

# Review of osteoimmunology and the host response in endodontic and periodontal lesions

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Both lesions of endodontic origin and periodontal diseases involve the host response to bacteria and the formation of osteolytic lesions. Important for both is the upregulation of inflammatory cytokines that initiate and sustain the inflammatory response. Also important are chemokines that induce recruitment of leukocyte subsets and bone-resorptive factors that are largely produced by recruited inflammatory cells. However, there are differences also. Lesions of endodontic origin pose a particular challenge since that bacteria persist in a protected reservoir that is not readily accessible to the immune defenses. Thus, experiments in which the host response is inhibited in endodontic lesions tend to aggravate the formation of osteolytic lesions. In contrast, bacteria that invade the periodontium appear to be less problematic so that blocking arms of the host response tend to reduce the disease process. Interestingly, both lesions of endodontic origin and periodontitis exhibit inflammation that appears to inhibit bone formation. In periodontitis, the spatial location of the inflammation is likely to be important so that a host response that is restricted to a subepithelial space is associated with gingivitis, while a host response closer to bone is linked to bone resorption and periodontitis. However, the persistence of inflammation is also thought to be important in periodontitis since inflammation present during coupled bone formation may limit the capacity to repair the resorbed bone.

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Periapical lesions of endodontic origin and periodontitis are two common conditions found in the oral cavity that share pathologic mechanisms involving interactions between immune cells and bone. Lesions of endodontic origin are associated with bacterial contamination and necrosis of the dental pulp, which typically progress through four stages: (1) exposure of the dental pulp to the oral cavity with subsequent bacterial colonization, (2) inflammation and necrosis of the dental pulp, (3) the development of inflammation in the periapical area, and (4) periapical resorption of bone and formation of granulomas or cysts. Osteolytic lesions in periodontitis are initiated by bacterial plaque in the gingival sulcus and on the tooth surface. Periodontitis similarly occurs in four stages: (1) bacterial accumulation of a biofilm and presence in the gingival sulcus (colonization), (2) bacterial penetration of epithelium and connective tissue in the gingiva adjacent to tooth surface (invasion), (3) stimulation of a host response that involves activation of the acquired and innate immune response

(inflammation), and (4) destruction of connective tissue attachment to the tooth surface and bone that is irreversible (irreversible tissue loss). Both oral diseases demonstrate similar patterns of development, bone resorption associated with bacteria that adhere to and invade soft tissue stimulating an inflammatory response and subsequent osteoclastogenesis.

## Lesions of endodontic origin

Endodontic lesions typically develop from exposure of the pulpal tissue to oral bacteria as a result of deficiencies in the integrity of a tooth. This may result from carious lesions that dissolve the mineralized dental tissue, fractures of the tooth structure, as well as iatrogenic and other circumstances that allow bacteria to penetrate into the pulpal tissues. In most cases, these events lead to infection within the dental pulp, which causes the development of inflammation that spreads from the exposed area. The inflammation is often followed by pulpal tissue necrosis, leading to chronic infection, the

spread of inflammation to the tooth apex, and bone resorption. The inflammatory response involves the recruitment and activation of leukocytes of both the innate and adaptive immune responses, with resultant osteoclastogenesis and formation of an osteolytic lesion at the apex of the tooth.

Inflammation and resorption of bone at the tooth apex, in most cases, is a consequence of the interaction between microbial infection and the host response. The critical role of bacteria in the development of periapical lesions has been demonstrated by mechanical exposure of the dental pulp to the oral cavity in germ-free animals. In these germ-free animals, pulp exposure heals with an initial or transitory inflammatory response within the pulpal tissue, followed by a reparative response from pulpal cells, and leading to the formation of a new dentin-like matrix bridging the exposed site. In contrast, mechanical pulp exposure in animals with normal oral bacteria causes an infection of the dental pulp, with pulpal tissue necrosis and chronic infection that prevents the repair process (1). The infection persists as the necrotic tissue of the dental pulp is inaccessible to leukocytes and, hence, represents a protected reservoir of bacteria (2, 3). The chronic inflammation stimulated by bacteria and their products in the periapical area of the tooth leads to localized bone resorption that is 'uncoupled' so there is no reparative bone formation without treatment. The result is formation and expansion of granulomas or cysts in the apical tissues (4).

Understanding the pathogenic mechanisms underlying the development of endodontic lesions is confounded by the persistence of a 'bacterial reservoir' that exists in the pulp canal and necrotic tissue. The bacterial presence stimulates an inflammatory response to resist infection. During this response a number of cell types present release cytokines, chemokines, leukotrienes, and prostaglandins into the area. These inflammatory mediators reinforce the recruitment of polymorphonuclear leukocytes (PMNs) and other leukocytes, creating an interesting dichotomy of activity and consequences as to the essential protective or destructive roles mediated (3). As would be expected, the host response plays a critical and protective role in lesions of endodontic origin in limiting the spread of infection into the fascial planes. Consistent with this expectation, specific inhibitors of inflammatory cytokines tend to cause the formation of larger osteolytic lesions since they compromise the ability of the host to protect itself from the reservoir of bacteria in the necrotic pulp. This increase in lesion dimensions occurs even though the blocked inflammatory cytokines also play an important role in osteoclastogenesis. The use of inhibitors or mice with targeted genetic deletions may not necessarily reveal the role of a particular cytokine or cell type that plays an important role in activating osteoclastogenesis since its inhibition or knockout may also increase

susceptibility to bacterial infection. If the impact on resistant infection is greater, the larger lesion will be produced even though direct effect on deletion or inhibition should reduce osteoclast formation. The reverse is also true. For example, an enhanced host response in an animal model for periapical endodontic lesions demonstrates increased numbers of PMNs and monocytes with a reduction in the extent of apical bone resorption, even though the host response may contribute to the bone resorption (5). In another example, the deletion of tumor necrosis factor (TNF) or IL-1 receptor signaling causes larger osteoclast lesion formation even though both cytokines stimulate bone resorption. This occurs because deletion of TNF or IL-1 signaling impairs the antibacterial activity of the host response that is critical in lesions of endodontic origin (6). In particular, IL-1 receptor signaling is needed to prevent the spread of infection from necrotic pulp into fascial planes and to protect the host from significant morbidity and mortality that would result (6). Thus, there is considerable complexity in examining the impact of cytokine signaling since cytokines have both destructive roles as well as an important protective function in antibacterial defense (6).

