Mod Rheumatol (2005)15:225–231
DOI 10.1007/s10165-005-0398-6

REVIEW ARTICLE

Hiroshi Takayanagi

Osteoimmunological insight into bone damage in rheumatoid arthritis

Received: March 10, 2005 / Accepted: March 14, 2005

Abstract Research into the bone destruction associated with rheumatoid arthritis has highlighted the importance of the interplay of the immune and skeletal systems. Arthritic bone destruction is attributable to the defective control of osteoclastogenesis by T cells. We revealed that excessive expression of receptor activator of NF-κB ligand (RANKL) and a paucity of interferon-γ underlie the enhanced osteoclastogenesis in arthritis. The interdisciplinary research field called osteoimmunology has attracted further attention after identification of a number of unexpected bone phenotypes in mice lacking immunomodulatory molecules. Accumulating evidence suggests that the immune and skeletal systems share not only cytokines but also various signaling molecules, transcription factors, and membrane receptors. Thus, bone turns out to be a dynamic tissue that is constantly renewed, where the immune system participates to a hitherto unexpected extent. This emerging field will be of great importance for a better understanding and treatment of rheumatic diseases.

Key words Arthritis · Osteoclast · Osteoimmunology · Receptor activator of NF-κB ligand (RANKL) · Stat1

Introduction to osteoimmunology

Homeostasis of the vertebrate skeletal system depends on a dynamic balance of bone-forming osteoblasts and bone-resorbing osteoclasts.[1] This balance must be tightly controlled by various regulatory systems such as the endocrine system. Excessive activity of osteoclasts leads to pathological bone resorption, as seen in a variety of osteopenic diseases: autoimmune arthritis, periodontitis, postmenopausal osteoporosis, Paget's disease, and bone tumors.[2] Therefore, elucidating regulatory mechanisms of osteoclast differentiation is critical for an understanding of the health and disease of the skeletal system.

Immune and skeletal systems have a variety of regulatory molecules, such as cytokines, in common. Furthermore, immune cells form in the bone marrow, interacting with bone cells. Consequently, the physiology and pathology of one system may very well affect the other: abnormal activation of the immune system leads to bone destruction in diseases like rheumatoid arthritis.[3–5] More recently, animal models deficient in immunomodulatory molecules have been found to frequently develop an unexpected skeletal phenotype.[6,7] Thus, the crosstalk between the immune and skeletal systems and the interdisciplinary field called osteoimmunology has attracted much attention in recent years.[8–11]

A key cytokine for osteoclastogenesis: RANKL

Receptor activator of NF-κB ligand (RANKL) is a tumor necrosis factor family cytokine essential for the induction of osteoclastogenesis.[12,13] RANKL was cloned as an activator of dendritic cells expressed by activated T cells, suggesting that this molecule is important in both the skeletal and immune systems.[14,15] RANKL binds to its receptor RANK and stimulates the osteoclastogenic signals in the monocyte/macrophage lineage. RANKL expression on osteoblasts and stromal cells is upregulated by a number of factors that activate bone resorption such as vitamin D3, prostaglandin E2, parathyroid hormone, and interleukin (IL)-1. The targeted disruption of RANKL results in defective formation of the lymph nodes and lymphocyte differentiation, as well as osteopetrosis, a sclerotic bone disease caused by impaired osteoclastic bone resorption.[16] Thus, this molecule

H. Takayanagi (✉)
Department of Cell Signaling, Graduate School, Tokyo Medical and Dental University, and Center of Excellence (COE) Program for Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
Tel. +81-3-5803-5471; Fax +81-3-5803-0192
e-mail: taka.csi@tmd.ac.jp

SORST, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi 332-0012, Japan

explicitly highlighted the close relationship between immune and bone systems.[17]

The role of osteoclast and RANKL in arthritic bone destruction

The molecular link between abnormal immune responses and bone damage has long been a mystery in autoimmune arthritis. Although there had already been circumstantial evidence that the osteoclasts generated from synoviocytes play a critical role,[3] it was not until RANKL was cloned and found to be overexpressed in arthritic joints that researchers widely accepted that osteoclasts indeed do play a critical role in arthritic bone destruction.[4,18] Finally, recent studies have provided genetic evidence that RANKL as well as osteoclasts are central to the inflammatory destruction of bone.[19,20] However, in spite of the critical function of RANKL in the enhanced osteoclastogenesis in arthritis, it remains unclear whether synovial mesenchymal cells (synovial fibroblasts) or T cells are the major RANKL-expressing cells.

