The interactions and shared mechanisms of T cells and osteoclasts

Hans-Jürgen Gober, and Hiroshi Takayanagi *
Department of Cell Signaling, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University and Global Center of Excellence (GCOE) Program for International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan

During evolution, the bony skeleton and the adaptive immune system have coevolved in vertebrate animals. The shared evolutionary origin and bone marrow microenvironments apparently provided the two systems with common molecules involved in individual cell lineage differentiation and function. In fact, the bone and immune systems share abundant molecules and regulatory mechanisms in the maintenance of physiologic function. Furthermore, dysregulated interactions between the immune and skeletal systems frequently result in pathological conditions affecting both systems. In humans, the most frequent skeletal pathologies are associated with bone loss through the excessive activity of osteoclasts. T cells represent the major regulatory players in bone destruction in inflammation. In this review we focus on the shared mechanisms and interactions between osteoclasts and T cells. Understanding these apparently different cell lineages in an integrated functional context should provide a molecular basis for developing novel therapeutic approaches to bone and inflammatory diseases.

Rec./Acc.7/14/2009, pp239-248

Introduction
The bony skeleton, which is essential in vertebrates, enables weight-bearing locomotive activity, the reversible storage of calcium and the harbouring of the hematopoietic stem cells from which immune cells are derived. Bone is continuously restructured, which demands the degradation of “old” and formation of “new” bone in a tightly controlled manner. This process, called bone remodeling, is dependent on the dynamic balance of bone formation and resorption, which are mediated by osteoblasts and osteoclasts, respectively. An impairment of this balance is often associated with bone diseases, such as osteoporosis and rheumatoid arthritis¹.

Osteoblasts are mesenchymal cells that secrete bone-matrix proteins and promote mineralization². Osteoclasts are large multinucleated cells of hematopoietic origin belonging to the monocyte-macrophage lineage, which resorb bone matrix through
Regulation of osteoclastogenesis by molecules shared with T cells

1) RANKL is essential for osteoclastogenesis and regulates immune tolerance

The close relationship between bone and the immune system came to be appreciated when the osteoclast differentiation factor expressed by osteoblasts was identified as receptor activator of nuclear factor-κB ligand (RANKL) \(^8\), a cytokine belonging to the TNF family and originally discovered in activated T cells \(^9\). RANKL is indispensable for osteoclastogenesis, since Rankl-deficient mice exhibit severe osteopetrosis due to a complete lack of osteoclasts. RANKL is the cytokine which induces osteoclast commitment in monocyte/macrophage lineage cells. Under physiological conditions, osteoclast formation is mediated by RANKL expressed in bone marrow mesenchymal cells. The expression of RANKL is upregulated in these cells by osteoclastogenic factors such as vitamin D\(3\), prostaglandin E\(2\), parathyroid hormone, interleukin (IL)-1, IL-6, IL-11, IL-17 and TNF-\(α\) \(^{10}\). RANKL is a membrane-associated cytokine, although a minor amount is secreted through enzymatic shedding of its ectodomain \(^{11}\). While the role of soluble RANKL in osteoclast differentiation is unclear, it may have relevance as a chemoattractant for cancer metastases, by enabling certain cancer cells to migrate and metastasize to bone tissue \(^{12}\). Among other effects, RANKL can promote bone resorption, which is necessary for the remodeling process. Hence, the balance of RANKL expression in the bone marrow microenvironment is crucial for maintaining normal bone homeostasis.

In the immune system, RANKL seems to be multifunctional and its role is dependent on the RANKL-expressing cell type. T cells expressing RANKL facilitate the survival of interacting dendritic cells \(^{13}\) and accelerate immune reactions in certain autoimmune conditions \(^{14}\). In contrast, keratinocytes express RANKL in response to ultraviolet stimulation of the skin, activating local resident dendritic cells and triggering the expansion of regulatory T (Treg) cells, which suppress immune reactions \(^{15}\). Moreover, RANKL was found to be required in fetal lymphoid tissue inducer cells in the development of secondary lymphoid organs \(^{16}\). Interestingly, RANKL expression in thymocytes is essential for the induction of autoimmune regulator (AIRE) in thymic epithelial cells, a factor required for the elimination of autoreactive thymocytes and the establishment of central tolerance \(^{17,18}\). However, RANKL-deficient mice do not exhibit a severe form of immunodeficiency \(^{19}\). In contrast to the skeletal system, the functional loss of RANKL in the immune system seems to be compensated by other molecules, such as CD40L \(^{20}\).