The control of the periapical infection seems to be a critical aspect of this process, since the absence of the pleiotropic enzyme inducible nitric oxide synthase (iNOS) also results in larger lesions with the recruitment of a greater number of inflammatory cells and frequently associated with periapical abscesses development (7). This contrasts with periodontal disease, in which a protected bacterial reservoir does not exist and the use of inhibitors or mice with targeted deletions of the host response typically do not compromise the antibacterial defenses sufficiently to complicate the analysis. Thus, lesions of endodontic origin appear to be at an increased susceptibility to bacterial infection with inhibition of the host response in contrast to periodontal disease.

### *Leukocytes and endodontic lesions*

The initiation of an inflammatory cascade in lesions of endodontic origin includes the complex interplay of multiple cell types involving the activation of endothelial cells, PMNs, macrophages, lymphocytes, and osteoclasts leading to rapid bone destruction. The complex host response involves cells of both the innate and adaptive immune response. The rapid destruction of bone found with endodontic lesions is initiated by multiple bacteria or their products including lipopolysaccharides (LPSs) (3). Bacteria are thought to stimulate resorption through the induction of proinflammatory cytokines such as IL-1 $\beta$ , IL-1 $\alpha$ , receptor activator of nuclear factor kappa-B ligand (RANKL), or TNF- $\alpha$  (8, 9). The initial activation of the host response occurs through stimulation of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) receptors (10). Both TLRs and

NODs are highly expressed on multiple cell types associated with the endodontic lesions including monocytes/macrophages, granulocytes, pulp fibroblasts, osteoclast precursors, and mesenchymal cells (10, 11). Activation of these receptors leads to the stimulation of multiple proinflammatory cytokines, including IL-1, TNF- $\alpha$ , and IL-6, and has been associated with enhanced RANKL production, osteoclastogenesis, and bone resorption (10, 11).

Multiple studies have reinforced the concept that the development of bone resorption in lesions of endodontic origin involves the adaptive immune response. The predominant cell types in endodontic lesions in a rat model were shown to be T-cells followed by B-cells and monocytes/macrophages (12). Multiple T-cell responses have been associated with endodontic lesions, including Th1 (IL-2 and IFN- $\gamma$ ), Th2 (IL-4 and IL-5), T-regulatory cells (Tregs; IL-10 and TGF- $\beta$ ), and Th17 (IL-17A) lymphocytes (13–15). In fact, the key transcription factors essential for Th1, Th2, and Tregs differentiation, T-bet, GATA-3, and FOXP3, respectively, have been found in periapical lesions (14) as well as IL-17A, the prototypical cytokine produced by Th17 cells (13).

The importance of the adaptive immune response in protecting the host during formation of endodontic lesions has been demonstrated in numerous studies. The exposure of the dental pulp in severe combined immunodeficient (SCID) mice showed periapical lesions of similar size to that found in normal control mice (16). However, approximately one-third of the immunodeficient mice with endodontic lesions developed orofacial abscesses. Interestingly, two studies identified contrasting results utilizing *nulnu* rats with a deficient T-cell response. While one study showed greater bone resorption following endodontic infections, suggesting a critical protective role, the other study failed to identify a difference in the amount of bone resorption (17, 18). Evidence of a protective role for IFN- $\gamma$ , the prototypical Th1-cytokine, was demonstrated as the absence of IFN- $\gamma$  resulted in increased bone resorption compared to wild-type mice (19). Emerging evidence suggests that the majority of Th17 cells also express IFN- $\gamma$ , supporting a role for both Th1 and Th17 proinflammatory responses in the pathogenesis of periapical periodontitis (13). An examination of the Th2 response with genetic deletion of IL-4 failed to identify an effect, suggesting more complex redundancies or that Th2 responses are not critical in protection or bone resorption (19). However, the anti-inflammatory cytokine IL-10 has been demonstrated to be a protective factor against periapical bone resorption. Periapical lesions in mice with genetic ablation of IL-10 were increased in size compared with wild-type mice, consistent with a protective role for IL-10 (19). Furthermore, IL-10 mRNA levels in human periapical granulomas have been positively correlated with the expression of proteins,

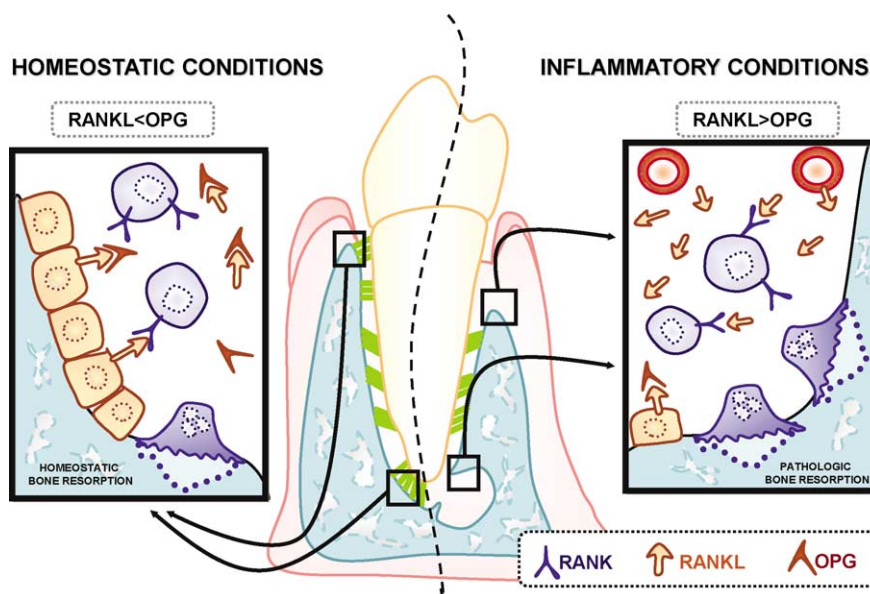
SOCS1 and SOCS3, which act as negative regulators of the inflammatory signaling (20). Interestingly, Tregs, as a potential source of IL-10, were found in the periapical lesions following endodontic infection consistent with a regulatory role in lesion development (15, 21).