T-cell inhibition of osteoclastogenesis by interferon (IFN)-γ

Because T-cell infiltration is a hallmark of rheumatoid synovium, we investigated the effect of activated T cells on osteoclast formation in vitro. However, the unexpected results revealed that activated T cells have a strong suppressive effect on osteoclastogenesis.[5] We therefore hypothesized that the T cells might have a negative regulatory mechanism to counterbalance the action of RANKL, since abnormal bone resorption is not observed during normal T-cell responses despite the expression of RANKL. Using mice lacking a receptor component for IFN-γ, we revealed that the IFN-γ produced by T cells strongly suppresses osteoclastogenesis by interfering with the RANKL signaling pathway.[5] Thus, activated T cells not only positively regulate, but also negatively affect osteoclastogenesis. Rather, it is more appropriate to say that T cells are not osteoclastogenic under most physiological conditions.

How is T-cell inhibition of osteoclastogenesis avoided in arthritis?

The question thus arises: how can antiosteoclastogenic T cells have a positive effect on osteoclastogenesis under pathological conditions? T cells must possess a specific pathological mechanism to suppress IFN-γ-mediated inhibition of osteoclastogenesis, which is normally dominant in activated T cells. This is why abnormal bone resorption is observed only in pathological conditions such as arthritis but not in normal immune responses.[8]

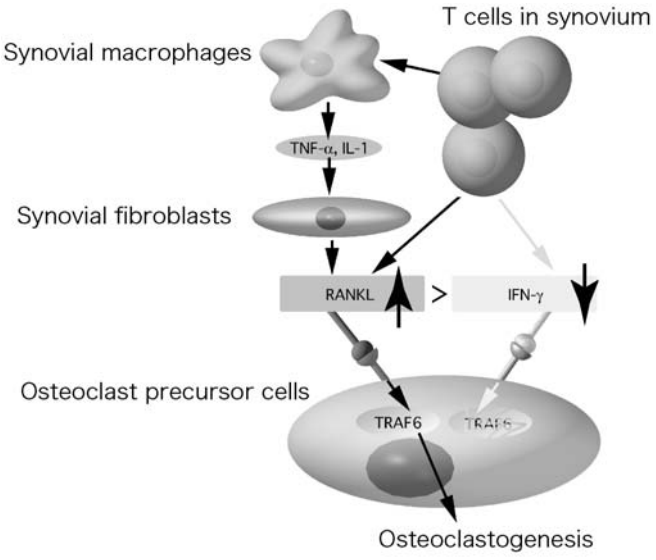

Fig. 1. Mechanism of arthritic bone destruction. Activated T cells have a positive effect on osteoclastogenesis in an indirect manner in vivo: they stimulate the macrophages to secrete proinflammatory cytokines such as tumor necrosis factor α (*TNF-α*) and interleukin-1 (*IL-1*), which strongly induce receptor activator of NF-κB ligand (*RANKL*) in synovial fibroblasts. In addition, T cells express RANKL themselves, but the contribution remains controversial. On the other hand, there is a very low level of interferon γ (*IFN-γ*), which is a major T-cell cytokine that inhibits osteoclastogenesis. This imbalance may be responsible for the aberrant activation of osteoclast formation in arthritis

A possible mechanistic scheme of the bone destruction initiated by T cells in arthritis is summarized in Fig. 1. RANKL is abundantly expressed in synovial fibroblasts stimulated with inflammatory cytokines such as IL-1 or tumor necrosis factor (TNF)-α in addition to RANKL in T cells. Interestingly, despite the significant T-cell infiltration observed in arthritic joints, IFN-γ expression in these T cells is suppressed.[21,22] Therefore, the paucity of IFN-γ and the enhanced expression of RANKL may underlie the activation of osteoclastogenesis in arthritis. It is currently unknown why the T cells which have infiltrated into rheumatoid synovium have such "frustrating features," i.e., an expression of surface markers for memory T cells, a low production of IFN-γ or IL-2, and hyporesponsiveness to in vitro restimulation.[22] We believe that synovial cells of mesenchymal origin are a major source of RANKL in arthritic joints, and that the RANKL expressed in T cells may have relatively limited contribution, as discussed below.