2) NFAT transcription factors promote T cell and osteoclast development

The discovery of RANKL subsequently led to investigation of the downstream signaling pathway of its receptor RANK in osteoclasts. Nuclear factor of activated T cells c1 (NFATc1), a transcription factor originally discovered in T cells \(^{21}\), was subsequently identified as the master regulator for osteoclast differentiation \(^{22}\). The induction of NFATc1 is necessary and sufficient to induce osteoclast commitment of monocyte/macrophage precursor cells \(^{23}\). While NFATc1 has an exclusive role in osteoclast development, it shares a redundant role with NFATc2 in T cell development and activation \(^{24}\). In osteoclastogenesis, however, the physiological role of NFATc2 seems to be limited to the initial induction of NFATc1 \(^{23}\). The activity of NFAT transcription factors is universally regulated by the phosphatase calcineurin through control of the phosphorylation status of NFAT \(^{25}\). In osteoclasts and T cells, the activity of this enzyme is regulated by calcium-calmodulin signaling \(^{26}\). Unlike NFATc2, which is constitutively transcribed in T cells,
transcription of the NFATc1 gene in osteoclasts needs to be induced by cytoplasmic-resident dimeric transcription factors belonging to the nuclear factor-κB (NF-κB) family and activator protein-1 (AP-1). The ligation of RANK by RANKL activates AP-1, which is a dimeric complex composed of c-fos and c-jun proteins. Mice lacking c-fos develop severe osteopetrosis in the absence of immunodeficiency. Furthermore, NF-κB in particular, a dimer composed of the subunits p50 and p52, is activated by RANKL in osteoclast lineage cells. Deficiency in p50 and p52 leads to osteopetrosis without any cell-intrinsic defects in T cell development. NF-κB activation is essential, but not sufficient for osteoclastogenesis, since several pro-inflammatory cytokines induce NF-κB activation in macrophages, but do not induce NFATc1 or osteoclast commitment.

3) Immunoreceptors and costimulatory signaling in osteoclastogenesis

NFAT activation requires cytoplasmic calcium mobilization, which cannot be triggered by the RANK signal alone. In T cells, induction of the calcium signal requires the involvement of immunoreceptor tyrosine-based activation motif (ITAM)-containing adapter proteins, which subsequently activate ζ-associated protein-70 (ZAP-70) and phospholipase C-γ (PLC-γ). While CD3ζ represents such an adapter protein in T cells, macrophages correspondingly express DNAX-activating protein 12 (DAP12) and Fc receptor common γ-subunit (FcRγ). Indeed, mice deficient in both of the adapter proteins DAP12 and FcRγ develop severe osteopetrosis associated with insufficient osteoclast formation and bone resorption. DAP12 was shown to be essential for efficient osteoclast differentiation in mice and humans. In contrast, the physiologic relevance of FcRγ in this context is relatively unclear, since FcRγ deficiency does not cause any bone defect in mice. There exist at least four immunoglobulin-like receptors in osteoclast lineage cells, including triggering receptor expressed in myeloid cells 2 (TREM-2), signal-regulatory protein β1 (SIRP β1), paired immunoglobulin-like receptor (PIR-A) and osteoclast-associated receptor (OSCAR), which have been reported to associate with DAP12 or FcRγ. However, while TREM-2 was shown to be required for efficient osteoclast formation in human, the in vivo relevance of other immunoreceptors and their corresponding ligands remain to be determined. In vitro osteoclast formation experiments showed that the ligands for DAP12- and FcRγ-associated receptors have a different distribution in osteoblasts and myeloid cell types. However, further studies are required to elucidate this issue. As ITAM-dependent signals alone cannot induce osteoclastogenesis, but rather support RANK signaling for efficient NFATc1 activation and osteoclast formation, this signal is called costimulatory signal for RANK (Fig.1A).

Since costimulatory signals in T cells are frequently accompanied by inhibiting receptors, which terminate or neutralize the initial activation, the involvement of analogous receptors in osteoclasts has been anticipated. Recently, the immunoreceptor tyrosine-based inhibiting motif (ITIM)-containing receptor PIR-B was found to inhibit ITAM-dependent osteoclast differentiation in vitro.

Little is known about the contribution of osteoclast costimulatory signals in pathologic bone resorption. Whereas osteoclast costimulatory signals do not always appear relevant in osteoporosis, such as in post-menopausal osteoporosis model mice, studies conducted on rheumatoid arthritis (RA) model mice revealed that PIR-A receptors are involved in bone loss in inflammation. The identification of ligands for the immunoreceptors involved in osteoclast costimulatory signaling and their tissue distribution is an important issue to understand the relevance of costimulation under physiologic and pathologic conditions.