### Cytokines

The initial rapid destruction of bone in the apical area of the root has been associated with the production of prostaglandins, in particular PGE<sub>2</sub>, through the cyclooxygenase pathway (22). These findings provide clarification to an earlier report that indomethacin reduces the extent of bone resorption in endodontic lesions (23). Endodontic lesions have been associated with multiple proinflammatory cytokines and chemokines. Cytokines that participate in the formation of osteolytic lesions are shown in Figs. 1 and 2. Interleukins (IL), particularly IL-1 $\alpha$  and IL- $\beta$  are produced in periapical lesions by several types of cells including macrophages, osteoclasts, PMNs, and fibroblasts (24, 25). The role for IL-1 in stimulating periapical bone destruction was demonstrated using interleukin-1 receptor antagonists to show a 60% reduction in lesion development (26). It appears that much of the induced osteoclastogenic activity in periapical lesions is specifically related to the formation of interleukin-1 $\alpha$  (27). However, when IL-1 receptor signaling is completely deleted there is increased lesion size and systemic morbidity (5). In addition, TNF- $\alpha$  expression has been identified in lesions of endodontic origin by cells such as PMNs, monocytes/macrophages, and fibroblasts and may contribute to lesion formation (3). The IL-6 has been observed in exudates from human periapical lesions, with osteoblasts, fibroblasts, macrophages, PMNs, and T lymphocytes identified as expressing IL-6 protein (28, 29). IL-6 has been shown to play a protective role since endodontic lesions in IL-6 deficient animals are increased in size compared with control mice (30). The role of cytokines in formation of endodontic and periodontal osteolytic lesions is shown in Tables 1 and 2.

Neutrophils are active in the development of bone loss associated with endodontic lesions. This has been demonstrated in animals with some neutropenia having a considerable decrease in periapical lesion formation (31). The recruitment of PMNs with chemokines has also been implicated in the pathogenesis of periapical lesions. IL-8/CXCL8 chemokine expression is prominent in periapical lesions, consistent with heavy infiltration by PMNs (32).

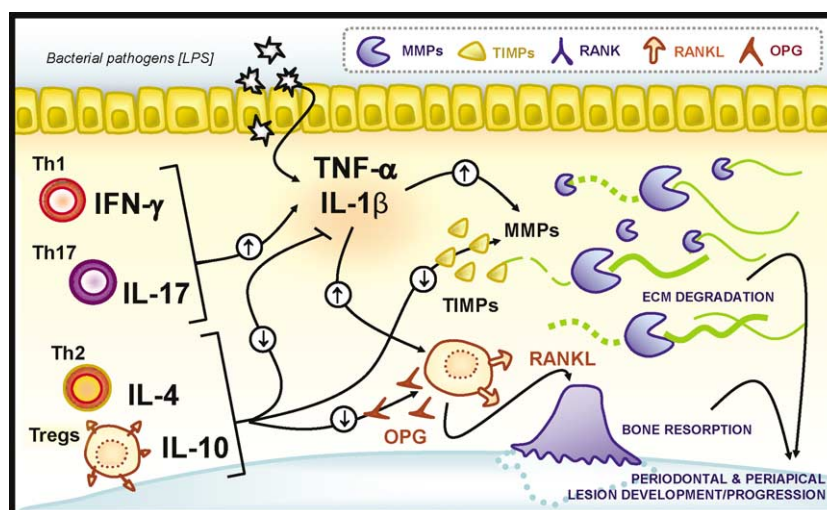
The recruitment of monocytes is critical in the antimicrobial defense in lesions of endodontic origin. Chemokines and chemokine receptors stimulate innate and adaptive immunity in the periapical environment and in the development of granulomas associated with these lesions (33). Genetic deletion of MCP-1/CCL2, identified in monocytes/macrophages and bone lining cells in



**Fig. 1.** RANKL/OPG balance is an important factor in regulating bone resorption in periodontal and periapical environments. Osteoclast differentiation and activation are driven by the interaction of RANK (receptor activator of nuclear factor- $\kappa$ B) with its ligand, RANKL. Osteoprotegerin, OPG, is a decoy receptor of RANKL that inhibits RANK-RANKL engagement. In homeostatic conditions (left side), RANKL and OPG levels are thought to be in balance so that there is limited osteoclastogenesis and bone resorption. With an inflammatory stimulus, the RANKL/OPG ratio increases in periodontal and periapical tissues and leads to stimulation of osteoclast activity and pathologic bone resorption.

endodontic lesions, significantly reduces monocytic infiltrate while increasing the amount of bone resorption (34, 35). Similarly, the absence of the MCP-1 receptor

(CCR2) or genetic ablation of CC chemokine receptor five (CCR5) results in an increased amount of apical bone resorption and is associated with higher levels of the



**Fig. 2.** Cytokine regulation of matrix degradation and bone resorption in periodontal and periapical environments. The presence of microbial pathogens in periodontal and periapical environments trigger an initial production of proinflammatory cytokines, such as TNF- $\alpha$  and IL1 $\beta$ , which stimulate expression and activation of matrix metalloproteinases (MMPs) that degrade extracellular connective tissue matrix. Cytokines such as TNF- $\alpha$  can stimulate osteoclastogenesis independently while other cytokines stimulate RANKL expression that leads to formation of osteoclasts and osteoclast activity. The combined innate and adaptive immune responses are likely to lead to the high levels of inflammation and bone resorption. These proinflammatory cytokines are thought to generate an amplification loop that contributes to periodontal and periapical lesion progression. Conversely, cytokines produced by Th2 cells and Tregs, such as IL-4 and IL-10 have the opposite effect, in part, through stimulating production of tissue inhibitors of matrix metalloproteinases (TIMPs) and OPG as well as restrain inflammatory cytokine production.

