

Biology of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and Osteoprotegerin (OPG) in Periodontal Health and Disease - A Review

V.A. Patil, M.H. Desai

Department of Periodontology, HKES's S. Nijalingappa Institute of Dental Sciences and Research, Sedam Road, Gulbarga - 585105

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Abstract:

Periodontitis is a destructive disease that targets tooth-supporting structures through complex and multifactorial pathogenic processes. Alveolar bone destruction, a hallmark of periodontitis progression and one of the major causes of tooth loss in human, is mediated by the host immune and inflammatory response to microbial challenge. The discovery of the receptor activator of nuclear factor-kappa B (RANK)/ RANK ligand (RANKL)/osteoprotegerin (OPG) axis has improved the knowledge of bone metabolism regulation. The RANKL/RANK interaction is needed for differentiation and maturation of osteoclast precursor cells to activate osteoclasts and for the survival of mature osteoclasts. Osteoprotegerin is the naturally occurring inhibitor of osteoclast differentiation. It binds to RANKL with high affinity and blocks RANKL from interacting with RANK. The RANKL/OPG ratio is increased in periodontitis compared to healthy individuals, suggesting that this molecular interaction may be important in modulating local bone loss.

In recent decades, studies on cellular and molecular mechanisms of bone loss in periodontitis have suggested that the use of host modulation agents is an important adjunctive therapy to scaling and root planning. This line of investigation is expected to help in the development of new therapeutic approaches to stop progressive bone resorption induced by oral bacterial challenge.

Key Words: Bone resorption, osteoprotegerin; periodontitis; RANKL.

Introduction:

The bone is an engineering feat; it is strong but light enough to permit locomotion, rigid to allow muscles to be fixed but capable of bending without breaking and its structure is programmed for variable loading. In addition to its structural role, bones also serve as a reservoir for calcium. This means that bone is malleable and its structure can be sampled, adapted or fine tuned for the process of remodeling.

As per glossary of periodontal terms (2001), periodontitis is defined as inflammation of the supporting tissues of the teeth. Usually a progressively destructive change leads to loss of bone and periodontal ligament. Balance between bone resorption by osteoclasts and bone formation by osteoblasts determines the level of bone mass. The remodeling of bone takes place in so called "bone multi-cellular units" (BMU's) and is initiated by recruitment, formation and activation of bone resorbing osteoclasts. They are activated by RANKL and by integrin-mediated signalling from bone matrix itself ¹.

Osteoclast differentiation is regulated by osteoblasts. Receptor activator of nuclear factor kappa B ligand and its decoy receptor, osteoprotegerin, are essential molecules for osteoclast differentiation supported by osteoblasts.

Our understanding of the molecular mechanisms that regulate osteoclasts formation and activation has advanced rapidly during the past decade since the discovery of RANKL/RANK signaling system and following the development in the late 1980's of *in vitro* assays that facilitated harvesting of large numbers of osteoclasts precursors (OCP's) from the bone marrow or spleen, which could then be cultured in the absence of osteoblasts/stromal cells. An early indication that RANKL and OPG could be of relevance to the periodontium came from *in vitro* work demonstrating that periodontal ligament cells do produce RANKL and OPG and they can as well support osteoclastogenesis through RANKL signalling². Both osteoblasts and periodontal ligament fibroblasts are stimulated with lipopolysaccharide and IL-1 express RANKL, suggesting that once the inflammation extends into the periodontal ligaments and alveolar bone, alveolar bone resorption might be accelerated through the increased expression of RANKL. The periodontopathic bacteria *A. actinomycetemcomitans*

Corresponding Author: Dr. Veena A. Patil,
Plot No. 27 & 28, Mankar Layout, Behind Ram Mandir, Gulbarga - 585105 (Karnataka)
Phone No.: 08472 - 254927, +91 9480285089
E-mail: veenaashokpatil@gmail.com

and *P. gingivalis* have unique mechanisms to induce RANKL in osteoblasts and gingival fibroblasts. Receptor activator of nuclear factor-kappa B ligand and OPG expression might also be related to the function of amelogenin, and regulation of odontoclast formation³. An appreciation of the relationship between immune processes and the bone metabolism in various inflammatory bone diseases has given rise to the field of osteoimmunology⁴.

This field has given a future outlook to study the basis of periodontal destruction. Hence, further research is required on osteoimmunology which will make the molecular mechanisms of bone destruction clear and a novel diagnostic parameter and therapies in periodontics can be devised.

