested a relationship between selected subgingival pathogens and the C-telopeptide pyridinoline cross-link, and concluded that under appropriate conditions, certain subgingival species may stimulate a host response that can lead to cytokine release, induction of osteoclast activity, and cleavage of pyridinoline cross-links in bone collagen.[80] A longitudinal study in ligature-induced experimental periodontitis in beagle dogs reported that GCF ICTP levels increased significantly 2 weeks following initiation of the disease.[74] This increase preceded the increase in bone-seeking radiopharmaceutical uptake (BSRU) by 2 weeks and the radiographical evidence of bone loss by 4 weeks. The authors concluded that GCF ICTP related positively to indices of active alveolar bone loss in experimental periodontitis and may serve as a marker for future alveolar bone loss. Still, its use clinically will depend on the results of human longitudinal studies.

Oringer et al. reported that GCF ICTP levels and subgingival plaque composition were not significantly different between implants and natural teeth and that elevated ICTP levels were associated with disease progression.[81] Longitudinal studies are required to determine whether elevated ICTP levels may predict the development of peri-implant bone loss.

Glycosaminoglycans (GAGs). The sulcular fluid appears to be rich in metabolic or degradative prod-

ucts of the proteoglycans found in the various periodontal tissues. Some site specificity for various glycosaminoglycans in the periodontium has been noted; the gingival connective tissue is rich in dermatan sulfate, while alveolar bone is rich in chondroitin sulfate.[82] Sulfated glycosaminoglycans in GCF have been associated with clinical situations where altered metabolic activity of the alveolar bone is evident, including early, advanced, and juvenile periodontitis, but not chronic gingivitis, where bone loss is not present.[83,84] This suggests that the presence of raised levels of sulfated GAGs in GCF reflect active destruction of periodontal tissue, likely alveolar bone. Further, the high concentration of chondroitin sulfate in crevicular fluid sampled from sites of active bone resorption has been interpreted to indicate that this GAG originates from the matrix of the alveolar bone.[83] Levels of chondroitin sulfate are significantly higher in diseased patients as compared to healthy patients.[85] Giannobile et al. reported elevated GCF GAG levels in increasing severity of disease and concluded that GAG analysis in GCF may be used to detect early preclinical changes in the periodontal tissues.[86] High levels of chondroitin sulfate are also found in non-inflamed sites undergoing orthodontic tooth movement, which implies an association between chondroitin sulfate in crevicular fluid and bone resorption.[87]

Two major glycosaminoglycan components, hyaluronan and chondroitin-4-sulfate, have been detected in peri-implant sulcular fluid.[88,89] High levels of chondroitin-4-sulfate have been found immediately following exposure and occlusal loading of implants.[90] This suggests that chondroitin-4-sulfate in gingival crevicular fluid may be a marker of bone breakdown, since there would not be a contribution by the periodontal ligament. The peri-implant sulcular fluid GAG profile may also be a useful method of monitoring implants to detect adverse tissue responses at an early stage. Dot blot assays and enzyme-linked immunosorbent assay provide an accurate assessment of the glycosaminoglycan level with the simplicity of a "chairside" application. The potential for chondroitin sulfate to be a useful marker of active bone resorption appears to be promising but requires further detailed investigation.

PHARMACOLOGICAL STRATEGIES FOR TREATING PERIODONTAL BONE LOSS

Pharmacologic strategies designed to treat periodontal bone destruction generally either target the bacteria in the lesion or the host response to the bacteria. Historically, most strategies have focused on the bacteria, whereas pharmacologic strategies that specifically inhibit the formation or activity of osteoclasts have been less intensively utilized. Still, many of these osteoclastic pathway inhibitors have proved valuable for treating systemic diseases associated with bone loss such as osteoporosis and Paget's disease.[12] Targeting the host response via inhibition of bone resorption may be accomplished by altering the differentiation of osteoclasts, the specific components necessary for the process of resorption, or the duration of their activity via reducing their lifespan (Fig. 4).

