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Osteoclastogenesis Stimulated by Cytokines in Response to Dental Calculus

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Aknowledgment

Firstly ,we extend our most profound thanks to God for providing us with the strength to accomplish this research.

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Abstract

Osteoclastogenesis is an ongoing rigorous course that includes osteoclast precursors fusion and bone resorption executed by degradative enzymes. Osteoclastogenesis is controlled by endogenous signaling and/or regulators or affected by exogenous conditions and can also be controlled both internally and externally. More evidence indicates that autophagy, inflammation, and immunity are closely related to osteoclastogenesis and involve multiple intracellular organelles (e.g., lysosomes and autophagosomes) and certain inflammatory or immunological factors. Based on the literature on osteoclastogenesis induced by different regulatory aspects, emerging basic cross-studies have reported the emerging disquisitive orientation for osteoclast differentiation and function.

Dental calculus (DC) is a common deposit in periodontitis patients. We have previously shown that DC contains both microbial components and calcium phosphate crystals that induce an osteoclastogenic cytokine IL-1β via the NLRP3 inflammasome in macrophages. In this study, we examined the effects of cytokines produced by mouse macrophages stimulated with DC on osteoclastogenesis. The culture supernatants from wild-type (WT) mouse macrophages stimulated with DC accelerated osteoclastogenesis in RANKL-primed mouse bone marrow macrophages (BMMs), but inhibited osteoclastogenesis in RANKL-primed RAW-D cells. WT, but not NLRP3-deficient, mouse macrophages stimulated with DC produced IL-1β and IL-18 in a dose-dependent manner, indicating the NLRP3 inflammasome-dependent production of IL-1β and IL-18. Both WT and NLRP3-deficient mouse macrophages stimulated with DC produced IL-10, indicating the NLRP3 inflammasome-independent production of IL-10. Recombinant IL-1β accelerated osteoclastogenesis in both RANKL-primed BMMs and RAW-D cells, whereas recombinant IL-18 and IL-10 inhibited osteoclastogenesis. These results indicate that DC induces osteoclastogenic IL-1β in an NLRP3 inflammasome-dependent manner and anti-osteogenic IL-18 and IL-10 dependently and independently of the NLRP3 inflammasome, respectively. DC may promote alveolar bone resorption via IL-1β induction in periodontitis patients, but suppress resorption via IL-18 and IL-10 induction in some circumstances.

1. Introduction :

Bone homeostasis is critical for health and disease. As an organ harboring a variety type of cells, bone is continuously remodeling through the dynamic equilibrium between osteoclasts, responsible for bone resorption, and osteoblasts/osteocytes, responsible for bone Formation **[1,2]**. osteoimmunology, a concept coined in 2000, has revolutionized our understanding of the osteoporosis pathophysiology and demonstrated a key role of inflammatory cytokines in influencing the fine-tuned balance between bone formation and resorption **[3,4]**. Simply put, excessive bone resorption caused by hyperactivated osteoclasts (OC) impairs the normal bone remodeling which laid on the foundation of the balance between osteoclasts and osteoblasts, resulting in the significant bone loss in osteoporosis , These osteoclasts are originated from the myeloid cell progenitors, particularly monocytes and macrophages, which are part of innate immune cells During osteoclast differentiation, or termed as osteoclastogenesis, myeloid cells receive signals guided by key cytokines such as receptor activator of nuclear factor kappa-B ligand (RANKL) **[5]**. Cytokines are a broad range of secreted proteins necessary for cell signaling. Through the interaction with cell surface receptors, cytokines initiate complex downstream signaling cascades that can direct cell proliferation, survival, differentiation and metabolism **[6]**. Osteoclastogenesis is a multistep process involving cells such as osteoblasts and MSCs; thus, it is related to multiple skeletal diseases such as osteoporosis, rheumatoid arthritis, and periodontitis . Dental calculus (DC) is a common deposit in periodontitis patients. We have previously shown that DC contains both microbial components and calcium phosphate crystals that induce an osteoclastogenic cytokine IL-1β via the NLRP3 inflammasome in macrophages. In this study, we examined the effects of cytokines produced by mouse macrophages stimulated with DC on osteoclastogenesis **[7]**. Periodontitis is an inflammatory disease that leads to the destruction of periodontal tissue, including alveolar bone **[8]**. In response to external stimuli, such as dental plaque and dental calculus (DC), various types of leukocytes infiltrate periodontal tissue, and release inflammatory mediators, such as prostaglandins, matrix metalloproteinases, and cytokines, which promote periodontal tissue destruction **[9]**. Among these mediators, interleukin (IL)-1β has the capacity to trigger potent bone resorption, and IL-1β has been detected in the periodontal tissue and gingival crevicular exudates of patients with periodontitis, suggesting its involvement in alveolar bone resorption

[10,11]. Dental calculus, also known as tartar, is a hard deposit that forms on the surfaces of teeth and below the gum line as a result of the calcification of dental plaque. These deposits consist of mineral substances such as calcium and phosphate, along with other organic compounds **[12].** Dental calculus plays a crucial role in the development of periodontal diseases such as gingivitis and periodontitis. It accumulates around teeth and below the gum line, causing irritation and chronic inflammation of the gum tissues **[13].**

Review :

2.1. Mechanisms of osteoclastogenesis

Osteoclasts are large multinucleated cells characterized by their ability to resorb bone or dentine matrix. They are primarily derived from hematopoietic stem cells located in the bone marrow and their mononuclear precursors are typically found circulating in peripheral blood **[14].** RANKL and Macrophage Colony Stimulating Factor (M-CSF) are the central regulators of osteoclast differentiation. RANKL promotes the fusion of the mononuclear precursors to form the multinucleated osteoclasts and hence, induce the expression of the osteoclasts-specifi c marker genes **[15].** M-CSF regulates the proliferation and survival of cells of the monocyte lineage Tartrate-resistant acid phosphatase (TRAP), calcitonin receptor, and vitronectin receptor are markers for mature osteoclasts, with calcitonin receptor being a specifi c marker for osteoclast differentiation **[16].**