Regulation of Osteoclast Formation and Activation:

Osteoclasts are multinucleated bone resorbing cells formed by cytoplasmic fusion of their mononuclear precursors, which are in the myeloid lineage of hematopoietic cells that also give rise to macrophages. The switch to osteoclasts differentiation requires expression of c-Fos (proto-oncogene) by the osteoclast precursors cells⁵. To resorb bone effectively, osteoclasts attach themselves firmly to the bone surface using specialized actin – rich podosomes, which they use to form tightly sealed circular extension of their cytoplasm with the underlying bone matrix. Within these sealed zones they form ruffled membranes that increase the surface area of the cell membrane for secretion of hydrochloric acid and the proteolytic enzyme cathepsin K. There they simultaneously destroy the inorganic and organic matter of bone, while protecting neighboring cells by this sealing mechanism. They are activated by RANKL and integrin mediated signaling pathway from the bone matrix¹. Osteoclasts are required during embryonic development for the removal of bone trabeculae formed under growth plates during endochondral ossification and thus for formation of the bone marrow cavity to facilitate normal hematopoiesis. Failure of osteoclast formation or activity results in osteoporosis, some forms of which are lethal because of attendant immunodeficiency and increased risk of fractures.

Osteoclasts work in packs within remodeling units under the of osteoblast lineage cells expressing macrophage colony stimulating factor (M-CSF) and RANKL. The strategy for acquiring OCP's from sources was developed in the knowledge that M-CSF expression

by osteoblast/stromal cells was required for progenitor cells to differentiate into osteoclasts, but that M-CSF on its own was unable to complete this process. This requirement for M-CSF was based on the observation that op/op mice, which do not express functional M-CSF, have osteopetrosis because of a lack of osteoclasts. Rodan & Martin⁶ proposed the novel hypothesis that osteoblast/stromal cells play a central role in the regulation of osteoclast formation and bone resorption. Many investigators had attempted to identify the osteoclast-activating factor that completed the differentiation of precursors that had been exposed to M-CSF.

Rankl:

Receptor activator of nuclear factor kappa-B ligand is known as tumor necrosis factor ligand superfamily member 11(TNFSF11), TNF-related activation-induced cytokine(TRANCE), osteoprotegerin ligand (OPGL) and also as osteoclast differentiation factor (ODF). Receptor activator of nuclear factor kappa-B ligand is a type II homotrimeric transmembrane protein that is expressed as a membrane- bound and a secreted protein, which is derived from the membrane form as a result of either proteolytic cleavage or alternative splicing⁷. Three human RANKL isoforms - hRANKL1, hRANKL2 and hRANKL3 are identified. hRANKL1 possesses intracellular, transmembrane and extracellular domains. Intracellular domains are absent in hRANKL2 and hRANKL3. hRANKL3 additionally does not possess transmembrane domains⁸. The proteolytic cleavage of RANKL requires ADAM (a disintegrin and metalloprotease domain) and matrix metalloproteases⁹. Receptor activator of nuclear factor kappa-B ligand expression is stimulated in osteoblast/stromal cells by most of the factors that are known to stimulate osteoclast formation and activity. It is highly expressed in lymph nodes, thymus and lung, and at low levels in a variety of other tissues including spleen and bone marrow¹⁰. In inflamed joints it is expressed by synovial cells and secreted by activated T cells. These sources of RANKL appear to be responsible, at least in part, for mediating the joint destruction in patients with rheumatoid arthritis¹¹. Tumor Necrosis Factor also mediates joint destruction in rheumatoid arthritis by systemically increasing the number of circulating OCPs, and by promoting their egress from the bone marrow into the peripheral blood and then to the inflamed joints, where it promotes fusion of these cells to osteoclasts along with RANKL and interleukin-1¹².

Receptor activator of nuclear factor kappa-B ligand like TNF, stimulates the release of immature progenitors into the circulation. However, RANKL does not induce OCP mobilization in protein tyrosine phosphatase-c deficient mice. Thus making osteoclasts defective in terms of bone adhesion and resorption¹³. Thus, RANKL induced osteoclast activation may regulate progenitor recruitment as part of homeostasis and host defense, linking bone remodeling with regulation of hematopoiesis. Preclinical studies in mice have shown that RANKL is also expressed in mammary epithelial cells during pregnancy and is required for lactational hyperplasia of mammary epithelial cells and milk production¹⁴. It is also expressed by some malignant tumor cells that also express RANK, and thus it may play a role in inducing tumor cell proliferation by an autocrine mechanism or in a paracrine manner if it is produced by accessory cells, such as activated T cells¹⁵. However, production by T cells of RANKL also induces expression of interferon- γ by activated osteoclasts through c-Fos to negatively regulate their formation¹⁶. This mechanism can be enhanced by T-cell produced interferon- γ , which degrades TNF receptor associated factor (TRAF), an essential adapter protein that is recruited to RANK to mediate RANK signaling¹⁷.