Contribution of T cells to pathogenesis and osteoclastogenesis in arthritis

Bone destruction in rheumatoid arthritis is initially triggered by the activation and infiltration of T cells, which eventually enhances the expression of RANKL in synoviocytes as well as in T cells themselves. T cells may

play an important role not only as an initial trigger but also a constant stimulator of bone destruction, mainly by inducing inflammatory cytokines (e.g., TNF-α, IL-1) and RANKL in synovial fibroblasts. Although RANKL is expressed in T cells,[23] T cells also produce inhibitors of RANKL such as IFN-γ[5] and IL-4.[24] Thus, the direct effect of T cells on osteoclastogenesis depends on a dynamic balance among the cytokines they produce. It is tenable that even if RANKL is expressed on T cells, IFN-γ and other cytokines inhibit RANKL signaling, impeding T cells from participating in the direct positive control of osteoclastogenesis. The distribution of T cells in synovium is not always adjacent to bone destruction sites, where osteoclasts are abundant, lending further support to the notion T cells mainly act on osteoclastogenesis in an indirect manner.

Are T cells absolutely required for osteoclastogenesis in rheumatoid arthritis? Lipopolysaccharide (LPS)-induced bone destruction or collagen-induced arthritis can be induced in mice lacking T cells.[25,26] Therefore, T-cell-mediated reactions are not essential for osteoclastogenesis, at least in some models of inflammatory bone destruction. Although the evidence for the considerable contribution of T cells to the exacerbation of bone destruction in rheumatoid arthritis must be admitted, the enhanced expression of RANKL in synoviocytes induced by synovial inflammation may still be part of the critical molecular basis for osteoclastogenesis in arthritis. The activation of T cells should be understood to be one of the causes initiating this inflammation, but the direct effect of RANKL expressed on T cells appears to have relatively weak influence on osteoclastogenesis in vivo. Needless to say, further studies are necessary to determine exactly how T cells contribute to osteoclastogenesis in various diseased conditions including rheumatoid arthritis.

Involvement of T cells in osteoporosis

Although it is well documented that IFN-γ has a bone protective effect in antigen-specific autoimmune arthritis,[27,28] recent studies suggest that IFN-γ may have a causal role in the bone loss associated with estrogen deficiency.[29,30] Pacifici and colleagues propose that IFN-γ activates antigen presentation through class II transactivator (CIITA) induction leading to the accumulation of a TNF-α-producing T-cell population, but there is little evidence that estrogen deficiency results in the activation of antigen-specific immune reactions. It is an interesting hypothesis that T-cell immunity is also involved in the pathogenesis of postmenopausal osteoporosis, but careful interpretation is still needed at this point since the antigens that activate T cells in estrogen deficiency are unknown, and the specific mechanism by which enhanced antigen presentation leads to the generation of bone-sparing T-cell population is not well understood.[31]

Negative feedback regulation of RANKL signaling by IFN-β

During the course of a genome-wide screening of the target genes induced by RANKL, multiple IFN-α/β–inducible genes in osteoclast precursor cells were detected. This led us to investigate the bone phenotype of mice deficient in an IFN-α/β receptor component, IFNAR1.[6] These mice spontaneously develop marked osteopenia (low bone mass) accompanied by enhanced osteoclastogenesis. We found that RANKL induces the *IFN*-β gene in osteoclast precursor cells and that IFN-β inhibits differentiation by interfering with the RANKL-induced expression of c-Fos, an essential transcription factor for osteoclastogenesis. Interestingly, unlike the case of induction by viruses, *IFN*-β gene induction by RANKL is not dependent on interferon regulatory factor (IRF)-3/IRF-7, but on c-Fos. Thus, a unique autoregulatory mechanism operates, wherein the RANKL-induced c-Fos induces its own inhibitor. A series of studies thus have placed both the IFN-α/β and -γ systems in the context of osteoimmunology, indicating that these cytokines are critical not only for immune responses but also for bone homeostasis under both physiological and pathological conditions.[5,32]

Novel function of Stat1 in bone remodeling

Despite the distinct mechanisms of regulating osteoclast differentiation by IFN-β and IFN-γ, Stat1 is critically involved in both mechanisms to suppress RANKL signaling (Fig. 2). These mechanisms are consistent with the canonical activation pathway of Stat1 dependent on its phosphorylation: Stat1 resides in the cytoplasm in the latent form, and the binding of IFNs to their receptors results in the activation of Jak family kinases, which include phosphorylate tyrosine 701 of Stat1. Phosphorylated Stat1 leads to the activation of transcription factors, which include ISGF3 (a heterotrimeric complex consisting of Stat1, Stat2, and IRF-9) and IFN-γ-activated factor (GAF; a Stat1 homodimer).[33,34] It was also reported that unphosphorylated Stat1 is involved in the transcriptional control,[35] but there has been no evidence that Stat1 has a physiological function in the cytoplasm.