**Table 1.** Cytokine effects on bone lesions associated with endodontic or periapical lesions

Cytokine	Effect of deletion or inhibition	Effect of cytokine on osteoclasts	Other effects of cytokine
IL-1 $\alpha$	Reduction in lesion size	Increase in immature osteoclasts and increase resorption by osteoclasts	Increased levels of MMP's 1, 3, 9, and 13
IL-1 $\beta$	Increase in lesion size	Increased levels of immature osteoclasts and increase resorption by osteoclasts	Increased expression of RANK, RANKL, and MMP-9
IL-1 receptor	Increased lesion size		Increased morbidity and mortality from endodontic infection
IL-2	Increase in lesion size	Unknown	Promotes T-cell growth and expansion and CMI
IL-4	No effect on lesion size	Suppresses osteoclast differentiation and osteoclast bone resorption	Promotes differentiation of CD4+ T-cells into T <sub>H</sub> 2s
TNF- $\alpha$	No effect on lesion size	Induces osteoclast differentiation, osteoclastogenesis, and bone resorption	Proinflammatory effects, promotes CMI
TNF receptor	Increased lesion size		Greater osteoclast activity and formation when deleted due to lack of protection
IL-6	Increase in lesion size	Increase in number of osteoclasts and increase resorption	Anti-inflammatory effects, stimulates release of acute phase proteins
IL-/CXCL8	Unknown	Increases osteoclast motility and decreases resorption	Stimulates PMN/monocyte, basophil, and T-cell recruitment
IL-10	Deletion results in increase in lesion size	No effect on osteoclast number, inhibits osteoclastogenesis	Promotes PMN infiltration
MCP-1	Increase in lesion size	Stimulates recruitment of precursors	Stimulates protective monocyte/lymphocyte recruitment
IL-17	Reduced lesion size	Increased levels associated with decreased numbers of osteoclasts	Stimulates monocyte/lymphocyte recruitment
INF- $\gamma$	No effect on lesion size Reduced levels of IL-6, IL-10, IL-12, TNF- $\alpha$	Decreases osteoclastogenesis	Promotes CMI
IL-12	No effect on lesion size	Decreases TNF- $\alpha$ and RANKL induced osteoclastogenesis	Increases IL-18
IL-18	No effect on lesion size	Decreases TNF- $\alpha$ induced osteoclastogenesis	Increases IL-12
Nitric Oxide	Increase in lesion size	Unknown	Induces IL-8, RANK production, macrophage, and osteoblast apoptosis

osteolytic factors, such as RANKL and cathepsin K (19, 36). Interestingly, activation of the MCP-1/CCL2-CCR2 axis appears to play an active role in mediating the migration of monocytes/macrophages while limiting the infiltration of PMNs (36).

The RANKL and osteoprotegerin (OPG) expression demonstrate a heterogeneous pattern in periapical granulomas, ranging from a high ratio of RANKL to OPG consistent with bone resorption to a low ratio seen in sites with minimal bone resorption (37). These disparate findings in levels of RANKL/OPG ratio may be indicative of an expanding lesion with active bone resorption or a stable lesion with minimal bone resorption (37, 38). A description of the role of the RANK-OPG axis in

stimulating osteoclastogenesis and bone resorption is shown in Fig. 1.

### Periodontal diseases

The periodontium is a complex set of tissues that are in close proximity with a complex biofilm harboring diverse and numerous bacterial species (39, 40). Periodontal diseases include gingivitis and periodontitis. While it is a consensus that periodontal diseases are stimulated by bacterial adherence to the tooth surface, there is controversy about which bacteria stimulate the irreversible breakdown of periodontal tissues in periodontitis (40, 41). Recent evidences from studies that do not rely upon bacterial culture techniques suggest that there are

**Table 2.** Comparison of inflammatory component roles in bone resorption

Inflammatory component	Deletion/inhibition effects on bone resorption (lesion size) <sup>a</sup>	
	Endodontic	Periodontal
IL-1	Reduced	Reduced
IL-1 receptor	Increased <sup>b</sup>	Not tested
TNF- $\alpha$	No effect	Reduced
TNF receptor	Increased	Reduced
IL-6	Increased	Reduced
Prostaglandins	Reduced	Reduced
IFN- $\gamma$	Increased	Reduced
PMNs/monocytes	Reduced <sup>b</sup>	Increased <sup>c</sup>
B- and T-cell (SCID mice)	No effect <sup>b</sup>	Reduced
IL-17	No effect	Increased

<sup>a</sup>Increased bone resorption is consistent with protective effects; reduced resorption consistent with a net destructive effect.

<sup>b</sup>With increased morbidity/mortality noted.

<sup>c</sup>Based on clinical findings of immunodeficiencies.

approximately 700 bacterial species in the oral cavity (39). As a general rule, the bacteria that might cause periodontitis have classically been identified as gram-negative anaerobic bacteria that survive in the gingival sulcus, the space between the tooth surface and the adjacent gingival epithelium (42). Much attention has been spent on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, which have been linked to localized aggressive ‘juvenile periodontitis’ and ‘adult periodontitis’, respectively (42, 43). However, recent approaches to bacterial identification have suggested that a reevaluation of pathogenic species is warranted.

The presence of periodontal pathogens is required but not sufficient for disease initiation. In fact, studies clearly demonstrate that cytokines induced by the host response play a critical role in periodontal tissue breakdown (44–47). Host-microbe interactions start in the gingival epithelium and stimulate an inflammatory response that confers efficient protection against bacteria (i.e. the systemic consequences of acute infection are rare). However, the host mediator release results in a clinical outcome represented by the onset of gingivitis. Because the gingival epithelium and underlying connective tissue are chronically exposed to bacteria or their products, both the innate and the acquired immune response are chronically activated in connective tissue adjacent to epithelium lining the gingiva. In most cases, tissue destruction caused by activation of the host response is reversible and associated with gingivitis. On the other hand, under certain conditions that are not fully understood, the disease can progress and cause destruction of the underlying connective tissue attachment of the

gingiva toward the tooth surface and from tooth to bone. Indeed, periodontitis is distinguished from gingivitis by the irreversible nature of the attachment loss. One of the most important uncertainties regarding periodontitis is its chronic nature. Periodontitis may represent a series of brief insults, or ‘bursts’, which accumulate and appear to be chronic over time with extended periods of remission. However, the length of time of the ‘burst’ is unknown. Alternatively, there may be relative constant stimulation over time, but it is not known how long the chronic destructive period lasts in the chronic model. In spite of evidences for both models (48–50), the nature of periodontal disease progression remains uncertain. This problem has plagued human studies since it is difficult to know whether an individual is undergoing active periodontal breakdown at any given point in time. Furthermore, the relative absence of longitudinal studies has made the interpretation of results with human patients difficult since relationships between a given variable and irreversible periodontal breakdown are difficult to establish in cross-section studies.