Rank:

Receptor activator of nuclear factor kappa-B is a type I homotrimeric transmembrane protein whose expression was initially detected only on OCPs, mature osteoclasts, and dendritic cells. Like RANKL, however, it is expressed widely. Receptor activator of nuclear factor kappa-B ligand protein expression has been reported in mammary gland and some cancer cells, including breast and prostate cancers, two types of tumors with high bone metastasis potential. Although no human have been identified to date with inactivating mutations or deletions of RANK, a deletion mutation occurred spontaneously in a line of transgenic mice, which consequently had all of the features of mice with targeted deletion of RANK, confirming the importance of RANK for osteoclast formation¹⁸. Activating mutations in exon 1 of RANK causes an increase in RANK-mediated nuclear factor-kappa B (NF kappa-B) signaling and a resultant increase in osteoclast formation and activity, account for the increased osteolysis seen in some patients with familial Paget's disease and have confirmed the importance of this system in humans¹⁹.

Osteoprotegerin:

Osteoprotegerin also known as osteoclastogenesis inhibitory factor (OCIF) is a basic glycoprotein which acts as a decoy receptor of RANKL. It is expressed in many tissues apart from osteoblasts, including heart, kidney, liver, spleen and bone marrow. Its expression is regulated by most of the factors that induce RANKL expression by osteoblasts. Although there are contradictory data, in general up regulation of RANKL is associated with down regulation of OPG, or at least lower induction of OPG, such that the ratio of RANKL to OPG changes in favor of osteoclastogenesis. Many reports have supported the assertion that the RANKL/OPG ratio is a major determinant of bone mass²⁰. An osteoprotective role for OPG in humans is supported by the report of homozygous deletions of 100 kilo bases of OPG in two patients with juvenile Paget's disease, an autosomal-recessive disorder characterized by increased bone remodeling, osteopenia and fractures²¹. It is also supported by the identification of an inactivating deletion in exon 3 of OPG in three siblings with idiopathic hyperphosphatasia, which is an autosomal-recessive bone disease characterized by increased bone turnover associated with deformities of long bones, kyphosis, and acetabular protrusion in affected children²². A recent surprising finding is that OPG expression is regulated by Wnt/ β -catenin signaling in osteoblasts, the same pathway that regulates osteoblastic bone formation²³. Thus, bone mass is determined by the combined efforts of osteoblasts and osteoclasts, and is regulated in osteoblasts by two major signaling pathways: RANKL/RANK and Wnt/ β -catenin. OPG also appears to protect large blood vessels from medial calcification, based on the observation of renal and aortic calcification occurring in OPG knockout mice²⁴. Furthermore, the absence of OPG in OPG/apolipoprotein E double knockout mice accelerates the calcific atherosclerosis that develops in apolipoprotein E knockout mice, suggesting that OPG protects against this complication of atherosclerosis²⁵. Whether OPG and RANKL signaling plays important roles in cardiovascular disease remain to be determined and is controversial. For example, there is also an association between high levels of OPG in serum and cardiovascular disease, diabetes, and chronic renal failure in humans²⁶. However, OPG in this latter setting does not appear to protect the skeleton against the increased bone resorption of secondary hyperparathyroidism mediated by PTH in patients with renal osteodystrophy or against vascular calcification. It is possible that OPG in the serum of such patients is bound to a plasma protein(s) and thus

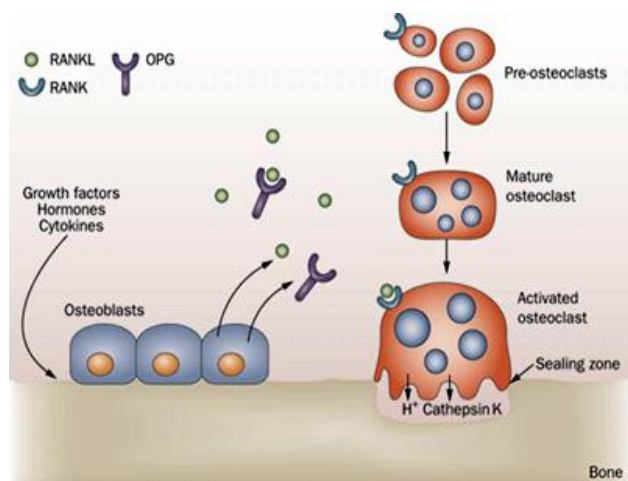


Fig. I: RANKL-RANK-OPG interplay

rendered inactive, but further studies will be required to determine the significance of these observations, which question whether the RANKL/OPG ratio in serum is indicative of bone mass/bone resorption in these settings²⁷.