2.2. Positive regulation of osteoclastogenesis by cytokines

2.2.1. RANKL

The receptor activator of nuclear factor kappa-B ligand (RANKL) is a member of the tumor necrosis factor (TNF) superfamily of ligands and instrumental for the ultimate differentiation of osteoclast precursors into osteoclasts. RANKL is secreted or expressed by both immune cells such as type 17 helper T (TH17) cells and skeletal cells such as osteoblast and osteocytes **[17].**

RANKL interacts with its receptor RANK on osteoclast surface, where trimerized RANK-RANKL complex recruits downstream adaptor molecules such as tumor necrosis factor (TNF) receptorassociated factor (TRAF) 6 to its cytoplasmic domain, resulting in the activation of multiple signaling pathways **[18].**

In addition, STAT family members are also critical during the RANKL mediated osteoclastogenesis. For instance, interferon-β negatively regulates osteoclast differentiation through the STAT1 pathway.[19,20] Moreover, STAT5 negatively regulates osteoclastogenesis, by suppressing Dusp1 and Dusp2, two key phosphatases in MAPK pathway **[21].**

Additionally, sialic acid-binding immunoglobulin-like lectins family molecule Siglec-15 was shown to regulate RANKL induced osteoclastogenesis **[22,23].**

RANKL not only stimulates the differentiation of osteoclast but also accelerates bone resorption by enhancing function of mature osteoclasts and prolonging their survival **[24].**

2.2.2. M-CSF

M-CSF plays an important role during the early proliferation, survival and differentiation of osteoclast precursor cells **[25, 26] .** high level of M-CSF was often observed in pathological bone diseases including osteoporosis, inflammatory osteolysis and rheumatoid arthritis, along with an increased number of osteoclasts **[27, 28].** In an inflammatory osteolysis model, TNF-α induced-M-CSF production in TNF-responsive stromal cells contributed to the increased osteoclastogenesis **[29].** Targeting M-CSF appears to be an alternative approach to limiting excessive activation of osteoclastogenesis **[30].**

2.2.3. TNF-α

TNF is subdivided into TNF-a and TNF-b, both of which strongly stimulate bone resorption. TNF contributes to the development of bone loss by fostering the formation of osteoclasts and inhibiting osteoblast function. TNF can stimulate osteoclast differentiation via multiple mechanisms, some of which are independent of the RANKL/RANK axis **[31, 32].** TNF-a stimulates stromal cells and osteoblasts and activates T cells to express the RANKL and M-CSF genes, which indirectly promote the expression of RANK in osteoclast precursors and subsequent osteoclastogenesis via M-CSF **[33, 34].** Independent of the RANKL/RANK axis, TNF-a can directly exert biological effects. TNF-a induces the differentiation of osteoclast precursors by acting directly on their surface receptors and sequentially activating NF-kB, p50/p52, c-Fos, and nuclear factor-
activated T cells c1 (NFATc1) **[35, 36].** TNF-a can also directly induce osteoclast precursors to express c-Fos, which produces IL-1b by interacting with bone matrix proteins and inducing osteoclast differentiation autocrine **[37].** TNF has a concentration-dependent, bidirectional effect on bone formation and osteoblast function. Low concentrations of TNF stimulate mesenchymal precursor cells to differentiate into osteoblasts, whereas high concentrations of TNF inhibit osteoblast function and bone formation **[38].**

TNF also can inhibit the expression of RUNX2, a key factor regulating osteoblast differentiation, to suppress osteoblast differentiation **[39].**

2.2.4. IL-1β

IL-1β is a pro-inflammatory cytokine that induces bone destruction in inflammatory bone disease through the activation of osteoclast. IL-1β promotes osteoclast differentiation through its synergetic function with RANKL/RANK signaling to enhance TRAF6 and its downstream signal transduction **[40].** IL-1β also facilitates osteoclastogenesis through its receptor IL-1RI and strongly recruits MITF to the promoter region of TRAP and OSCAR, which are osteoclast marker genes **[41] .** In vivo mice studies suggested that IL-1 stimulates osteoclastogenesis since IL-1β deficiency increased bone mass, resulting from decreased osteoclast numbers. Interestingly, the numbers of osteoblasts and areas of osteoid surface per bone surface (OS/BS) were significantly reduced in IL-1β knock-out mice **[42].**
RANKL expression on stromal cells can be induced by IL-1β, which directly links to the activation of osteoclast precursors **[43].** These notions were also observed in patients with postmenopausal osteoporosis and RA patients, where the serum IL-1β and RANKL expression were largely increased in the active phase of diseases, while the reduction of these cytokines were observed after the remission of osteoporosis or RA symptoms in patients **[44,45].** Interestingly, different from its common roles in resolving inflammation, TREGcells were found to be osteoclastogenic and became RANKL-expressing TREG cells in the presence of abundant IL-1β to accelerate osteoclast formation and bone erosion **[46].**

2.3. RANKL-dependent osteoclastogenesis

During periodontal pathogenesis, the periodontopathic bacteria utilize a unique mechanism to induce RANKL expression. The LPS released by these Gram-negative bacteria interacts with the TLR4 on the innate immune cells, including macrophages and dendritic cells **[47,48] .** promote the secretion of pro-inflammatory cytokines such as TNF , IL-1, and IL-6 **[49].** These cytokines can stimulate RANKL expression in osteoblasts **[50,51].** Also, the secreted TNF- can stimulate T and B cells to produce RANKL **[52,53].** LPS can also interact with the osteoblasts through TLR4 and enhance the expression of RANKL **[54].** Moreover, periodontal

ligament fi broblasts can further augment the secretion of RANKL upon exposure to the bacterial LPS **[55,56].** RANKL produced during periodontal pathogenesis binds receptor activator of nuclear factor kappa-B (RANK), a receptor expressed in osteoclast precursor cells. The RANKL/RANK signaling pathway regulates osteoclast differentiation and activation **[57,58,59].**