Animal models have established a clear causal relationship between bacteria and periodontitis. In an animal model, a ligature is tied around the teeth allowing plaque accumulation and bacterial penetration, which leads to subsequent inflammation and alveolar bone resorption (51). In fact, gnotobiotic rats treated identically do not exhibit periodontal bone loss (52), demonstrating the essential role of bacteria in this model. Additional evidence is provided by the treatment with antibiotics or topical application of antimicrobial agents, which reduce bone resorption in the ligature model, while increased colonization by gram negative bacteria enhances bone resorption (51). In other animal models, the inoculation of periodontal pathogens into the oral cavity of rodents induces bone loss. In several studies, the introduction of *P. gingivalis* by oral lavage stimulates alveolar bone resorption (51). Similarly, introduction of *A. actinomycetemcomitans* in rodents leads to colonization and loss of the alveolar bone (43, 51). Thus, experiments with animal models support human studies demonstrating the role of bacteria in inflammation onset and periodontitis.

Since the presence of bacteria is required, but not sufficient to trigger periodontitis development, the recognition of microbial components as ‘danger signals’ by host cells and the subsequent production of inflammatory mediators is an essential step in periodontitis pathogenesis. Indeed, one of the critical components of the host response to bacteria or their products is a family of receptors called the toll-like receptors (TLRs). The TLRs activate the innate immune response binding to various microbial components (i.e. diacyl lipopeptides, peptidoglycan, LPS, flagellin, bacterial DNA, etc.) (53). After TLR activation, an intracellular signaling cascade

leads to the activation of transcription factors, such as nuclear factor- $\kappa$  B (NF- $\kappa$ B), activator protein-1 (AP-1), and subsequent production of various cytokines and chemokines (53). Recent studies describe a role for both TLR-2 and TLR-4 in the recognition of *A. actinomycetemcomitans*, whose impact range from stimulating inflammatory cytokine expression and inflammatory cell migration to inducing osteoclastogenesis and alveolar bone loss (54, 55). Besides TLRs, the nucleotide-binding oligomerization domain (NOD) receptors and the inflammasome system have been pointed out as potential accessory molecules that trigger the host response against periodontal pathogens (56).

Animal models also provided the initial clear evidences for the role of some host immune inflammatory factors in the progression of periodontal diseases. When the host response is altered by treatment with specific inflammatory inhibitors or genetic manipulation, the severity of periodontal connective tissue and bone loss stimulated by periodontal bacteria is clearly reduced. The first concrete evidence that inhibition of an inflammatory response reduces periodontal diseases was carried out in a dog model (57) in which the inhibition of prostaglandins significantly reduced alveolar bone loss. Subsequent studies have used a number of techniques to demonstrate that cytokines play an important role in periodontitis. Non-human primates treated with inhibitors to two major proinflammatory cytokines, IL-1 and TNF, exhibit reduced periodontal bone loss and loss of attachment compared to control animals (44, 45, 58). Similarly, RANKL inhibition decreases alveolar bone loss in several models of periodontal disease (46, 59, 60). It is hypothesized that periodontal disease progression is due to a combination of several factors, including the presence of periodontopathic bacteria, high levels of proinflammatory cytokines and prostaglandins, the production and activation of MMPs and RANKL, and relatively low levels of interleukin-10 (IL-10), transforming growth factor- $\beta$  (TGF- $\beta$ ), tissue inhibitors of metalloproteinase (TIMPs), and osteoprotegerin (OPG) (61). A description of inflammatory cytokines and cell types that participate in bone resorption and destruction of connective tissue matrix is shown in Fig. 2.

### Inflammatory mediators

The cyclooxygenase enzymes, COX-1 and COX-2, catalyze the conversion of arachidonic acid to prostaglandins. COX-1 is constitutively expressed and leads to the generation of prostaglandins that are particularly important in homeostasis. COX-2 is inducible and leads to the formation of prostaglandins involved in inflammatory processes. Prostaglandins (PGs), potent stimulators of bone formation and resorption, are produced by bone cells, fibroblasts, gingival epithelial cells, endothelial cells, and inflammatory cells (62). Prostaglandin E2 produc-

tion is elevated in individuals with periodontitis compared with healthy subjects (63). When applied topically to the gingival sulcus, prostaglandin E2 induces a marked increase in osteoclasts. Moreover, it is synergistic with lipopolysaccharide stimulating osteoclastogenesis (64).

Both PGE-2 and leukotriene B-4 were found in gingival crevicular fluid of individuals with localized aggressive periodontitis. Furthermore, *P. gingivalis* stimulates increased PGE-2 levels and increased COX-2 expression by infiltrated leukocytes in vivo (65). In an animal ligature induced periodontitis model, both a non-selective COX inhibitor and a selective COX-2 inhibitor reduced osteoclast numbers and alveolar bone loss when compared to non-treatment (66). Many clinical trials have explored the use of a COX-2 inhibitor as an adjunct to periodontal therapy. These inhibitors improved the clinical outcome after periodontal therapy compared to periodontal therapy alone (67). However, they are not clinically used in the treatment of human patients due to side effects. Lipoxins and resolvins, products of omega-3 fatty acids, induce resolution of inflammation, and to protect against periodontal bone loss stimulated by bacteria in animal models (68).

The induction of experimental periodontitis is associated with the expression of innate immune cytokines (69). IL-1 stimulates the expression of proresorptive cytokines, such as RANKL and TNF- $\alpha$  and proteinases that participate in periodontal connective tissue destruction and bone resorption. In the periodontium, IL-1 is produced by several types of cells including PMNs, monocytes, and macrophages (70, 71). In patients with periodontitis, IL-1 $\beta$  expression is elevated in gingival crevicular fluid at sites of recent bone and attachment loss (71, 72). The IL-1 $\beta$  is also found to be higher in gingiva from individuals with a history of periodontitis than in samples from healthy individuals (72).

