

Review in histology and cell biology

## Osteoimmunology represents a link between skeletal and immune system

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### Abstract

There is a complex interplay between the cells of the immune system and bone. These interactions are not only mediated by the release of cytokines and chemokines but also by direct cell-cell contact. Studies of intracellular signaling mechanisms in osteoclasts have revealed that numerous immunomodulatory molecules are involved in the regulation of bone metabolism. Recently, it was proposed that immunoreceptors found in the immune cells are also an essential signal for osteoclasts activation, along with receptor activator of NF- $\kappa$ B (RANK) ligand (RANKL). Collectively, these and similar observations regarding cross-regulation between the immune and skeletal systems constitute the field of osteoimmunology. Here we briefly highlight core areas of interest and selected recent advances in this field.

### Key words

Osteoimmunology, RANKL, RANK, Bone, Immune system

### Introduction

The term osteoimmunology was used for the first time in 2000 (Arron et al., 2000) to describe the interaction of cells of the immune and skeletal systems. In fact, one year before, receptor activator of NF- $\kappa$ B (RANK) ligand (RANKL) was found in T lymphocytes and described as a regulator of dendritic cell and osteoclast function, having an important role in promoting osteoclastogenesis (Rho et al, 2004; Takayanagi, 2012). Immune and skeletal systems have several regulatory factors in common, such as cytokines, transcription factors and receptors. Consequently, these two systems interact with each other in both physiological and pathological conditions. In particular, pathological activation of one system affects the other, such as in the case of rheumatoid arthritis where abnormal activation of the immune system affects bone remodelling leading to pathological bone erosions. During chronic inflammation, the balance between bone formation and resorption is skewed towards osteoclast-mediated resorption. Moreover, in inflamed joints osteoclasts are located at the interface between the inflamed synovium and bone and, as in physiological conditions, the RANKL/RANK/OPG system is the major player in bone resorption (Herman et al., 2008).

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## **RANK/RANKL/OPG system**

### Rank receptor

RANK belongs to the TNFR superfamily. It is synthesized as a type I transmembrane protein of 616 amino acids and assembles into a functional trimer. RANK is expressed by several tissues, such as skeletal muscle, thymus, liver, colon, mammary glands, prostate, pancreas, and by several cell types, such as cells of the monocyte/macrophage lineage including precursors and mature osteoclasts, B and T lymphocytes, dendritic cells, fibroblasts, and articular chondrocytes. The activation of RANK stimulates the differentiation of osteoclastic precursors into mature osteoclasts as well as the activation of mature osteoclasts.

### Rankl cytokine

RANKL is also called TNF-related activation-induced cytokine, TRANCE; osteoclast differentiation factor, ODF; and osteoprotegerin ligand, OPGL. It is a type II transmembrane protein belonging to the TNF superfamily, whose gene was cloned fifteen years ago by four different groups contemporaneously (Anderson et al., 1997; Wong et al., 1997; Lacey et al., 1998; Yasuda et al., 1998). It exists predominantly in a membrane-bound form, with a short cytoplasmic N-terminal domain and a single transmembrane region, but a soluble form can be generated through alternative splicing (Ikeda et al., 2001) or through the cleavage by matrix metalloproteinases and ADAMs (disintegrin and metalloproteinase domain-containing proteins) (Lum et al., 1999; Hikita et al., 2007; Hikita et al., 2009). RANKL aggregates into homotrimers through conserved and specific residues in the extracellular domain, and trimerization is essential for the activation of its cognate receptor RANK (Lam et al., 2001). Recently, the crystal structure of human RANKL in complex with its decoy receptor osteoprotegerin (OPG) has been determined, showing that in this case a different mode of interaction takes place, directly blocking the accessibility of residues of RANKL important for RANK recognition (Luam et al., 2012; Nelson et al., 2012). RANKL is broadly expressed, including both skeletal and extra-skeletal sites and many diverse cell types: mammary epithelial cells, keratinocytes, vascular endothelial cells, and synovial fibroblasts (Lacey et al., 1998; Narducci et al., 2009; Nicolin et al., 2008). In the bone, it is produced mainly by cells of mesenchymal origin, osteoblasts, hypertrophic chondrocytes, and bone marrow (BM) stromal cells; recently, osteocytes have been identified as another source of this cytokine (Atkins et al., 2000). In particular, concerning the immune system RANKL is produced by several cell types including monocytes, neutrophils, dendritic cells, B and T lymphocytes. In this way, immune cells have the ability to induce osteoclast differentiation and, consequently, bone resorption. Also, these cells are known for producing a variety of pro-inflammatory cytokines that contribute to bone damage by potentiating the effects of the RANK–RANKL signalling (Herman et al., 2008).