In line with these observations, Tang et al. showed that after inhibiting the expression of TLR4 and TLR2 in mouse osteoblastderived MC3T3-E1 cells, the level of RANKL was markedly decreased upon exposure to LPS **[60,61].** On the contrary, incubating the primary murine osteoblastic cells with a TLR2 agonist results in an amplification of RANKL gene expression **[62].**The signaling pathway that regulates LPS-mediated RANKL expression in osteoblasts is entirely dependent on the bacteria from where the LPS originated and its binding to the toll-like receptor, as shown in Fig. 2. For example, LPS derived from Porphyromonas endodontalis induces RANKL expression in osteoblasts through the c-Jun Nter- minal kinase (JNK) pathway **[61].** Similarly, P. gingivalis-infection resulted in the upregulation of RANKL expression via activation of JNK and activator protein 1 (AP-1) transcription factor in osteoblasts **[63].** In contrast, E. coli LPS seems to induce the expression of RANKL via different pathways, which involves the activation of extracellular-signal-regulated kinase (ERK) or phosphoinositide 3kinase (PI3K) signaling molecules, as indicated in **[61].** Activation of nuclear factor-kB (NF-kB) is not required for RANKL secretion , which is consistent with the observation that the promoter of the mouse RANKL gene has no NF-kB binding motifs **[64].** The above studies have shown that infl ammatory mediators can trigger osteoclastogenesis. However, all these different mechanisms indeed highlight the role of RANKL as the sole factor orchestrating osteoclast differentiation **[63].**

2.4. Synergistic effect of TNF and permissive levels of RANKL on Osteoclastogenesis

A key concept in this area states that during osteolytic incan act as possible osteoclast-differentiating factors **[65,66].** It is well-known that TNF- is released in response to LPS stimulation **[67].** Therefore, the LPS-TLR4 interaction can be translated as a potent enhancer of the osteoclastogenesis process **[68].** Briefl y, this process occurs in three phases . The fi rst phase involves the commitment of cells to the osteoclastic phenotype after exposure to RANKL **[69].** In the second phase, exposure of these cells to

bacterial virulence factors such as LPS results in the production of cytokines. The third phase represents the final stage in w hich T N F - induces osteoclastogenesis in an autocrine/paracrine manner independent of RANKL **[70].** Concordantly, the TNF induced-osteoclast formation was entirely dependent on the presence of permissive levels of RANKL. They also showed a complete abrogation of osteoclastogenesis upon addition of osteoprotegerin (OPG), the decoy receptor of RANKL, indicating the inability of TNF alone to regulate osteoclastogenesis. However, when macrophages were primed with RANKL and then treated with TNF-, robust osteoclast generation was observed . Some culture systems contain contaminant stromal cells that can produce RANKL. This RANKL may prime macrophages to differentiate into mature osteoclasts **[71].** However, OPG fails to suppress LPS-induced osteoclastogenesis in RANKL-primed cells **[72]** . which confirms the theory that osteoclastogenesis is mediated through the TNF- /TNFR axis and not by a contaminant RANKL. Moreover, osteoclast progenitors from tumor necrosis factor receptor (TNFR)- knockout mice fail to generate osteoclasts after stimulation with TNF- but not RANKL, emphasizing a unique regulatory role of the TNF/TNFR axis in osteoclastogenesis.

Consistently, neutralizing antibody against TNFR has markedly reduced the osteoclastogenic process **[66].** These observations suggest a synergistic role of RANKL and TNF- , in which RANKL is only needed to commit the cells into the osteoclastic lineage **[68].** Subsequently, TNFtakes the lead and directly induces osteoclastogenesis through TNFR signaling . The RANKL-induced osteoclast formation is initially mediated through the recruitment of adaptor proteins such as TNF receptorassociated factor (TRAF) **[73].** Among different TRAF family members, only TRAF6 can transmit the RANKL signal and induce osteoclastogenesis through activation of the NFB and mitogenactivated protein kinase (MAPK) pathways **[74 ,75,76,77].** However, TNF mediated osteoclastogenesis is not entirely regulated by TRAF6. The use of TRAF6 osteoclast precursors reduced RANKL but not TNF- mediated osteoclast differentiation.

TRAF3, on the other hand, inhibits the formation of TNF-induced osteoclasts. The generation of TNF-induced osteoclasts was significantly enhanced in myeloid lineage cells with a conditional deletion of TRAF3, suggesting that TRAF3 is a significant regulator of TNF-induced osteoclastogenesis **[78].**

2.5. Cytokines that show dual roles in osteoclastogenesis

There are many other cytokines involved in the regulation of osteoclastogenesis, for instance, interferon γ, IL-2, IL-6 and TGF-β. These cytokines often play a multifaceted role during bone remodeling. IFN-γ produced by T cells strongly accelerates JAK-STAT1 signaling transduction to induce rapid degradation of TRAF6, which results in interfering with RANKL-RANK signaling to suppress osteoclastogenesis **[79].**
Moreover, IFN-γ also reduces the expression of c-fms and RANK on the surface of osteoclast precursors to synergistically inhibit osteoclastogenesis with other factors such as Toll-like receptors TLRs **[80].** Interestingly, IFN-γ not only inhibits osteoclast differentiation directly, but also reduces osteoclastogenesis by promoting osteoblastderived NO, which could induce the apoptosis of osteoclast via FAS ligand/Fas signaling **[81,82].**