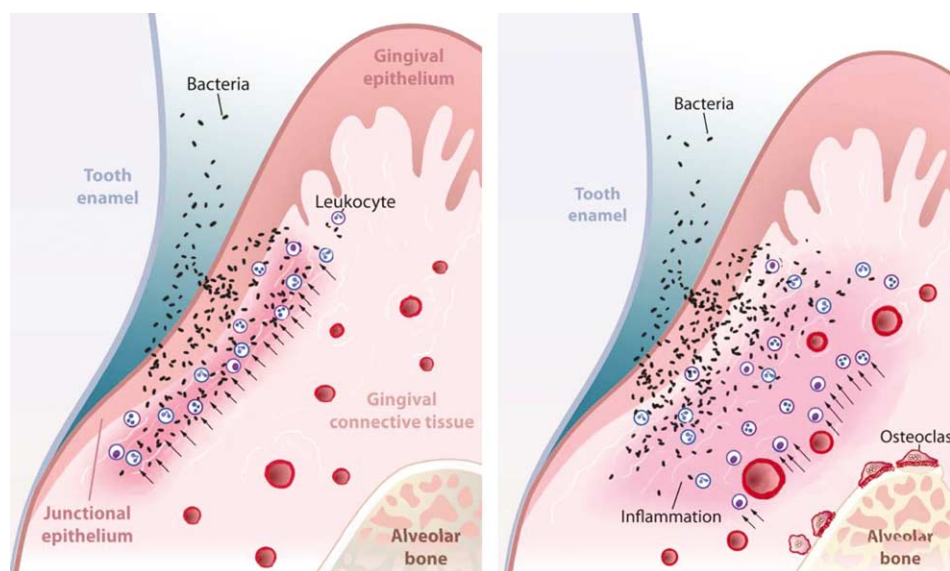
Using a non-human primate model, Delima et al. showed that IL-1 inhibition significantly reduced inflammation, connective tissue attachment loss, and bone resorption induced by periodontal pathogens when compared to controls (45). In other studies, IL-1 receptor deficient mice had less *P. gingivalis* LPS-induced osteoclastogenesis compared to similarly treated wild-type mice (73). In a different approach, the exogenous application of recombinant human IL-1 $\beta$  in a rat model of experimental periodontitis accelerated alveolar bone destruction and inflammation over a 2-week period (74). In addition, transgenic mice overexpressing IL-1 $\alpha$  in gingival epithelium developed a periodontitis-like syndrome, leading to the loss of attachment and destruction of periodontal bone (75). Taken together, these studies strongly support the role of IL-1 in promoting alveolar bone destruction in periodontitis.

The TNF refers to two associated proteins, TNF- $\alpha$  and TNF- $\beta$ . TNF- $\alpha$  levels are upregulated in gingival crevi-

cular fluid at sites where bone and attachment loss have recently occurred (71, 76–78). TNF- $\alpha$  was also found to be higher in diseased periodontal tissue samples than in tissue samples from healthy individuals (72). A cause and effect relationship between TNF- $\alpha$  and periodontal bone loss has been demonstrated. Administration of recombinant TNF- $\alpha$  accelerates periodontal destruction in a rat periodontitis model (79). On the other hand, *P. gingivalis* induced osteoclastogenesis is reduced in TNF receptor-deficient mice compared to wild-type controls, indicating that osteoclast formation is dependent on TNF- $\alpha$  regulated pathway as part of the host response to bacterial challenge (80). Garlet et al. recently showed that TNFR-1 knockout mice developed significantly less inflammation, indicated by chemokine and chemokine receptors downregulation, and less alveolar bone loss in association with RANKL downregulation in response to *A. Actinomyces-temcomitans* oral inoculation (81). Furthermore, mRNA levels of IL-1 $\beta$ , IFN- $\gamma$ , and RANKL in gingival tissues were significantly lower in TNFR-1 knockout mice than in wild-type infected mice. In contrast, *A. actinomyces-temcomitans* levels quantified by real-time PCR were significantly greater in TNF receptor ablated mice than in wild-type controls and were associated with lower levels of the PMN-related antimicrobial mediator myeloperoxidase in experimental mice (81). Thus, the absence of TNFR-1 resulted in a lower production of cytokines in response to *A. actinomyces-temcomitans* infection even in the presence of higher levels of periodontal pathogens. Based on similar studies, it can be implied that the local production of TNF- $\alpha$  plays a role in upregulating the

host response to bacteria and stimulating bone resorption during periodontitis. It is also possible that oral bacteria in addition to causing local pathology may contribute to systemic conditions by enhancing cytokine production subsequent to bacteremias. Interestingly, *P. gingivalis* LPS stimulates a strong local inflammatory response but a weak systemic inflammatory response (82). Inhibition of IL-1 and TNF- $\alpha$  together significantly reduces the progression of inflammation toward bone, osteoclastogenesis, and periodontal tissue destruction (44). In the gingiva, a higher expression of IL-6 is found in gingival crevicular fluid and in the gingiva in mononuclear cells and T-cells from periodontitis patients than in healthy controls (83, 84). The LPS from the periodontal pathogen *A. actinomyces-temcomitans* induces IL-6 expression, osteoclastogenesis, and bone loss (85). Oral inoculation of *P. gingivalis* in mice with genetically deleted IL-6 have decreased bone loss compared to wild-type mice, suggesting that IL-6 contributes to the progression of bacteria-induced bone loss (47).

After a host response triggered by microbial recognition, the spatial orientation of the subsequent leukocyte infiltration into periodontal tissues is likely to contribute to periodontal disease. Histologically, the inflammatory infiltrate is observed even in the presence of minimal clinical signs of inflammation, but when inflammation is restricted to the connective tissue closest to the gingival epithelium, gingivitis is present (86). However, in animal models, when the inflammatory infiltrate moves closer to bone, osteoclastogenesis is induced and periodontal bone loss takes place (44). This suggests that the periodontal



**Fig. 3.** Spatial relationship between an inflammatory infiltrate and periodontal bone loss. In periodontitis, bacteria attach to the tooth surface and invade the adjacent epithelium and connective tissue. This causes formation of an inflammatory infiltrate indicated by the black arrows. If the inflammatory infiltrate is at a distance from bone (left panel), osteoclastogenesis is not stimulated. However, if the infiltrate moves closer to bone (right panel), osteoclasts are induced and bone resorption occurs.



disease development might be determined by the progression of the inflammatory infiltrate toward bone. The impact of spatial location of the inflammatory infiltrate on bone resorption is shown in Fig. 3.