Anti-Inflammatory Agents

Agents that block cytokine production or activity are the early strategies to inhibit bone resorption. Historically, non-steroidal anti-inflammatory agents (NSAIDs) have provided promising results in slowing periodontal destruction.[91] Adverse reactions, however, have limited their widespread use.[92] Studies of

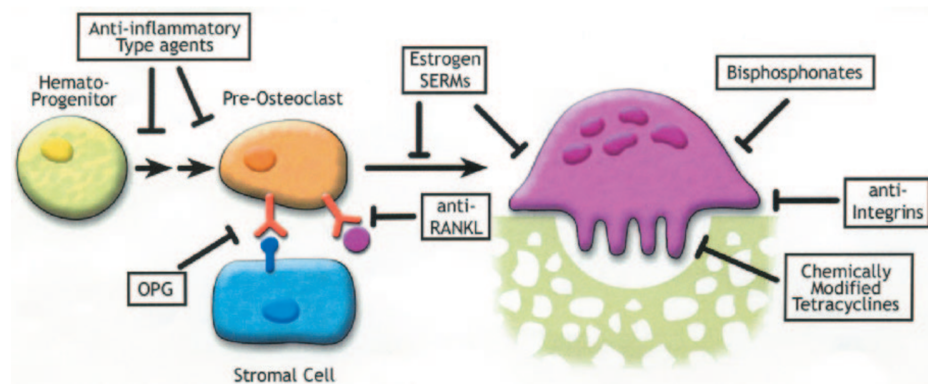

Figure 4.
Potential therapeutic strategies to treat bone resorption. Agents that block the differentiation or activity of osteoclasts are potential therapeutic agents. OPG inhibits the differentiation of osteoclasts through its action as a decoy receptor that blocks RANKL and RANK juxtacrine interaction. Antibodies to RANKL can also block this interaction. Estrogen and SERMs may inhibit the activity of osteoclasts but also promote apoptosis of osteoclasts, thus reducing their active lifespan. Bisphosphonates also promote osteoclast apoptosis. Chemically modified tetracylines reduce the protease degradation of the organic matrix, and anti-integrins block the initial osteoclast adhesion to the matrix.

their systemic use are well known, and recently, the promise of avoiding adverse systemic effects has been addressed by the use of localized application of NSAIDs.[93] Inhibition of cyclooxygenase-2 (COX-2), a mediator of proinflammatory prostaglandin activity, prevented alveolar bone loss in a rat model of experimental periodontitis.[94] The use of selective COX-2 inhibitors in a wide range of conditions from rheumatoid arthritis to cardiovascular disease and cancer has dramatically expanded in the past few years.[92] Furthermore, interesting recent data suggest that specifically blocking IL-1 and TNF dramatically reduces the loss of alveolar bone in a monkey model.[95,96] The soluble antagonists utilized for these studies consist of the extracellular portion of the type I IL-1 receptor that acts as an IL-1 receptor antagonist, and a fusion protein of the extracellular domain of the TNF receptor-2 linked to the Fc domain of human IgG1 that acts as a TNF receptor antagonist. Blocking agents were administered by intrapapillary injection 3 times per week over a 6-week period with significant reductions in radiographic alveolar bone loss. These types of strategies may provide future therapeutic modalities to treat periodontal bone resorption, but many more studies will be required.

Bisphosphonates

Bisphosphonates are preferentially taken up by bone tissue in relatively high concentrations and inhibit osteoclast formation and function.[12] The accumulation of high levels of bisphosphonates in the bone could potentially make them available to the surrounding tissue. Bisphosphonates have proved to be very powerful inhibitors of bone resorption when assessed under a variety of conditions, both in culture and in vivo.[97-99] It is thought that bisphosphonates bind to the bone surface and act directly on osteoclasts to inhibit their resorptive activity and to promote their apoptosis. In addition, there is evidence to suggest that bisphosphonates also affect protein production in osteoblasts.[97] Previous findings have identified a potential role for bisphosphonates by inhibiting periodontal disease in animal models or altering MMP production from human periodontal ligament cells.[98,99] Furthermore, bisphosphonate-complexed implants result in better osteoconduction and repair in animal models, suggesting that the inhibition of bone resorption facilitates bony healing.[100] The inhibitory effect of bisphosphonates on the activity of both MMP-1 and MMP-3 have been shown in cultured periodontal ligament cells.[99] It is suggested that these compounds possibly affect matrix metalloproteinases released from resident cells in the periodontal attachment apparatus, including periodontal ligament. The inhibition of MMP activity at the bone surface may block an initiating step in the bone resorption process.