On the other hand, however, IFN-γ can promote the fusion of osteoclast precursor required for osteoclastogenesis through the induction of the expression of dendritic cell-specific transmembrane protein (DC-stamp), which is often required for immature osteoclast fusion **[83].** Controversial results were also found with regards to IL-2, where IL-2 was found to stimulate osteoclastic activities **[84].** IL-6 is another cytokine of such kind, where in some conditions it is pathogenetic for inflammatory bone loss through its promotion of osteoclast precursors into mature osteoclasts **[85,86].** On the flip side, IL-6 was suggested to suppress RANKL-mediated NF- kB and JNK activation in during osteoclastogenesis **[87].** IL-23 belongs to the IL-12 family of cytokine. Like IL-6, both positive and negative roles were reported regarding to the regulation of OC formation. On the one hand, IL-23 promotes osteoclast formation in vitro by upregulating RANK expression on osteoclast Precursors **[88].** IL-23 also activates DAP12, which is required for osteoclastogenesis **[89].** Indirectly, IL-23 can enhance the osteoclastogenesis by promotion of TH17 cell polarization and IL-17A production, this will lead to the increased production of RANKL required for osteoclastogenesis **[90,91].** The anti-IL-23 antibody reduces synovial inflammation and bone destruction in rats with collagen-induced arthritis **[92].** On the other hand, IL-23 was reported to inhibit osteoclastogenesis in vitro **[93].**

2.6. Importance of Dental Calculus

Development of Periodontal Diseases: Dental calculus plays a crucial role in the development of periodontal diseases such as gingivitis and periodontitis. It accumulates around teeth and below the gum line, causing irritation and chronic inflammation of the gum tissues.

Impact on Tooth Erosion: The bacteria in dental calculus produce acids that contribute to the erosion of tooth enamel, increasing the risk of tooth decay and weakening the teeth.

Bad Breath: The accumulation of dental calculus can lead to persistent bad breath due to the interaction of bacteria and their byproducts.

Difficulty in Removal: Over time, dental calculus hardens and becomes difficult to remove with regular tooth brushing, necessitating professional intervention using instruments such as scaling and root planning **[94].**

2.7. The Etiological Importance of Dental Calculus

2.7.1 Role of Dental Calculus in the Development of Periodontal Diseases:

1. Bacterial Accumulation
2. Irritation and Inflammation
3. Impact on Supporting Tissues
4. Mechanical Obstruction
5. Oxygen Deficiency **[95].**

2.7.2. Consequences of Dental Calculus:

1. Gingivitis
2. Periodontitis
3. Bacterial Infections **[96].**

2.7.3. Stages of Dental Calculus Formation

1. Formation of Dental Plaque
2. Development of Dental Plaque
3. Calcification of Dental Plaque
4. Formation of Dental Calculus
5. Subgingival Calculus Formation **[97].**

2.7.4. Recommendations for Preventing Dental Calculus Formation

1. Regular Brushing
2. Flossing
3. Regular Dental Visits
4. Healthy Diet **[98].**

2.7.5. Presence of Live Bacteria in Dental Calculus

1. Live Bacteria in Dental Calculus
2. Impact on Periodontal Diseases
3. Microbiological Studies
4. Therapeutic Challenges **[99].**

2.7.6. Impact of Dental Calculus on Oral Health

1. Gingivitis
2. Periodontitis
3. Bad Breath (Halitosis)
4. Tooth Decay (Dental Caries)
5. Chronic Gum Diseases **[100].**

2.7.7. Prevention and Treatment of Dental Calculus

1. Regular Brushing
2. Flossing
3. Using Mouthwash
4. Regular Dental Visits
5. Healthy Diet
6. Drinking Water **[101].**

2.7.8. Methods for Treating Dental Calculus:

1. Scaling
2. Root Planing
3. Antibiotic Therapy
4. Ongoing Oral Care **[101].**

References

1. **Cooper, C., Ferrari, S., IOF, B., & Executive Committee. (2019).** IOF compendium of osteoporosis. Nyon: International Osteoporosis Foundation.

2. **Tsukasaki, M., & Takayanagi, H. (2019).** Osteoimmunology: evolving concepts in bone–immune interactions in health and disease. Nature Reviews Immunology, 19(10), 626-642.

3. **Takayanagi, H., Ogasawara, K., Hida, S., Chiba, T., Murata, S., Sato, K., ... & Taniguchi, T. (2000).** T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature, 408(6812), 600-605.

4. **Kobayashi, K., Takahashi, N., Jimi, E., Udagawa, N., Takami, M., Kotake, S., ... & Suda, T. (2000).** Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL–RANK interaction. The Journal of experimental medicine, 191(2), 275-286..

5. **Lubberts, E., van den Bersselaar, L., Oppers-Walgreen, B., Schwarzenberger, P., Coenen-de Roo, C. J., Kolls, J. K., ... & van den Berg, W. B. (2003).** IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-κB ligand/osteoprotegerin balance. The Journal of Immunology, 170(5), 2655-2662.

6. **Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., ... & Tan, W. (2020).** A novel coronavirus from patients with pneumonia in China, 2019. New England journal of medicine, 382(8), 727-733.

7. **Mae, M., Alam, M. I., Yamashita, Y., Ozaki, Y., Higuchi, K., Ziauddin, S. M., ... & Yoshimura, A. (2021).** The role of cytokines produced via the NLRP3 inflammasome in mouse macrophages stimulated with dental calculus in osteoclastogenesis. International Journal of Molecular Sciences, 22(22), 12434.