To investigate the physiological function of Stat1 in the skeletal system, we examined the bone phenotype of *Stat1*$^{-/-}$ mice.[7] Consistent with the observation that Stat1 is required for the IFN-β-mediated inhibition of osteoclastogenesis, we found an increased osteoclast number and enhanced osteoclastic bone resorption in *Stat1*$^{-/-}$ mice. However, the *Stat1*$^{-/-}$ mice had an increased bone mass, unlike the *IFNAR1*$^{-/-}$ mice that exhibited osteopenia. This unexpected bone phenotype of the *Stat1*$^{-/-}$ mice prompted us to examine the status of bone-forming osteoblasts in these mice. Bone morphometric analysis revealed a notable increase in bone formation rate and other osteoblast parameters such as osteoid surface/thickness and osteoblast sur-

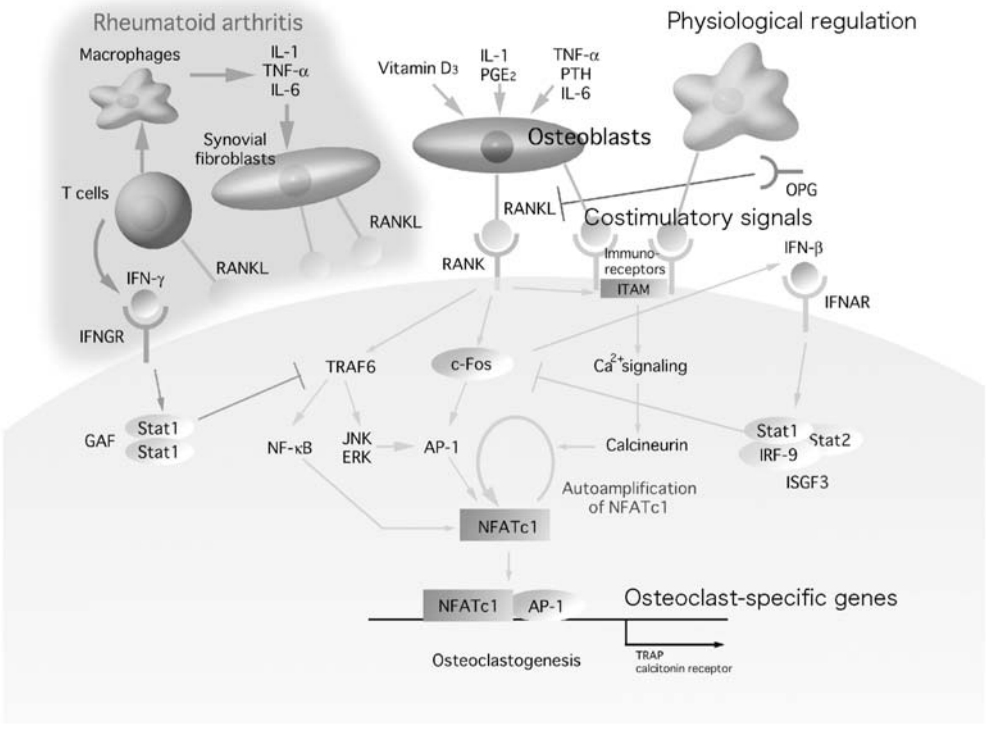

Fig. 2. Signaling crosstalk between the immune and skeletal systems in osteoimmunology. Receptor activator of NF-κB ligand (*RANKL*) binds to its receptor, RANK, and activates essential signaling for osteoclastogenesis including TNF receptor-associated factor 6 (TRAF6), c-Fos, and calcium pathways. The transcription factor nuclear factor of activated T cells c1 (NFATc1) integrates these pathways, acting as a master switch for osteoclast differentiation. Immunoglobulin-like receptors associated with immunoreceptor tyrosine-based activation motif (ITAM)-harboring adaptors are a novel type of essential receptor for osteoclastogenesis, acting as costimulatory molecules for RANKL. Under inflammatory conditions, the interferon (*IFN*)-γ produced by activated T cells inhibits RANKL signaling by downregulation of TRAF6 expression. In addition to osteoprotegerin (*OPG*), IFN-β, which is induced by RANKL, is an important physiological regulator of RANKL signaling, by inhibiting the expression of c-Fos. Immunomodulatory factors often influence RANKL expression in mesenchymal cells such as osteoblasts and modulate RANKL signaling through complex signaling crosstalk

face, suggesting that excessive osteoblast differentiation is responsible for the increased bone mass. In vitro osteoblast differentiation was significantly enhanced in the absence of Stat1. These results indicate that Stat1 interferes with osteoblast differentiation and bone formation.

Cytoplasmic attenuation of Runx2 by Stat1

To gain insights into the mechanism by which Stat1 interferes with the osteoblast differentiation program, we examined the role of Stat1 in signaling events induced by bone morphogenetic protein (BMP)-2, a potent osteogenic cytokine. Briefly, BMP-2 binds to its receptor, which induces the phosphorylation of Smad family proteins such as Smad1, Smad5, and Smad8, resulting in the formation of a trimeric complex with Smad4.[36] The Smad complex translocates to the nucleus and cooperates with another class of transcription factor, Runx2, to activate the transcription of osteoblast-specific genes.