4) Costimulation regulates the threshold for TCR and RANK signaling

The term costimulation was originally introduced in T cell biology to describe the requirement of naive T cells to receive a second signal besides T cell receptor (TCR) ligation for their activation and commitment to functional effector T cells. Costimulation in this context thus has an exclusive function, since only professional antigen-presenting cells (APCs) can induce adequate expression of costimulatory ligands in response to innate stimuli. Multiple costimulatory receptors have been identified in T cells, and unlike in osteoclasts, several inhibitory receptors have also been revealed. Among all the costimulatory and inhibitory receptors in T cells, CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) are the most prominent and well characterized. Both interact with the ligands B7.1 and B7.2 expressed on APCs. The signaling of CD28 differs from osteoclast costimulatory receptors in some aspects. The signaling by CD28 does not require an adapter protein, but the cytoplasmic domain directly recruits phosphatidylinositol 3 kinase (PI3K) upon activation. While ligation of CD28 lowers the threshold for the TCR signal to support T cell activation, ligation of CTLA-4 increases this threshold by recruitment of protein tyrosine phosphatase-2 (SHP-2) (Fig.1B).

In T cells, the requirement for costimulation helps to maintain peripheral tolerance, since the expression of costimulatory ligands
and activation of naïve T cells is tightly regulated\(^5\). Similarly, the requirement for costimulation in osteoclasts may restrict the generation of osteoclasts to bone tissue, since RANKL is expressed in many tissues outside of bone. Moreover, similar to T cells, where costimulation lowers the threshold for T cell activation, recent studies performed in our lab suggest that costimulation in osteoclast lineage cells lowers the threshold for RANKL-induced NFAT activation and osteoclast formation (Fig.2).

**Regulation of osteoclastogenesis by T cells**

1) Cell contact-dependent interaction between T cells and osteoclast lineage cells

Although RANKL was originally discovered in activated T cells, the direct involvement of T cell-derived RANKL in osteoclast differentiation has thus far not been clearly illustrated. Studies using species-mixed T cell-macrophage cultures\(^6\) and
Fig. 2 Costimulation is required for T cell maturation and osteoclast differentiation
(A) Stimulation of TCR in absence of costimulation is insufficient to induce maturation of naïve T cells into an effector phenotype. Instead, naïve T cells become anergic or undergo apoptosis. (B) In the presence of sufficient costimulation provided by ligands expressed on the APC, naïve T cells mature into cytotoxic or helper (T<sub>H</sub>) cells. In mature T cells, costimulation lowers the threshold for antigen-dependent T cell activation. (C) Macrophages cannot differentiate into osteoclasts in the absence of costimulation, even in the abundant presence of RANKL. (D) Costimulation permits RANKL-dependent osteoclast development and lowers the threshold for RANK-signaling and osteoclast commitment.

paraformaldehyde-fixed T cells<sup>41</sup> did not provide evidence for a physiological interaction between T cells and osteoclast precursor cells. On the other hand, the role of T cell-derived RANKL in alveolar bone destruction was demonstrated in a periodontitis mouse model<sup>42</sup>, although it did not prove the requirement for a direct supporting effect of T cell-expressed RANKL on osteoclast differentiation. Most of the cytokines released by activated T cells, such as interferon-gamma (IFN-γ), inhibit osteoclast differentiation<sup>43</sup>, pointing out the difficulty in obtaining evidence for the supporting effect of inflammatory T cells on osteoclast differentiation. Recently it was reported that the chemokine interferon-γ-inducible protein 10 (IP-10) suppresses IFN-γ production and simultaneously induces RANKL expression in T<sub>H</sub> cells in sufficient amounts to support osteoclastogenesis <em>in vitro</em><sup>44</sup>. However, further studies are required to address the role of IP-10 in bone destruction in inflammation. Studies conducted on the role of T cell-derived RANKL in osteoclast development under non-inflammatory conditions revealed conflicting results: Osteopetrosis in RANKL-deficient mice is partly recovered by crossing these mice with transgenic mice that express RANKL on T<sub>H</sub> cells<sup>45</sup>. In contrast, osteopetrosis in humans with a mutation in RANKL was not cured by bone marrow transfer, suggesting that RANKL on the hematopoietic cells is not sufficient for the physiological level of osteoclast formation<sup>46</sup>. To date there is no direct proof for the involvement of T cell-derived RANKL in osteoclastogenesis under conditions resembling the physiologic interactions between T cells and osteoclast precursor cells. Further studies will be required to elucidate the role of T cell-derived RANKL, in particular mice with a T cell-specific ablation of RANKL would be useful to help address this issue.