Among the mediators potentially involved in leukocyte diapedesis and subsequent spatial localization in periodontal environment, chemokines have been investigated with special interest in the last decade. Chemokines are small chemotactic cytokines that stimulate the recruitment of inflammatory cells (33, 87). They are divided into two major families based on their structure, CC and CXC chemokines, which basically bind to the two major classes of receptors, CC chemokine receptors (CCR) and CXC chemokine receptors (CXCR). Chemokines are produced by several resident and inflammatory cell types in the periodontium (33). Some chemokines can stimulate one or more steps of bone resorption, including the recruitment, differentiation, or fusion of precursor cells to form osteoclasts or enhance osteoclast survival (33, 87). They could also affect periodontal bone loss by recruiting cells, such as neutrophils, which protect against bacterial invasion.

Chemokines are found in gingival tissue and crevicular fluid. The IL-8/CXCL8, a chemoattractant of PMNs, is found at higher levels in gingival crevicular fluid prior to clinical signs of inflammation following cessation of tooth brushing. Moreover, in subjects with a history of periodontitis, IL-8/CXCL8 in gingiva and gingival crevicular fluid are increased and correlated with disease severity (88).

One of the most abundant expressed chemokines is macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ /CCL3), which is localized in the connective tissue subjacent to gingival epithelium (89). MIP-1 $\alpha$ /CCL3-positive cells increase with increasing severity of periodontal disease. It is a ligand for the chemokine receptors CCR1 and CCR5 and is associated with the recruitment of monocytes/macrophages and dendritic cells via CCR1 and lymphocytes polarized into Th1 phenotype by CCR5 (90). Thus, MIP-1 $\alpha$ /CCL3 has a potential role in stimulating bone resorption through effects on macrophages and Th1 cells. Moreover, CCR1+ and CCR5+ cell populations may affect resorption since they include osteoclast precursors (91, 92). This is consistent with findings that MIP-1 $\alpha$ /CCL3 directly stimulates osteoclasts differentiation (93) and stromal-cell derived factor-1 (SDF1/CXCL12) regulates osteoclast function and is found in the periodontium (94).

A number of chemokines have been detected in gingiva or in gingival crevicular fluid including regulated upon activation normal T-cell expressed and secreted (RANTES/CCL5). RANTES/CCL5 is found in greater levels in active periodontal lesions compared to inactive sites (89, 95). That RANTES/CCL5 may be involved in periodontal bone resorption is supported by findings that

it binds to CCR1 and CCR5 (91, 96). Monocyte chemoattractant protein-1 (MCP-1/CCL2) may also contribute to periodontitis. MCP-1/CCL2 levels are directly correlated with gingival inflammation (97, 98). It stimulates monocyte/macrophage recruitment and activity and has been implicated as a chemoattractant for osteoclast precursors (91, 96).

RANKL stimulates osteoclastogenesis and bone resorption. A number of studies have established that RANKL inhibition decreases periodontal bone resorption (46, 59, 60) and establishes a role for RANKL in periodontitis. Osteoprotegerin (OPG) is a molecule that is also upregulated by inflammatory conditions and blocks RANKL by binding to it. A high ratio of RANKL/OPG creates proresorptive conditions while a low RANKL to OPG ratio is antiresorptive. During bacteria stimulated periodontal bone loss there is an initial increase in the ratio of RANKL/OPG (69). After the initial bone loss, antiresorptive factors are produced including OPG, as well as IL-4 and IL-10, reducing the RANKL/OPG ratio (69). This relationship is shown in Fig. 2. The RANKL/OPG ratio has been examined in gingival tissues or gingival crevicular fluid. It has been shown that periodontitis is associated with an increase in RANKL. The RANKL/OPG ratio greater than 1 predominates in chronic periodontitis lesions while a ratio of 0.5 or less is found in chronic gingivitis lesions (37).

RANKL is upregulated in both pathologic and physiologic bone resorption. In pathologic inflammatory bone disease, RANKL expression has been shown to have the highest level in B-cells, followed by T-cells, and then monocytes (99). This indicates that activated T- and B-cells can be the cellular source of RANKL for bone resorption in diseased gingival tissue. In physiologic bone, remodeling bone-lining cells such as osteoblasts or their precursors appear to be an important source of RANKL.

The IFN- $\gamma$  is a lymphokine, produced by lymphocytes and natural killer cells that has been implicated in periodontal bone loss. Its expression is associated with Th1 lymphocytes. Mice with a genetic ablation of IFN- $\gamma$  have less *P. gingivalis* induced bone loss compared to wild-type controls (47). T-cells are an important source of IFN- $\gamma$  in periodontitis (84) and have been linked to increased RANKL expression (100).

Lymphocytes also produce cytokines that are anti-inflammatory, such as IL-4 and IL-10 (101). These cytokines are associated with a Th2 response and reduce the severity of experimental periodontitis (102). However, a direct link between Th1 lymphocytes enhancing periodontal disease and Th2 lymphocytes reducing it is not necessarily straightforward since there are components of a Th2 response that are also prodestructive (103).

Innate immune cells have been shown to play an important role in periodontal bone resorption (61, 104).

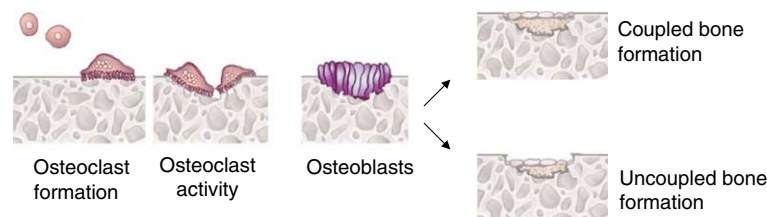
Monocytes and macrophages produce several cytokines and lytic enzymes that stimulate the breakdown of connective tissue and bone resorption (61). PMNs have been shown to have both protective and destructive effects (105). A protective role is inferred from findings that neutrophil disorders including cyclic neutropenia, Chédiak-Higashi syndrome, and leukocyte adhesion deficiency syndrome promote periodontal diseases (105). The production of reactive oxygen species and cytokines implicates them in the destructive phase. Monocytes and PMNs produce a respiratory burst that generates superoxides, hydrogen peroxide, hydroxyl radicals, hypochlorous acid, and chloramines. These products contribute to bacterial killing based on evidence that impaired production of iNOS and MPO are associated with increased levels of periodontal pathogens (81, 106). In addition, PMNs and monocytes/macrophages release elastases and collagenases that break down connective tissue (107) and are linked to the development of periodontal lesions (107).