[37] The expression of Stat1 resulted in a strong inhibition of the Runx2-dependent activation of the *osteocalcin* promoter, without affecting the Smad1-dependent promoter activation, suggesting that Stat1 selectively interferes with the transcriptional activity of Runx2. Indeed, an electrophoretic mobility shift assay (EMSA) using the Runx2-binding probe revealed that the DNA-binding activity of Runx2 is upregulated in *Stat1*$^{-/-}$ osteoblasts.[7] Although a study using Runx2 transgenic mice suggested that the abnormal expression of Runx2 at the late stage of osteoblast differentiation may affect bone formation,[38] Stat1 mice provide an interesting model in which the temporal Runx2 expression is normal but its activity is enhanced.

Is the inhibitory effect of Stat1 on Runx2 activity dependent on its activation by phosphorylation? In the context of the signaling of IFNs and other cytokines, Stat1 is activated by phosphorylation at tyrosine 701. Interestingly, the phosphorylation of tyrosine 701 of Stat1 was barely detectable in osteoblasts irrespective of BMP-2 stimulation. In addition, the inhibitory effect on Runx2 was exerted by a mutant form of Stat1 in which tyrosine 701 is converted to phenylalanine, showing that the inhibition of Runx2 activity by Stat1 is independent of phosphorylation.

Finally, we investigated the association between Stat1 and Runx2, and found that the inhibitory effect of Stat1 on Runx2 is dependent on the physical interaction between the two molecules. The overexpression of Runx2 resulted in the

nuclear localization of the protein, but the coexpression of Stat1 with Runx2 maintained Runx2 in the cytoplasm, inhibiting the nuclear localization and transcriptional activity of Runx2. Thus, unphosphorylated Stat1 residing in the cytoplasm associates with Runx2 and attenuates the activity of Runx2. The mode of action of Stat1 is unique in that it interferes with another transcription factor, Runx2, in the cytoplasm in its transcriptionally latent form.

NFATc1 is the master transcription factor for osteoclastogenesis

To gain insights into the mechanism underlying the RANKL-specific induction of the osteoclast differentiation program, we further pursued a genome-wide screening approach to identify the genes specifically induced by RANKL in BMMs.[39] In this screening, we found that *NFATc1*, a member of the NFAT family of transcription factor genes,[40] is the most strongly induced transcription factor gene following RANKL stimulation. The transcription factors of the NFAT family, originally discovered in the context of T cell activation,[41] are also involved in the function and development of diverse cells in other biological systems, where they are under the control of a calcium-regulated phosphatase, calcineurin.[42,43]

RANKL also induces and activates NFATc1 through calcium signaling, and calcineurin inhibitors such as FK506 and cyclosporin A strongly inhibit osteoclastogenesis. Interestingly, the FK506-mediated inhibition of NFATc1 activity results in a defective induction of the mRNA of *NFATc1*, indicating that *NFATc1* induction is dependent on its own activity: NFATc1 autoamplifies its own gene, possibly by binding to its own promoter. We believe that this autoamplification of the *NFATc1* gene is the most specific event in osteoclast differentiation.