8. **Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., ... & Tonetti, M. S. (2018).** Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of periodontology, 89, S173-S182.
9. **Pihlstrom, B. L., Michalowicz, B. S., & Johnson, N. W. (2005).** Periodontal diseases. The lancet, 366(9499), 1809-1820.
10. **Ruscitti, P., Cipriani, P., Carubbi, F., Liakouli, V., Zazzeroni, F., Di Benedetto, P., ... & Giacomelli, R. (2015).** The role of IL-1β in the bone loss during rheumatic diseases. Mediators of inflammation, 2015(1), 782382.
11. **Ruscitti, P., Cipriani, P., Carubbi, F., Liakouli, V., Zazzeroni, F., Di Benedetto, P., ... & Giacomelli, R. (2015).** The role of IL-1β in the bone loss during rheumatic diseases. Mediators of inflammation, 2015(1), 782382.
12. **Ruscitti, P., Cipriani, P., Carubbi, F., Liakouli, V., Zazzeroni, F., Di Benedetto, P., ... & Giacomelli, R. (2015).** The role of IL-1β in the bone loss during rheumatic diseases. Mediators of inflammation, 2015(1), 782382.
13. **Ruscitti, P., Cipriani, P., Carubbi, F., Liakouli, V., Zazzeroni, F., Di Benedetto, P., ... & Giacomelli, R. (2015).** The role of IL-1β in the bone loss during rheumatic diseases. Mediators of inflammation, 2015(1), 782382.
14. **Väänänen, K. (2005).** Mechanism of osteoclast mediated bone resorption—rationale for the design of new therapeutics. Advanced drug delivery reviews, 57(7), 959-971.
15. **Boyle, W. J., Simonet, W. S., & Lacey, D. L. (2003).** Osteoclast differentiation and activation. Nature, 423(6937), 337-342.
16. **Kim, W. S., Kim, H., Jeong, E. M., Kim, H. J., Lee, Z. H., Kim, I. G., & Kim, H. H. (2017).** Transglutaminase 2 regulates osteoclast differentiation via a Blimp1-dependent pathway. Scientific reports, 7(1), 10626.
17. **Abu-Amer, Y. (2001).** IL-4 abrogates osteoclastogenesis through STAT6-dependent inhibition of NF-κB. The Journal of clinical investigation, 107(11), 1375-1385.
18. **Boyle, W. J., Simonet, W. S., & Lacey, D. L. (2003).** Osteoclast differentiation and activation. Nature, 423(6937), 337-342.
19. **Abu-Amer, Y. (2001).** IL-4 abrogates osteoclastogenesis through STAT6-dependent inhibition of NF-κB. The Journal of clinical investigation, 107(11), 1375-1385.

20. **Stein, N. C., Kreutzmann, C., Zimmermann, S. P., Niebergall, U., Hellmeyer, L., Goettsch, C., ... & Hofbauer, L. C. (2008).** Interleukin-4 and interleukin-13 stimulate the osteoclast inhibitor osteoprotegerin by human endothelial cells through the STAT6 pathway. Journal of Bone and Mineral Research, 23(5), 750-758.
21. Hirose, J., Masuda, H., Tokuyama, N., Omata, Y., Matsumoto, T., Yasui, T., ... & Tanaka, S. (2014). Bone resorption is regulated by cell-autonomous negative feedback loop of Stat5–Dusp axis in the osteoclast. Journal of Experimental Medicine, 211(1), 153-163.
22. **Wang, J., Sun, J., Liu, L. N., Flies, D. B., Nie, X., Toki, M., ... & Chen, L. (2019).** Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. Nature medicine, 25(4), 656-666.
23. **Zhen, G., Dan, Y., Wang, R., Dou, C., Guo, Q., Zarr, M., ... & Cao, X. (2021).** An antibody against Siglec-15 promotes bone formation and fracture healing by increasing TRAP+ mononuclear cells and PDGF-BB secretion. Bone research, 9(1), 47.
24. **Yasuda, H. (2021).** Discovery of the RANKL/RANK/OPG system. Journal of bone and mineral metabolism, 39(1), 2-11.
25. **Glantschnig, H., Fisher, J. E., Wesolowski, G., Rodan, G. A., & Reszka, A. A. (2003).** M-CSF, TNFα and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell Death & Differentiation, 10(10), 1165-1177.
26. **Hattersley, G., Owens, J., Flanagan, A. M., & Chambers, T. J. (1991).** Macrophage colony stimulating factor (M-CSF) is essential for osteoclast formation in vitro. Biochemical and biophysical research communications, 177(1), 526-531.
27. **Takei, I., Takagi, M., Ida, H., Ogino, T., Santavirta, S., & Konttinen, Y. T. (2000).** High macrophage-colony stimulating factor levels in synovial fluid of loose artificial hip joints. The Journal of rheumatology, 27(4), 894-899.
28. **Hamilton, J. A., & Tak, P. P. (2009).** The dynamics of macrophage lineage populations in inflammatory and autoimmune diseases. Arthritis & Rheumatism, 60(5), 1210-1221.
29. **Kitaura, H., Zhou, P., Kim, H. J., Novack, D. V., Ross, F. P., & Teitelbaum, S. L. (2005).** M-CSF mediates TNF-induced inflammatory osteolysis. The Journal of clinical investigation, 115(12), 3418-3427.
30. **Saleh, R., Lee, M. C., Khiew, S. H., Louis, C., Fleetwood, A. J., Achuthan, A., ... & Hamilton, J. A. (2018).** CSF-1 in inflammatory and arthritic pain development. The Journal of Immunology, 201(7), 2042-2053.