Dendritic cells of monocytic lineage are another group of innate immune cells that function to present antigen to lymphocytes and also promote inflammation by the production of chemokines and cytokines (108). They have been implicated in periodontal disease (108–111). Oral bacteria induce dendritic cells to produce cytokines such as IL-1 $\beta$ , IL-12, IFN- $\gamma$ , TNF- $\alpha$ , and TNF- $\beta$  (109, 110). Dendritic cells can form to osteoclasts (112). For example, *A. actinomycetemcomitans* stimulates dendritic cells in vitro to form osteoclasts in a RANKL dependent manner (113).

Oral bacteria stimulate cells of the adaptive immune response as shown by the presence of activated T- and B-lymphocytes in periodontal disease tissues (61). As discussed above, lymphocytes produce cytokines that promote bone resorption directly through RANKL or indirectly through IFN- $\gamma$ . There is evidence to suggest that lymphocytes are involved in mediating bacteria stimulated periodontal bone resorption. When severe

combined immunodeficient (SCID) mice that lack B- and T-lymphocytes are challenged with *P. gingivalis*, there is considerably less bone resorption than in wild-type normal mice (114). Moreover, (SCID) mice engrafted with human CD4(+) T-cells from individuals with aggressive early onset periodontal disease and subsequently challenged with *A. actinomycetemcomitans* exhibit enhanced periodontal bone loss (46). This bone loss is mediated by RANKL. Similarly, the adoptive transfer of B-cells from *A. actinomycetemcomitans* immunized rats followed by an injection of *A. actinomycetemcomitans* into the gingiva, stimulates greater alveolar bone resorption than control mice that have received B-cells from non-immunized mice (59). The increased resorption was shown to be RANKL mediated (59). The results of these experiments indicate that cells of the adaptive immune response significantly contribute to periodontitis.

In addition to Th1 and Th2 CD4+ lymphocytes, there are two other T-cell subsets that have been identified, Th17 and Tregs (regulatory T-cells). The Th17 lymphocytes produce IL-17, which in turn stimulate RANKL-mediated osteoclastogenesis (115). They have been implicated in rheumatoid arthritis, periodontal disease, and loosening of joint prostheses (115). Under experimental conditions, IL-17 appears to have an important protective function since genetic deletion of IL-17 receptors enhance periodontal bone loss stimulated by *P. gingivalis* in vivo (116). This may be due to the role of IL-17 in stimulating chemokines that induce recruitment of neutrophils. However, humans with periodontitis have increased levels of Th17 cells and IL-17 mRNA, compared to healthy tissues suggesting but not proving that IL-17 contributes to the destructive process (117). The Tregs modulate activation, proliferation, and effector function of conventional T-cells (118) and have been identified in periodontal tissues (119–121). Because they are associated with the production of IL-10, TGF- $\beta$ , and the inhibitory molecule CTLA-4, Tregs may reduce periodontal disease progression (119).



**Fig. 4.** The role of coupling in periodontal lesion development. Bone formation occurs after bone resorption so that the two processes are coupled. Thus, the resorption pit is occupied by osteoblasts that form new bone. In a normal healthy individual, the amount of bone formed equals the amount resorbed. In pathologic bone resorption, the amount of bone that forms is less than that resorbed so that there is net bone loss. This may be due to the impact of inflammation on bone formation. Inflammation could potentially interfere with coupling by reducing proliferation of osteoblast precursors, inhibiting differentiation of osteoblasts, decreasing osteoblast numbers by stimulating apoptosis, or by interfering with the production of bone matrix.

### Uncoupled bone formation and periodontitis

Bone remodeling involves a process of bone resorption followed by bone formation, a process referred to as coupling (122). Bacteria induced bone resorption in a healthy adult should be followed by an equivalent amount of bone formation. In periodontitis, there is a failure to form an adequate amount of new bone following resorption resulting in net bone loss. Thus, a critical aspect of periodontitis is uncoupling so that bacteria induced bone loss is not followed by an equivalent amount of new bone formation resulting in net bone loss. The impact of uncoupling on creating net bone loss is shown in Fig. 4.

The same process that stimulates bone resorption, inflammation, may be responsible for uncoupling. It is possible that under conditions where inflammation is in close proximity to and along the bone, it will affect osteoblast numbers or function and interfere with the coupling process. In an experimental model, the injection of *P. gingivalis* into connective tissue induces bone resorption followed by bone formation (123, 124). If the inflammation is prolonged by induction of the adaptive immune response, the capacity to form new bone is diminished and uncoupling occurs (123). Similarly, prolonged inflammation in diabetic animals interferes with bone formation in the periodontium following bacteria stimulated bone resorption (124). This interpretation is additionally supported by evidence that the application of cytokines in vivo stimulates bone resorption but also limits bone formation. Therefore, several lines of animal experimentation support the concept that inflammation uncouples bone formation from bone resorption. Thus, inflammation may not only stimulate the formation of osteoclasts and bone resorption, but also affect bone by altering the function of osteoblasts and limiting reparative bone formation.

### Conclusions

Polymicrobial infection in lesions of endodontic origin stimulates bone resorption by interacting with the leukocytes of the innate and adaptive immune responses. In endodontic lesions, the presence of inflammation suppresses bone formation so that lesion resolution does not occur until the causal bacteria are entombed by treatment and the inflammation subsides. Periodontitis is caused by a host response to the presence of bacteria or their products that invade connective tissue. The host defense, including innate and adaptive immunity, is responsible for combating bacteria invading the periodontal tissue. In humans, plaque accumulation occurs even in health so that there is a continuous state of inflammation in gingival tissue adjacent to teeth. By using animal models and specific inhibitors, both the innate and adaptive immune response have been conclusively shown to participate in the formation of period-

ontal lesions. Cytokines generated that induce osteolytic lesions are shown in Fig. 2 and a comparison of the effect of cytokine deletion or inhibition in the formation of lesions of endodontic origin and in periodontitis is shown in Table 2. It is also possible that the inflammation associated with periodontal bone resorption affects coupled bone formation contributing to net bone loss (see Fig. 4).

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