The necessary and sufficient role of the *NFATc1* gene in osteoclastogenesis has been demonstrated by the observation that *NFATc1*$^{-/-}$ embryonic stem (ES) cells cannot differentiate into osteoclasts and that the ectopic expression of NFATc1 causes BMMs to undergo osteoclast differentiation in the absence of RANKL. Therefore, we propose that NFATc1 comprises the master switch for the terminal differentiation of osteoclasts (Fig. 2).[11,39]

NFATc1 has promise as a therapeutic target for inflammatory destruction of bone

Leflunomide is one of the disease-modifying antirheumatic drugs that have been shown to inhibit bone destruction in clinical trials. Leflunomide prevents the proliferation of activated lymphocytes by inhibiting a key enzyme of de novo pyrimidine synthesis, dihydroorotate dehydrogenase. However, it was unclear whether the drug suppresses bone destruction by acting directly on osteoclasts.

We evaluated the effect of leflunomide on RANKL-induced osteoclast differentiation and found that leflunomide inhibits osteoclast differentiation due to a blockade of de novo pyrimidine synthesis.[26] Leflunomide also inhibits the RANKL-induced calcium signaling in osteoclast precursor cells, hence strongly inhibiting the induction of *NFATc1*. Importantly, a marked expression of NFATc1 in osteoclasts in rheumatoid joints was detected, revealing the clinical relevance of NFATc1 for the bone destruction which occurs in arthritis. Thus, the RANKL-dependent *NFATc1* induction pathway presents itself as an auspicious target for pharmacological intervention.

Calcium signaling and immunoreceptor tyrosine-based activation motif (ITAM)-harboring adaptors

Despite the revelation of the importance of the calcium-NFAT pathway, it remained unclear how RANKL activates calcium signals leading to the induction of *NFATc1*, because RANK belongs to the TNF receptor family that is not directly related to calcium signaling. Therefore, we became interested in a report by Kaifu et al., that in vitro osteoclast differentiation is severely inhibited in mice deficient in DAP12, a membrane adaptor molecule containing ITAM that activates calcium signaling in immune cells.[44] In spite of the in vitro blockade of osteoclast differentiation, DAP12-deficient (*DAP12*$^{-/-}$) mice exhibit only mild osteopetrosis and possess a normal number of osteoclasts in bone tissue, suggesting that DAP12-mediated signals play a critical role in the RANKL/M-CSF-induced culture system but other molecules can help overcome DAP12 deficiency in vivo. Consistent with a normal number of osteoclasts in vivo, we observed that *DAP12*$^{-/-}$ BMMs undergo osteoclast differentiation when the BMMs are cocultured with osteoblasts. This indicates that osteoblasts stimulate the signal that compensates for the loss of DAP12.

We hypothesized that the compensating molecule is FcRγ, and generated mice deficient in both molecules (*DAP12*$^{-/-}$*FcRγ*$^{-/-}$ mice). *DAP12*$^{-/-}$*FcRγ*$^{-/-}$ mice exhibit severe osteopetrosis due to a defect in the differentiation of osteoclasts.[45] Another group also independently generated the same double knockout mice and reported a similar phenotype.[46] The retroviral transfer of *DAP12* into *DAP12*$^{-/-}$ *FcRγ*$^{-/-}$ cells, but not the *DAP12* mutant deficient in ITAM, efficiently rescued osteoclast differentiation, suggesting that the ITAM signal is indispensable for RANKL-induced osteoclastogenesis. In addition, it was found that calcium signaling and *NFATc1* induction was impaired in *DAP12*$^{-/-}$ *FcRγ*$^{-/-}$ cells. This indicates that the ITAM signal is critical for calcium signaling in the osteoclast lineage as well as in lymphocytes.

Immunoglobulin-like receptors and costimulatory signals for osteoclastogenesis

FcRγ or DAP12 associates with several specific immunoreceptors for cell activation in myeloid lineage

cells and NK cells.[47,48] Among the multiple candidate immunoreceptors, we identified the pairing of PIR-A and OSCAR with FcRγ, and that of TREM-2 and SIRPβ1 with DAP12 in osteoclast lineage cells. The triggering of either receptor by crosslinking with an antibody accelerated RANKL-induced osteoclast differentiation, indicating the activating role of these immunoglobulin-like receptors in osteoclastogenesis. However, in the absence of RANKL, the stimulation of these receptors alone was not able to induce osteoclast differentiation, suggesting that these receptor-mediated signals act cooperatively with RANKL but cannot substitute for the signal. Therefore, we propose that such immunoreceptor-ITAM signaling should be labeled "costimulatory signals" for RANKL, since their function is analogous to that of costimulatory signals for the activation of immune cells such as T cells. It is now clear that RANKL and M-CSF are not sufficient for osteoclastogenesis. Immunoreceptors need to be stimulated to activate the requisite ITAM-dependent costimulatory signals.[45]

Conclusion

The discovery of the RANKL–RANK system has brought about rapid progress in the understanding of the regulatory mechanisms of osteoclast differentiation exerted by the immune system. However, osteoclast differentiation is not only regulated by RANKL. Novel regulators include IFN-β, IFN-γ, and other immunomodulatory cytokines. NFATc1, the master transcription factor for osteoclastogenesis, led us to realize the importance of calcium-mobilizing immunoreceptors in addition to RANK and the M-CSF receptor. The osteoimmunological insight will be increasingly beneficial in developing novel therapeutic strategies for rheumatic diseases in the near future.

Acknowledgments This work was supported in part by the PRESTO and SORST programs of JST, grants for the Genome Network Project from Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT), grants for the 21st century COE program, Grants-in-Aid for Scientific Research from JSPS and MEXT, Health Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan, grants from the Mitsubishi Foundation, The Kato Trust for Nambyo Research, Takeda Science Foundation, Daiwa Securities Health Foundation, The Naito Foundation, Kowa Life Science Foundation, Suzuken Memorial Foundation, Kato Memorial Bioscience Foundation, and Uehara Memorial Foundation.

References

1. Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. Dev Cell 2002;2:389–406.
2. Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science 2000;289:1508–14.
3. Takayanagi H, Oda, H, Yamamoto S, Kawaguchi H, Tanaka S, Nishikawa T, et al. A new mechanism of bone destruction in rheumatoid arthritis: synovial fibroblasts induce osteoclastogenesis. Biochem Biophys Res Commun 1997;240:279–86.
4. Takayanagi H, Iizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, et al. Involvement of receptor activator of nuclear factor κB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 2000;43:259–69.
5. Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, et al. T cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature 2000;408:600–5.
6. Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, et al. RANKL maintains bone homeostasis through c-Fos-dependent induction of *interferon-β*. Nature 2002;416:744–9.
7. Kim S, Koga T, Isobe M, Kern BE, Yokochi T, et al. Stat1 functions as a cytoplasmic atttenuator of Runx2 in the transcriptional program of osteoblast differentiation. Genes Dev 2003;17:1979–91.
8. Arron JR, Choi Y. Bone versus immune system. Nature 2000;408: 535–6.
9. Alliston T, Derynck R. Interfering with bone remodelling. Nature 2002;416:686–7.
10. Baron R. Arming the osteoclast. Nat Med 2004;10:458–60.
11. Takayanagi H. Mechanistic insight into osteoclast differentiation in osteoimmunology. J Mol Med 2005; 2005;83:170–9.
12. Lacey DL, Timms E, Tan HL, Kelly MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165–76.
13. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998;95:3597–602.
14. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997;390:175–9.
15. Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, et al. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 1997;272:25190–4.
16. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999;397: 315–23.
17. Theill LE, Boyle WJ, Penninger JM. RANK-L and RANK: T cells, bone loss, and mammalian evolution. Annu Rev Immunol 2002;20:795–823.
18. Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E, et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum 2000;43:250–8.
19. Pettit AR, Ji H, von Stechow D, Muller R. Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001;159:1689–99.
20. Redlich, K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, et al. Osteoclasts are essential for TNF-α-mediated joint destruction. J Clin Invest 2002;110:1419–27.
21. Firestein GS, Zvaifler NJ. How important are T cells in chronic rheumatoid synovitis? Arthritis Rheum 1990;33:768–73.
22. Kinne RW, Palombo-Kinne E, Emmrich F. T-cells in the pathogenesis of rheumatoid arthritis: villains or accomplices? Biochim Biophys Acta 1997;1360:109–41.
23. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402: 304–9.
24. Mirosavljevic D, Quinn JM, Elliott J, Horwood NJ, Martin TJ, Gillespie MT. T-cells mediate an inhibitory effect of interleukin-4 on osteoclastogenesis. J Bone Miner Res 2003;18:984–93.
25. Plows D, Kontogeorgos G, Kollias G. Mice lacking mature T and B lymphocytes develop arthritic lesions after immunization with type II collagen. J Immunol 1999;162:1018–23.
26. Urushibara M, Takayanagi H, Koga T, Kim S, Isobe M, Morishita Y, et al. The antirheumatic drug leflunomide inhibits osteoclastogenesis by interfering with receptor activator of NF-κB ligand-stimulated induction of nuclear factor of activated T cells c1. Arthritis Rheum 2004;50:794–804.
27. Manoury-Schwartz B, Chiocchia G, Bessis N, Abehsira-Amar O, Batteux F, Muller S, et al. High susceptibility to collagen-induced arthritis in mice lacking IFN-γ receptors. J Immunol 1997;158: 5501–6.