Several studies using animal models have evaluated the effects of bisphosphonates on the periodontium. In naturally occurring periodontitis in dogs, bisphosphonates had no effect in preventing periodontal attachment loss, but the bisphosphonate-treated group was found to have increased bone above the placebo control.[98] In ligature-induced periodontitis in monkeys, bisphosphonate treatment reduced the amount of attachment loss and prevented loss of bone density.[101,102] In other animal studies, bisphosphonates had little or no effect on preventing attachment loss and only a modest effect on the density of alveolar bone.[103] In a pilot clinical trial, the efficacy of the bisphosphonate drug alendronate in slowing alveolar bone loss due to periodontitis was investigated.[104] Over a 9-month period, alendronate reduced the risk of progressive alveolar bone loss. More recently, short-term results indicated a protective effect of the bisphosphonate alendronate in periodontal patients with type 2 diabetes.[105] The use of bisphosphonates to prevent and/or treat periodontitis must be considered very carefully at this time.

Chemically Modified or Low-Dose Tetracyclines

Tetracyclines, broad-spectrum antibiotics, are used extensively in the management of periodontal disease because of their ability to inhibit bacterial protein synthesis. However, newer applications of tetracyclines have focused on the ability of these agents to block tissue-destructive enzymes, such as the matrix metalloproteinases.[76,106] A group of tetracyclines including chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, and minocycline chelate the cations of metalloproteinases that are required for action. Other non-microbial mechanisms attributed to tetracyclines include inactivation of enzymes that activate metalloproteinases, scavenging reactive oxygen species, blockade of secretion of lysosomal proteinases, and modulation of osteoclast functions, including the induction of osteoclast apoptosis.[107] The mechanism by which tetracyclines inhibit matrix metalloproteinases appears to be independent of their antibacterial activity. In a variety of studies, low-dose tetracyclines have been shown to reduce collagenase activity in gingival tissue extracts and gingival crevicular fluid.[106,109] In a clinical study, neither placebo nor low-dose tetracycline

treatment resulted in significant differences in attachment level compared to baseline values.[108] This study found that patients receiving low-dose doxycycline exhibited a small gain in attachment levels, whereas the placebo group experienced a small loss in attachment. Because of the undesirable effects of long-term tetracycline therapy, chemically modified tetracyclines were developed to eliminate the antimicrobial properties while maintaining activities on matrix metalloproteinases. This modification produces a molecule that has no antimicrobial activity but inhibits collagenase activity and reduces tissue breakdown.[109] Currently, chemically modified tetracyclines appear to have promising therapeutic potential in the treatment of periodontitis.

Estrogens and Selective Estrogen Receptor Modulators (SERMs)

Estrogen deficiency leads to accelerated bone resorption characteristic of postmenopausal osteoporosis. Estrogen withdrawal is associated with large increases in bone resorption, with increased osteoclast numbers due to enhanced osteoclast formation and activity and reduced osteoclast apoptosis. Estrogen replacement therapy has long been considered the first line therapy for preventing osteoporosis in women. Treatment with estrogens clearly inhibits bone loss as well as bone turnover and increases bone mineral density.[12] There is substantial evidence that estrogen inhibits both osteoclast activity and differentiation by regulating production of stimulatory and inhibitory cytokines by osteoblasts and monocytes.[110,111] The molecular mechanism of action of estrogens on bone, as well as other tissues, is not fully understood. The discovery of the agents able to exert full or partial estrogen effects on various tissues led to the development of a new class of drugs known as selective estrogen receptor modulators (SERMs).[12] The mechanism by which SERMs inhibit bone resorption is likely to be the same as estrogen's mechanism, by blocking production of cytokines that promote osteoclast differentiation and by promoting osteoclast apoptosis.