Possible Therapeutic Approaches for Inhibition of Periodontal Bone Loss by Targeting Rankl:

We know that the production of RANKL from bacterial antigen-specific T and B cells seems to trigger periodontal bone loss and that the suppression of antigen-specific T and B cell responses may sufficiently facilitate down-modulation of periodontal bone destruction. Thus, we might envision an ideal therapy that suppresses the bone destructive consequences of antigen-specific T and B cells while, at the same time, permitting the homeostatic bone remodeling mediated by RANKL-produced from osteoblasts and bone marrow stromal cells. Indeed, interference with co-stimulatory molecules, which are required for induction of antigen-specific T and B cells, abrogated periodontal bone resorption. In other words, systemic administration of CTLA4Ig, a functional antagonist of CD28 binding to B7²⁸ or anti-CD40 ligand antibody to inhibit CD40/CD40L²⁹, could theoretically abrogate bone resorption in an animal periodontal model. In addition to these approaches, we are now challenged to establish new therapeutic regimens to mediate the antigen-specific T and B cells. For example, one approach that is attracting wide attention involves the utilization of regulatory CD4⁺ T cells, which play a suppressive role in inflammation caused by the activation of adaptive T cell responses³⁰. Accordingly, the therapeutic application of T cells to modulate periodontal bone loss

can be researched. This line of investigation is expected to help in the development of new therapeutic approaches to stop progressive bone resorption induced by oral bacterial challenge.

Discussion

As compared to healthy gingival tissues, periodontitis affected tissues had higher RANKL and lower OPG molecules³¹. Studies on RANKL/OPG ratio, demonstrated 2.2-fold increase in chronic periodontitis when compared to healthy individuals³² or equally elevated levels in chronic and aggressive periodontitis, compared to individuals who were healthy or had gingivitis³³. Other studies also demonstrate the correlation between RANKL/OPG ratio in GCF and gingival inflammation, which indicates that this ratio has specificity to determine periodontal bone destruction³⁴. With regards to microbiota the presence of *P.gingivalis* in subgingival biofilms at chronic periodontitis sites, positively correlated with RANKL tissue gene expression levels or the RANKL/OPG ratio but not with OPG³². Similarly a positive correlation was found between RANKL total amounts in GCF and the subgingival presence of *P. gingivalis* and *T.denticola* in chronic periodontitis but no correlations were found in healthy individuals³⁵. Hence, these studies warrant that if OPG is increased or RANKL inhibited, it will have a positive effect on the periodontium. Thus this approach was used in an experimental study, where concomitant administration of OPG in T-cell specific *A. actinomycetemcomitans* induced periodontitis model in mice, diminished alveolar bone destruction and reduced osteoclast numbers³⁶. In another periodontitis model, effects of an anti-RANKL antibody on periodontal bone resorption were tested. Results showed that antibody to RANKL can inhibit *A. actinomycetemcomitans*-specific T cell-induced periodontal bone resorption by blockade and reduction of tissue RANKL, providing an immunological approach to ameliorate immune cell-mediated periodontal bone resorption³⁷. Thus it can be implied that inhibition of RANKL by OPG or direct can have a beneficial effect on the outcome of periodontal disease treatment and also prevent its recurrence and prevent further alveolar bone resorption.

Conclusion:

From this review it can be concluded that RANKL-OPG axis is one of the most important factor to determine the ongoing periodontal disease activity.

The RANKL–RANK–OPG axis is clearly involved in the regulation of bone metabolism in periodontitis, in which an increase in relative expression of RANKL or a decrease in OPG can tip the balance in favor of osteoclastogenesis and the resorption of alveolar bone. Post treatment high RANKL/OPG ratio indicates that the molecular mechanisms of bone resorption are still active, and the affected periodontal sites are at a potential risk for developing the disease again. Hence additional therapies to modulate the immune system which will target the RANKL–OPG axis to provoke a host response which will be beneficial in the long term periodontal management. Hence in addition to the long term clinical and prospective studies more studies are required, which will monitor the periodontal disease progression and the progressive changes in the RANKL–OPG level before and after treatment and during the maintenance phase also. In addition it can be concluded that any kind of interference with the RANKL–RANK–OPG axis may have a protective effect on periodontal bone loss. Such interference may form the basis for rational drug therapy in periodontics in the future.

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