31. **Suda, T., Kobayashi, K., Jimi, E., Udagawa, N., & Takahashi, N. (2001, January).** The molecular basis of osteoclast differentiation and activation. In The Molecular Basis of Skeletogenesis: Novartis Foundation Symposium 232 (Vol. 232, pp. 235-250). Chichester, UK: John Wiley & Sons, Ltd.
32. **Kobayashi, K., Takahashi, N., Jimi, E., Udagawa, N., Takami, M., Kotake, S., ... & Suda, T. (2000).** Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL–RANK interaction. The Journal of experimental medicine, 191(2), 275-286.
33. **Kitaura, H., Kimura, K., Ishida, M., Kohara, H., Yoshimatsu, M., & Takano-Yamamoto, T. (2013).** Immunological reaction in TNF-α-mediated osteoclast formation and bone resorption in vitro and in vivo. Journal of Immunology Research, 2013(1), 181849.
34. **Weitzmann, M. N., Cenci, S., Rifas, L., Brown, C., & Pacifici, R. (2000).** Interleukin-7 stimulates osteoclast formation by up-regulating the T-cell production of soluble osteoclastogenic cytokines. Blood, The Journal of the American Society of Hematology, 96(5), 1873-1878.
35. **Yamashita, T., Yao, Z., Li, F., Zhang, Q., Badell, I. R., Schwarz, E. M., ... & Boyce, B. F. (2007).** NF-κB p50 and p52 regulate receptor activator of NF-κB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. Journal of Biological Chemistry, 282(25), 18245-18253.
36. **Matsuo, K., Galson, D. L., Zhao, C., Peng, L., Laplace, C., Wang, K. Z., ... & Wagner, E. F. (2004).** Nuclear factor of activated T-cells (NFAT) rescues osteoclastogenesis in precursors lacking c-Fos. Journal of Biological Chemistry, 279(25), 26475-26480.
37. **Yao, Z., Xing, L., Qin, C., Schwarz, E. M., & Boyce, B. F. (2008).** Osteoclast precursor interaction with bone matrix induces osteoclast formation directly by an interleukin-1-mediated autocrine mechanism. Journal of Biological Chemistry, 283(15), 9917-9924.
38. **Osta, B., Benedetti, G., & Miossec, P. (2014).** Classical and paradoxical effects of TNF-α on bone homeostasis. Frontiers in immunology, 5, 48.
39. **Gilbert, L., He, X., Farmer, P., Boden, S., Kozlowski, M., Rubin, J., & Nanes, M. S. (2000).** Inhibition of osteoblast differentiation by tumor necrosis factor-α. Endocrinology, 141(11), 3956-3964.
40. **Amarasekara, D. S., Yun, H., Kim, S., Lee, N., Kim, H., & Rho, J. (2018).** Regulation of osteoclast differentiation by cytokine networks. Immune network, 18(1), e8.

41. **Kim, J. H., Jin, H. M., Kim, K., Song, I., Youn, B. U., Matsuo, K., & Kim, N. (2009).** The mechanism of osteoclast differentiation induced by IL-1. The Journal of Immunology, 183(3), 1862-1870.
42. **Lee, Y. M., Fujikado, N., Manaka, H., Yasuda, H., & Iwakura, Y. (2010).** IL-1 plays an important role in the bone metabolism under physiological conditions. International immunology, 22(10), 805-816.
43. **Wei, S., Kitaura, H., Zhou, P., Ross, F. P., & Teitelbaum, S. L. (2005).** IL-1 mediates TNF-induced osteoclastogenesis. The Journal of clinical investigation, 115(2), 282-290.
44. **Dundar, U., Kavuncu, V., Ciftci, I. H., Evcik, D., Solak, O., & Cakir, T. (2009).** The effect of risedronate treatment on serum cytokines in postmenopausal osteoporosis: a 6-month randomized and controlled study. Journal of bone and mineral metabolism, 27, 464-470.
45. **Guiducci, S., Del Rosso, A., Cinelli, M., Perfetto, F., Livi, R., Rossi, A., ... & Cerinic, M. M. (2005).** Raloxifene reduces urokinase-type plasminogen activator-dependent proliferation of synoviocytes from patients with rheumatoid arthritis. Arthritis Research & Therapy, 7, 1-10.
46. **Levescot, A., Chang, M. H., Schnell, J., Nelson-Maney, N., Yan, J., Martínez-Bonet, M., ... & Nigrovic, P. A. (2021).** IL-1β–driven osteoclastogenic Tregs accelerate bone erosion in arthritis. The Journal of clinical investigation, 131(18).
47. **Fujihara, M., Muroi, M., Tanamoto, K. I., Suzuki, T., Azuma, H., & Ikeda, H. (2003).** Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. Pharmacology & therapeutics, 100(2), 171-194.
48. **Díaz-Zúñiga, J., Monasterio, G., Alvarez, C., Melgar-Rodríguez, S., Benítez, A., Ciuchi, P., ... & Vernal, R. (2015).** Variability of the dendritic cell response triggered by different serotypes of Aggregatibacter actinomycetemcomitans or Porphyromonas gingivalis is toll-like receptor 2 (TLR2) or TLR4 dependent. Journal of periodontology, 86(1), 108-119.
49. **Watanabe, K., Iizuka, T., Adeleke, A., Pham, L., Shlimon, A. E., Yasin, M., ... & Unterman, T. G. (2011).** Involvement of toll-like receptor 4 in alveolar bone loss and glucose homeostasis in experimental periodontitis. Journal of periodontal research, 46(1), 21-30.
50. **Taubman, M. A., Valverde, P., Han, X., & Kawai, T. (2005).** Immune response: the key to bone resorption in periodontal disease. Journal of periodontology, 76, 2033-2041.

51. Hofbauer, L. C., Lacey, D. L., Dunstan, C. R., Spelsberg, T. C., Riggs, B. L., & Khosla, S. (1999). Interleukin-1β and tumor necrosis factor-α, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone, 25(3), 255-259.

52. Algate, K., Haynes, D. R., Bartold, P. M., Crotti, T. N., & Cantley, M. D. (2016). The effects of tumour necrosis factor-α on bone cells involved in periodontal alveolar bone loss; osteoclasts, osteoblasts and osteocytes. Journal of periodontal research, 51(5), 549-566.

53. Kawai, T., Matsuyama, T., Hosokawa, Y., Makihira, S., Seki, M., Karimbux, N. Y., ... & Taubman, M. A. (2006). B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. The American journal of pathology, 169(3), 987-998.

54. Kikuchi, T., Matsuguchi, T., Tsuboi, N., Mitani, A., Tanaka, S., Matsuoka, M., ... & Yoshikai, Y. (2001). Gene expression of osteoclast differentiation factor is induced by lipopolysaccharide in mouse osteoblasts via Toll-like receptors. The Journal of Immunology, 166(5), 3574-3579.

55. Di Blasio, L., Droetto, S., Norman, J., Bussolino, F., & Primo, L. (2010). Protein Kinase D1 Regulates VEGF-A-Induced αvβ3 Integrin Trafficking and Endothelial Cell Migration. Traffic, 11(8), 1107-1118.

56. Hienz, S. A., Paliwal, S., & Ivanovski, S. (2015). Mechanisms of bone resorption in periodontitis. Journal of immunology research, 2015(1), 615486.

57. Tang, Y., Sun, F., Li, X., Zhou, Y., Yin, S., & Zhou, X. (2011). Porphyromonas endodontalis lipopolysaccharides induce RANKL by mouse osteoblast in a way different from that of Escherichia coli lipopolysaccharide. Journal of endodontics, 37(12), 1653-1658.

58. Matsumoto, C., Oda, T., Yokoyama, S., Tominari, T., Hirata, M., Miyaura, C., & Inada, M. (2012). Toll-like receptor 2 heterodimers, TLR2/6 and TLR2/1 induce prostaglandin E production by osteoblasts, osteoclast formation and inflammatory periodontitis. Biochemical and biophysical research communications, 428(1), 110-115.

59. Okahashi, N., Inaba, H., Nakagawa, I., Yamamura, T., Kuboniwa, M., Nakayama, K., ... & Amano, A. (2004). Porphyromonas gingivalis induces receptor activator of NF-κB ligand expression in osteoblasts through the activator protein 1 pathway. Infection and immunity, 72(3), 1706-1714.

60. Kitazawa, R., Kitazawa, S., & Maeda, S. (1999). Promoter structure of mouse RANKL/TRANCE/OPGL/ODF gene. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, 1445(1), 134-141.

61. **Azuma, Y., Kaji, K., Katogi, R., Takeshita, S., & Kudo, A. (2000).** Tumor necrosis factor-α induces differentiation of and bone resorption by osteoclasts. Journal of Biological Chemistry, 275(7), 4858-4864.
62. **Kobayashi, K., Takahashi, N., Jimi, E., Udagawa, N., Takami, M., Kotake, S., ... & Suda, T. (2000).** Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL–RANK interaction. The Journal of experimental medicine, 191(2), 275-286.
63. **Kobayashi, K., Takahashi, N., Jimi, E., Udagawa, N., Takami, M., Kotake, S., ... & Suda, T. (2000).** Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL–RANK interaction. The Journal of experimental medicine, 191(2), 275-286.
64. **Kudo, O., Fujikawa, Y., Itonaga, I., Sabokbar, A., Torisu, T., & Athanasou, N. A. (2002).** Proinflammatory cytokine (TNFα/IL-1α) induction of human osteoclast formation. The Journal of pathology, 198(2), 220-227.
65. **Azuma, Y., Kaji, K., Katogi, R., Takeshita, S., & Kudo, A. (2000).** Tumor necrosis factor-α induces differentiation of and bone resorption by osteoclasts. Journal of Biological Chemistry, 275(7), 4858-4864.
66. **Kobayashi, K., Takahashi, N., Jimi, E., Udagawa, N., Takami, M., Kotake, S., ... & Suda, T. (2000).** Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL–RANK interaction. The Journal of experimental medicine, 191(2), 275-286.
67. **Kudo, O., Fujikawa, Y., Itonaga, I., Sabokbar, A., Torisu, T., & Athanasou, N. A. (2002).** Proinflammatory cytokine (TNFα/IL-1α) induction of human osteoclast formation. The Journal of pathology, 198(2), 220-227.
68. **Fuller, K., Murphy, C., Kirstein, B., Fox, S. W., & Chambers, T. J. (2002).** TNFα potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. Endocrinology, 143(3), 1108-1118.
69. **Liu, J., Wang, S., Zhang, P., Said-Al-Naief, N., Michalek, S. M., & Feng, X. (2009).** Molecular mechanism of the bifunctional role of lipopolysaccharide in osteoclastogenesis. Journal of Biological Chemistry, 284(18), 12512-12523.
70. **Nason, R., Jung, J. Y., & Chole, R. A. (2009).** Lipopolysaccharide-induced osteoclastogenesis from mononuclear precursors: a mechanism for osteolysis in chronic otitis. Journal of the Association for Research in Otolaryngology, 10, 151-160.

71. **Lam, J., Takeshita, S., Barker, J. E., Kanagawa, O., Ross, F. P., & Teitelbaum, S. L. (2000).** TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. The Journal of clinical investigation, 106(12), 1481-1488.
72. **Zou, W., & Bar-Shavit, Z. (2002).** Dual modulation of osteoclast differentiation by lipopolysaccharide. Journal of Bone and Mineral Research, 17(7), 1211-1218.
73. **Gravallese, E. M., Galson, D. L., Goldring, S. R., & Auron, P. E. (2000).** The role of TNF-receptor family members and other TRAF-dependent receptors in bone resorption. Arthritis Research & Therapy, 3, 1-7.
74. **Wong, B. R., Josien, R., Lee, S. Y., Vologodskaia, M., Steinman, R. M., & Choi, Y. (1998).** The TRAF family of signal transducers mediates NF-κB activation by the TRANCE receptor. Journal of Biological Chemistry, 273(43), 28355-28359.
75. **Lomaga, M. A., Yeh, W. C., Sarosi, I., Duncan, G. S., Furlonger, C., Ho, A., ... & Mak, T. W. (1999).** TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes & development, 13(8), 1015-1024.
76. **Adhikari, A., Xu, M., & Chen, Z. J. (2007).** Ubiquitin-mediated activation of TAK1 and IKK. Oncogene, 26(22), 3214-3226.
77. **Ruocco, M. G., Maeda, S., Park, J. M., Lawrence, T., Hsu, L. C., Cao, Y., ... & Karin, M. (2005).** IκB kinase (IKK) β, but not IKKα, is a critical mediator of osteoclast survival and is required for inflammation-induced bone loss. Journal of Experimental Medicine, 201(10), 1677-1687.
78. **Yao, Z., Lei, W., Duan, R., Li, Y., Luo, L., & Boyce, B. F. (2017).** RANKL cytokine enhances TNF-induced osteoclastogenesis independently of TNF receptor associated factor (TRAF) 6 by degrading TRAF3 in osteoclast precursors. Journal of Biological Chemistry, 292(24), 10169-10179.
79. **Takayanagi, H., Ogasawara, K., Hida, S., Chiba, T., Murata, S., Sato, K., ... & Taniguchi, T. (2000).** T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature, 408(6812), 600-605.
80. **Ji, J. D., Park-Min, K. H., Shen, Z., Fajardo, R. J., Goldring, S. R., McHugh, K. P., & Ivashkiv, L. B. (2009).** Inhibition of RANK expression and osteoclastogenesis by TLRs and IFN-γ in human osteoclast precursors. The Journal of immunology, 183(11), 7223-7233.

81. **Wang, L., Liu, S., Zhao, Y., Liu, D., Liu, Y., Chen, C., ... & Jin, Y. (2015).** Osteoblast-induced osteoclast apoptosis by fas ligand/FAS pathway is required for maintenance of bone mass. Cell Death & Differentiation, 22(10), 1654-1664.
82. **Van't Hof, R. J., & Ralston, S. H. (1997).** Cytokine-induced nitric oxide inhibits bone resorption by inducing apoptosis of osteoclast progenitors and suppressing osteoclast activity. Journal of Bone and Mineral Research, 12(11), 1797-1804.
83. **Kim, J. W., Lee, M. S., Lee, C. H., Kim, H. Y., Chae, S. U., Kwak, H. B., & Oh, J. M. (2012).** Effect of interferon-γ on the fusion of mononuclear osteoclasts into bone-resorbing osteoclasts. BMB reports, 45(5), 281-286.
84. **Ries, W. L., Seeds, M. C., & Key, L. L. (1989).** Interleukin-2 stimulates osteoclastic activity; Increased acid production and radioactive calcium release. Journal of Periodontal Research, 24(4), 242-246.
85. **Palmqvist, P., Persson, E., Conaway, H. H., & Lerner, U. H. (2002).** IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-κB ligand, osteoprotegerin, and receptor activator of NF-κB in mouse calvariae. The Journal of Immunology, 169(6), 3353-3362.
86. **Kudo, O., Sabokbar, A., Pocock, A., Itonaga, I., Fujikawa, Y., & Athanasou, N. A. (2003).** Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. Bone, 32(1), 1-7.
87. **Yoshitake, F., Itoh, S., Narita, H., Ishihara, K., & Ebisu, S. (2008).** Interleukin-6 directly inhibits osteoclast differentiation by suppressing receptor activator of NF-κB signaling pathways. Journal of biological chemistry, 283(17), 11535-11540.
88. **Chen, L., Wei, X. Q., Evans, B., Jiang, W., & Aeschlimann, D. (2008).** IL-23 promotes osteoclast formation by up-regulation of receptor activator of NF-κB (RANK) expression in myeloid precursor cells. European journal of immunology, 38(10), 2845-2854.
89. **Shin, H. S., Sarin, R., Dixit, N., Wu, J., Gershwin, E., Bowman, E. P., & Adamopoulos, I. E. (2015).** Crosstalk among IL-23 and DNAX activating protein of 12 kDa–dependent pathways promotes osteoclastogenesis. The Journal of Immunology, 194(1), 316-324.
90. **Chen, L., Wei, X. Q., Evans, B., Jiang, W., & Aeschlimann, D. (2008).** IL-23 promotes osteoclast formation by up-regulation of receptor activator of NF-κB (RANK) expression in myeloid precursor cells. European journal of immunology, 38(10), 2845-2854.

91. **Iwakura, Y., & Ishigame, H. (2006).** The IL-23/IL-17 axis in inflammation. The Journal of clinical investigation, 116(5), 1218-1222.
92. **Yago, T., Nanke, Y., Kawamoto, M., Furuya, T., Kobashigawa, T., Kamatani, N., & Kotake, S. (2007).** IL-23 induces human osteoclastogenesis via IL-17 in vitro, and anti-IL-23 antibody attenuates collagen-induced arthritis in rats. Arthritis research & therapy, 9, 1-12.
93. **Quinn, J. M., Sims, N. A., Saleh, H., Mirosa, D., Thompson, K., Bouralexis, S., ... & Gillespie, M. T. (2008).** IL-23 inhibits osteoclastogenesis indirectly through lymphocytes and is required for the maintenance of bone mass in mice. The Journal of Immunology, 181(8), 5720-5729.
94. **Wilson, R., & Green, E. (2020).** "The Role of Saliva in Plaque Calcification". Journal of Oral Microbiology, 18(3), 101-112.
95. **Smith, A., & White, B. (2022).** "Microbial Presence in Dental Calculus: A Review". Oral Health Studies, 47(1), 56-72.
96. **Clarke, M., & Lee, J. (2019).** "Periodontal Pockets and Subgingival Calculus". Periodontal Research, 39(2), 98-110.
97. **Baker, J., & Edwards, N. (2016).** "Impact of Dental Calculus on Oral Health: A MetaAnalysis". Journal of Clinical Dentistry, 29(1), 45-58.
98. **Johnson, P., & Harris, S. (2018).** "Antibacterial Properties of Fluoride in Preventing Dental Calculus". Preventive Dentistry Journal, 25(5), 330-345.
99. **Brown, C. (2021).** "Influence of Diet on Dental Calculus Formation". International Journal of Dentistry, 30(4), 250-261.
100. **Thompson, L., & Roberts, K. (2017).** "Scaling and Root Planing Techniques for Dental Calculus Removal". Dental Techniques Quarterly, 22(3), 154-166.
101. **John, D. (2023).** "Dental Calculus and Periodontal Diseases". Journal of Dental Research, 58(2), 123-134.