Recently, a cell contact-dependent and suppressing effect of T<sub>reg</sub> cells on osteoclast differentiation has been reported. The inhibiting receptor CTLA-4, which is abundantly expressed in T<sub>reg</sub> cells, was found to have a direct inhibiting effect on osteoclast differentiation<sup>47</sup>. However, the relevance of CTLA-4 remains controversial, since studies performed on human cells revealed that the cytokines released by T<sub>reg</sub> cells, such as transforming growth factor-beta (TGF-β), are responsible for their suppressing effect on osteoclast differentiation<sup>48</sup> (Fig. 3).
Clinical implications

In recent years, biological agents and small molecule inhibitors were developed for the treatment of inflammatory diseases accompanied by bone destruction which target molecules expressed in T cells and osteoclasts. A specific antibody to RANKL is undergoing clinical trials for postmenopausal osteoporosis and
RA with promising results\(^{44}\). In addition, an anti-RANKL antibody has been demonstrated to have an apparent preventive effect on tumor bone metastasis in animal studies\(^{11,55}\). Remarkably, treatment with an anti-RANKL antibody has not been reported to show any adverse effects on immune functions in the clinical trials. Furthermore, a fusion protein comprising CTLA-4 and human immunoglobulin has been reported to be effective in clinical trials for RA treatment\(^{50}\). The inhibition of IL-17 demonstrated successful results in the treatment of an experimental arthritis model\(^{57}\), but the clinical relevance of the newly recognized cytokine IL-17 has yet to be tested in human trials. Although it is difficult to specifically target transcription factors and signaling molecules in therapy, NF-κB inhibitors are also under development\(^{50}\). Some currently prescribed anti-rheumatic drugs have been shown to inhibit osteoclastogenesis in vitro by suppressing the induction of NFATc\(^{19,69}\). Specific inhibitors for costimulatory signaling in osteoclasts are not in development yet, although a small compound that inhibits Syk is undergoing a clinical trial\(^{41}\).

Despite these successful results in clinical trials, it is important to keep in mind that undesirable side effects might occur, even in therapies expected to target exclusively either the immune or the skeletal system. Bisphosphonate drugs, which are used to inhibit excessive bone resorption, expand and activate a subpopulation of T cells unique to humans, resulting in a flu-like syndrome\(^{60}\). Treatment with cyclosporine A, used to inhibit immune reactions after organ transplantation, frequently cause osteopenia. Via its molecular target calcineurin, cyclosporine A suppresses the activity of the NFAT transcription factors required for osteoblastic bone formation\(^{63}\). Therapies aimed at inhibiting the activity of TNF-α in RA patients have reactivated latent tuberculosis with lethal outcomes\(^{66}\). An unexpected effect was observed in an animal model of RA treated with a cathepsin K inhibitor. Cathepsin K, abundantly expressed in osteoclasts, carries out a previously unknown function in dendritic cells, and is required for the development of inflammatory T\(\text{h}17\) cells\(^{65}\). Drugs inhibiting cathepsin K were found to ameliorate inflammation in addition to suppressing osteoclastic bone resorption\(^{66}\).

## Conclusion

Osteoclasts and T cells share abundant common molecules and regulatory mechanisms. The term “osteoinmunology” was coined to highlight the reciprocal interactions between immune and skeletal systems\(^{67}\). Researchers have long been aware that both systems utilize an overlapping network of cytokines for intercellular communication, and several cytokines released by immune cells modulate bone homeostasis. Therefore, the evidence that the physiology and pathology of one system can affect the other is compelling. In this review we have focused on the relationship between T cells and osteoclasts, including the molecular mechanisms shared by these two cell types. The involvement of T cells in the bone destruction localized at inflammatory sites has long been acknowledged, but the mechanism could not be explained with the previous model of effector T\(\text{h}\) cell phenotypes. The recent characterization of T\(\text{h}17\) cells and their functional antagonists, T\(\text{reg}\) cells, has afforded a new perspective on the regulation of osteoclast-mediated bone resorption by T cells. A number of biological agents and small molecule drugs have recently been developed, including some which are currently tested in clinical trials, to ameliorate inflammation and bone destruction by targeting certain molecules required for T cell or osteoclast function. However, the molecular mechanisms shared between T cells and osteoclasts indicate that it is essentially impossible to understand the mode of action of drugs targeted to either system without taking into consideration the effects on both. A full-scale understanding of the relationship between osteoclasts and T cells will ultimately lead to novel strategies for the treatment of the inflammatory diseases associated with bone destruction.

## Acknowledgments

This work was supported in part by Grant-in-Aid for Creative Scientific Research, Grant-in-Aid for Challenging Exploratory Research, Grant-in-Aid for JSPS Fellows from the Japan Society for the Promotion of Science (JSPS), Grants-in-Aid for Global Center of Excellence (GCOE) Program from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT). It was also supported by grants from Yokoyama Foundation for Clinical Pharmacology, Takeda Science Foundation, and the Ichiro Kanemara Foundation for the Promotion of Medical Science and Medical Care.

## References

5. Janeway CA Jr: The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol