
Special Issue: Osteoimmunology and vascular biology

Brief Review

Introduction to the special issue “The interaction among bone, immunology and vascular biology”

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Bone enables locomotive activity, the storage of calcium, and the harboring of the hematopoietic stem cells from which blood and immune cells are derived. Bone is a dynamic organ under the active control of bone cells as well as hematopoietic cells and the vascular system. The immune, skeletal and vascular systems share various molecules, including cytokines, signaling molecules, transcription factors and membrane receptors. The dynamic interplay among these systems is observed in a variety of diseases, including rheumatoid arthritis and various cancers in addition to normal development. The framework of the osteo-vascular-immune system has become increasingly important for an independent understanding the biology of each system and will help provide a scientific basis for future therapeutic approaches to diseases related to these systems.

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Introduction

The endocrine system has traditionally been understood to be the main regulatory system of bone cells, including osteoblasts, osteoclasts, osteocytes and chondrocytes; however, bone contains not only bone cells, but also immune cells and a wealth of vessels¹⁾. Accumulating evidence shows that bone, immune and

vascular cells interact with one another and share a number of regulatory molecules, including cytokines, receptors, signaling molecules and transcription factors. Furthermore, immune cells are themselves derived from bone marrow or vascular cells, and thus develop in the same microenvironment as bone cells^{2,3)}. The dynamic interplay among these systems is observed in a variety

of diseases, including rheumatoid arthritis and cancer, in addition to normal development and physiological maintenance. The framework of the osteo-vascular-immune system has become recognized as increasingly important for the understanding the biology of each system and will provide a scientific basis for future therapeutic approaches to diseases related to these systems. Here, we summarize recent progress in the newly established interdisciplinary field of osteoimmunology and provide perspective on the relationship with vascular biology¹⁻³.

The crucial role of RANKL in physiological and pathological bone resorption

In 1998, the osteoclast differentiation factor was identified as a transmembrane protein of the tumor necrosis factor (TNF) superfamily^{2, 4}. Interestingly, immunologists cloned the same molecule as a stimulator of dendritic cells expressed by T cells, and called it either receptor activator nuclear factor- κ B ligand (RANKL) or TNF-related activation-induced cytokine (TRANCE)^{2, 4}. RANKL expression is upregulated by certain osteoclastogenic factors, such as vitamin D₃, prostaglandin E₂, parathyroid hormone, interleukin (IL)-1, IL-6, IL-11, IL-17 and TNF- α ^{5, 6}. However, the major source of RANKL *in vivo* remains unclear, since RANKL is expressed by several different cell types in both the bone and bone marrow, including osteoblasts, osteocytes, stromal cells and lymphocytes^{2, 4}. Recently, we demonstrated that osteocytes embedded within the bone matrix express a much higher amount of RANKL and have a much greater capacity to support osteoclastogenesis than either osteoblasts or stromal cells⁷. Furthermore, the crucial role of RANKL that is expressed by osteocytes was confirmed by the severe osteopetrotic phenotype observed in mice specifically lacking RANKL in osteocytes. These results clearly indicate that the osteocytes are the major source of RANKL in the bone remodeling process *in vivo*⁷. In arthritis models, RANKL-deficient mice are protected from bone loss, indicating that RANKL plays a crucial role in the bone destruction that occurs in arthritis⁸. RANKL has been shown to function as a chemotactic factor that recruits RANK-expressing tumor cells, suggesting an additional and unexpected pathological role in malignant tumor^{9, 10}.

The master transcription factor for osteoclastogenesis, NFATc1

The RANKL receptor, RANK, lacks intrinsic enzymatic activity in its intracellular domain, and transduces signals by recruiting adaptor molecules². TNF receptor-associated factor (TRAF) 6 is the main adaptor molecule that links RANK to the

differentiation and function of osteoclasts¹¹⁻¹³. The trimerization of TRAF6 leads to the activation of nuclear factor- κ B (NF- κ B) and mitogen-activated kinases (MAPKs)¹⁴. The activator protein 1 (AP-1) transcription factor complex is also essential for osteoclastogenesis¹⁵. RANKL potently induces nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), the master regulator of osteoclast differentiation, and this induction is dependent on both the TRAF6-NF- κ B and the c-Fos pathways¹⁶. The NFAT family of transcription factors was originally discovered in T cells, but this family is involved in the regulation of various biological systems^{17, 18}. The activation of NFAT is mediated by a specific phosphatase, calcineurin, which is activated by calcium-calmodulin signaling.

Phospholipase C γ (PLC γ), which mediates Ca²⁺ release from intracellular stores, is crucial for the activation of the key transcription factor NFATc1 via calcineurin¹⁶. The activation of PLC γ by RANK requires the protein tyrosine kinase Syk, along with immunoreceptor tyrosine-based activation motif (ITAM)-bearing molecules, such as DNAX-activating protein (DAP12) and the Fc receptor common gamma chain (FcR γ)¹⁹. As ITAM signals are essential for osteoclastogenesis, but by themselves cannot induce the process, these signals should properly be considered costimulatory signals for RANK. Thus, the ITAM signal is critical for calcium signaling in the osteoclast lineage as well as in lymphocytes. It is surprising and of considerable interest that most of the molecules involved in osteoclastogenic signalling have been identified in the immune system.

The crucial role of osteoclasts and Th17 in the bone loss which occurs in arthritis

In rheumatoid arthritis (RA), it has long been a challenging question how abnormal T-cell activation induces bone damage². We demonstrated efficient osteoclastogenesis in primary synovial cell cultures obtained from RA patients²⁰. Moreover, high RANKL expression has been detected specifically in the synovium of RA patients^{21, 22}. As RANKL is expressed in activated T cells, T cells might directly induce osteoclast differentiation by directly acting on osteoclast precursor cells under pathological conditions^{23, 24}. However, the interferon- γ (IFN- γ) produced by T cells potently suppresses RANKL signaling²⁵. The effects of T cells on osteoclastogenesis would be expected to be dependent on the balance between positive and negative factors expressed by the T cells. As the CD4⁺ T helper (Th) cell subsets Th1 and Th2 produce IFN- γ and IL-4, respectively, both of which are anti-osteoclastogenic, it has been a paradox how the activated CD4⁺ T cells in arthritis enhance osteoclastogenesis in the pres-

ence of these cytokines.

Data from our laboratory indicate that an IL-17 producing T_H cell subset (T_H17 cells) represents the long-sought-after T_H Oc cell subset among the known $CD4^+$ T cell lineages, whereas T_H1 and T_H2 cells in contrast have anti-osteoclastogenic effects²⁵. It has been reported that IL-17 expression is increased in RA joints²⁶. It is well established that IL-17 induces local inflammation in autoimmune diseases through inflammatory cytokine production²⁷⁻²⁹. Moreover, IL-17 induces RANKL on mesenchymal cells²⁶. Therefore, the infiltration of T_H17 cells into the inflammatory lesion links the abnormal T-cell response to the bone damage which takes places in arthritis, and the pathogenesis of RA should be reconsidered as a T_H17 -type disease from both the immunological and bone points of view.

The role of bone cells in the control of hematopoietic stem cells

These findings collectively indicate that the bone and the immune systems share a number of critically important molecules, and the interaction of bone and immune cells involved in the regulation of the skeletal system. It has been suggested that osteoblasts comprise a niche that is required for the maintenance of hematopoietic stem cells^{30,31}. Since it has been shown that the localization of CXCL12-expressing (CAR) cells (which are critically important for the colonization of hematopoietic stem cells) does not always match with that of osteoblasts, and CAR cells surround the vessels in bone marrow³², it remains unclear how bone-forming activity is related to the function of the hematopoietic niche, thus making osteoblast function in the regulation of hematopoietic stem cells an intriguing subject for future study. Osteoclasts are also suggested to have a role in the mobilization of hematopoietic cells from the bone marrow³³. If osteoclasts are indeed required for the immune response, it would further highlight bone as an immunological organ, but a great deal of work lies ahead before this can be definitively concluded. In addition, a recent report showed that osteoblasts provide an osteoclast niche, suggesting the basic importance of osteoblasts in the maintenance of other bone marrow cells³⁴.

Conclusion

Endochondral bone formation is dependent on the resorption of calcified cartilage after the invasion of vessels into the bone ossification center. The bone and immune systems share a remarkable array of molecules and regulatory mechanisms, but the vascular system also plays a role in this intimate relationship. It remains unclear how distinct functions are affected

through similar mechanisms. As the bone, the immunity and the vascular system are so intermingled, all factors that regulate one system should be investigated for their effect on other systems. This approach will be facilitated by the increasing availability of genetically modified mice. Such animal models will surely lead to a deeper understanding of the molecular basis for the cell lineage specifications, and, perhaps most importantly, cell type-specific treatments, despite the similarity and indeed, overlap of the three systems.

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