It has been speculated that estrogen deficiency and osteopenia/osteoporosis play a role in the progression of oral bone loss following menopause. Various reports also have linked estrogen deficiency and osteoporosis to increased oral bone resorption, attachment loss, and tooth loss.[112] It also has been demonstrated that in estrogen-deficient postmenopausal women, there is increased loss of interproximal alveolar bone height compared to estrogen-sufficient patients over a 1-year period. Reinhardt et al. found lower IL-1β levels in GCF of estrogen-sufficient early postmenopausal females with periodontitis as compared to estrogen-deficient patients.[113] In another study, the same group reported that estrogen supplementation was associated with reduced gingival inflammation and reduced frequency of clinical attachment loss in osteopenic/osteoporotic women in early menopause.[114] Van Steenberghe et al. compared lumbar spine bone mineral density with mandibular bone mass assessed by standardized intraoral radiographs.[115] A significant but moderate correlation was observed during the 5-year period. In contrast, Nordyred, in a cross-sectional study, reported no difference in clinical attachment level or alveolar bone loss.[116] Estrogen replacement therapy was reported to be associated with less gingival bleeding after correction for age. However, data in the periodontal literature indicate that estrogen supplementation may reduce the rate of clinical attachment loss in osteopenic/osteoporotic women.[114]

Osteoprotegerin (OPG)

There are a few studies in the literature relating OPG and its potential role in periodontal tissues. Shiba et al. investigated the expression of OPG levels in PDL cells and observed no age-related changes in the expression of OPG, although OPG mRNA levels were expected to decrease.[117] Wada et al. reported that conditioned medium from human periodontal ligament cells contained OPG and that periodontal ligament cells synthesized enough bioactive OPG to inhibit osteoclastic differentiation and function.[118] In a recent landmark study, Teng et al. investigated the role of RANKL in alveolar bone destruction during periodontal infection using an animal model.[26] Microbial stimulation by *A. actinomycetemcomitans* induced RANKL expression on the surface of $CD4^+$ cells, and in vivo inhibition of RANKL function with the decoy receptor OPG diminished alveolar bone destruction and reduced the number of periodontal osteoclasts after microbial challenge. These results indicate that the alveolar bone destruction observed in periodontitis is due, at least in part, to the action of osteoclasts and is mediated by RANKL. In other words, *A. actinomycetemcomitans* activates $CD4^+$ T cells in the periodontium and induces them to express RANKL, the key mediator of alveolar bone destruction in microorganism-induced infection. Thus, inhibition of the function of RANKL may have therapeutic value to prevent or interrupt alveolar bone and/or tooth loss in humans.

FUTURE STRATEGIES

The periodontal therapist has a challenge treating bone loss due to periodontal disease. When considering different therapeutics, one needs to keep the goal in view of the presenting periodontal status. Inhibitors of resorption are most effective when administered prior to the time when a patient would be susceptible to bone loss, i.e., as a preventive measure. Since we are as yet unable to accurately predict these periods, we are continually challenged with the decision of appropriate therapeutics. Preventing bone loss in a patient who will not likely experience bone loss has little value. Preventing further bone loss in a patient who has already experienced bone loss is obviously not as valuable as preventing the initial bone loss, but is still a worthy goal. Patients who have already experienced bone loss may be best suited to a regenerative procedure followed by a preventive strategy to reduce their chances of recurrent bone loss. All of these scenarios would benefit from a better understanding of prognostic features of our patients' susceptibility to bone loss. Improved understanding of prognosis will likely arise through better molecular identification of disease susceptibility genes. In the meantime, much can still be learned regarding the preventive strategies to abate bone resorption. Rodan and Martin provide compelling discussion of new targets of pharmacologic modulation of osteoclast formation and activity that may be applicable to the treatment of periodontal disease.[12] Blocking the action of RANKL through the use of OPG was discussed above, but other strategies such as the use of antibodies to RANKL are being explored with promise. Blocking the adhesion of osteoclasts to their target matrix through the use of agents that disrupt integrins has been reported to inhibit bone resorption and may provide viable options after clinical investigation.[119] The utilization of anti-inflammatory agents such as antagonists to IL-1 and TNF-α also must be considered since results from animal studies suggest they may also show promise in humans.[95] Recently, strategies other than tetracyclines for inhibiting MMPs have been described in animal models.[120] Peptides that specifically inhibit MMPs reduce MMP activity and bone loss and may have therapeutic benefit with further study.

We need to continue seeking viable antiresorptives while, at the same time, striving to better identify patients who are appropriate candidates for these types of therapeutics. As these 2 goals converge, we will be in a much better position to effectively intervene in the osseous destruction associated with periodontal diseases.

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