28. Vermeire K, Heremans H, Vandeputte M, Huang S, Billiau A, Matthys P. Accelerated collagen-induced arthritis in IFN-γ receptor-deficient mice. J Immunol 1997;158:5507–13.
29. Gao Y, Qian WP, Dark K, Toraldo G, Lin AS, Guldberg RE, et al. Estrogen prevents bone loss through transforming growth factor beta signaling in T cells. Proc Natl Acad Sci USA 2004;101:16618–23.
30. Cenci S, Toraldo G, Weitzmarnn MN, Roggia C, Gao Y, Qian WP, et al. Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-©-induced class II transactivator. Proc Natl Acad Sci USA 2003;100:10405–10.
31. Teitelbaum SL. Postmenopausal osteoporosis, T cells, and immune dysfunction. Proc Natl Acad Sci USA 2004;101:16711–2.
32. Takayanagi H, Kim S, Koga T, Taniguchi T. Stat1-mediated cytoplasmic attenuation in osteoimmunology. J Cell Biochem 2005;94: 232–40.
33. Taniguchi T, Takaoka A. The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. Curr Opin Immunol 2002;14:111–6.
34. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998;67:227–64.
35. Chatterjee-Kishore M, Wright KL, Ting JP, Stark GR. How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene. EMBO J 2000;19:4111–22.
36. Massague J. How cells read TGF-β signals. Nat Rev Mol Cell Biol 2000;1:169–78.
37. Ito Y, Miyazono K. RUNX transcription factors as key targets of TGF-β superfamily signaling. Curr Opin Genet Dev 2003;13:43–7.
38. Liu W, Toyosawa S, Furuichi T, Kanatani N, Yoshida C, Liu Y, et al. Overexpression of Cbfa1 in osteoblasts inhibits osteoblast maturation and causes osteopenia with multiple fractures. J Cell Biol 2001;155:157–66.
39. Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling for terminal differentiation of osteoclasts. Dev Cell 2002;3:889–901.
40. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol 1997;15:707–47.
41. Shaw JP, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR. Identification of a putative regulator of early T cell activation genes. Science 1988;241:202–5.
42. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 2000;1:11–21.
43. Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. Cell 2002;109 Suppl:S67–79.
44. Kaifu T, Nakahara J, Inui M, Mishima K, Momiyama T, Kaji M, et al. Osteopetrosis and thalamic hypomyelinosis with synaptic degeneration in DAP12-deficient mice. J Clin Invest 2003;111:323–32.
45. Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, et al. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. Nature 2004;428:758–63.
46. Mocsai A, Humphrey MB, Van Ziffle JA, Hu Y, Burghardt A, Spusta SC, et al. The immunomodulatory adapter proteins DAP12 and Fc receptor γ-chain (FcRβ) regulate development of functional osteoclasts through the Syk tyrosine kinase. Proc Natl Acad Sci USA 2004;101:6158–63.
47. Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. Nat Rev Immunol 2001;1:41–9.
48. Takai T. Roles of Fc receptors in autoimmunity. Nat Rev Immunol 2002;2:580–92.