### OPG cytokine

First in 1997 two independent groups identified a potent inhibitor of osteoclastogenesis named osteoprotegerin (OPG) (Lacey et al., 1998; Simonet et al., 1997) by

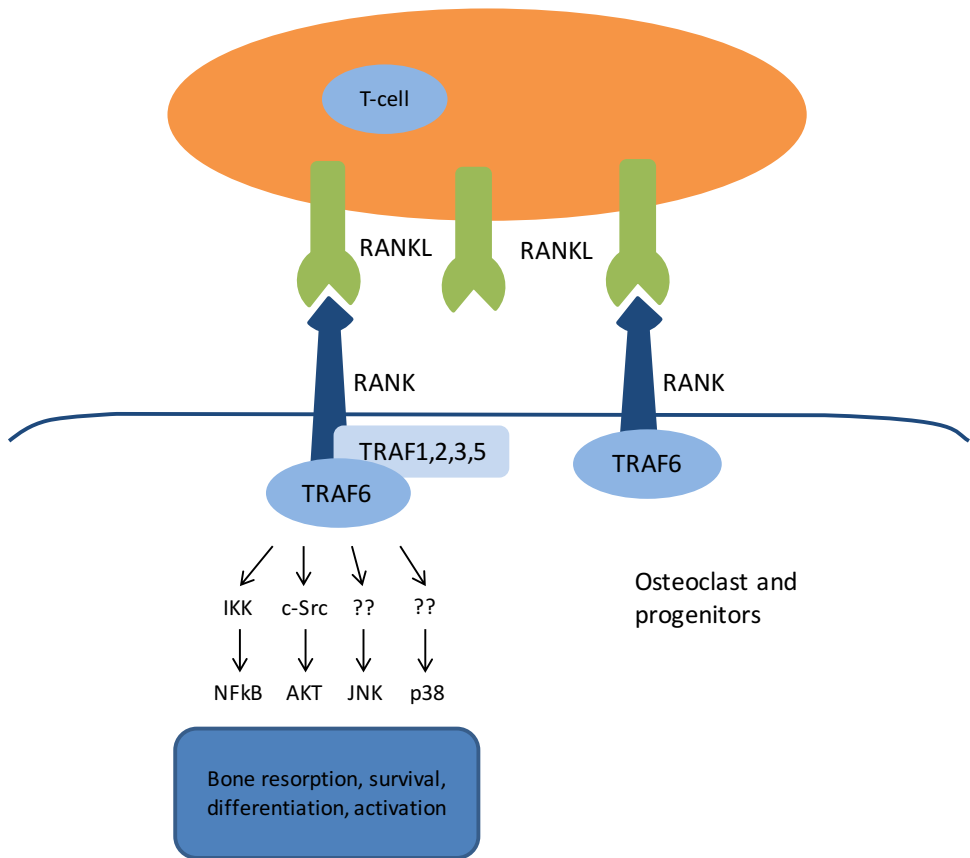
Amgen investigators in the USA and osteoclastogenesis inhibitory factor (OCIF) (Tsu-da et al., 1997; Matsuzaki et al., 1998) by investigators in Japan. OPG belongs to the TNF receptor (TNFR) superfamily, but differs from the common membrane TNFR in that it is soluble. OPG is produced as a pro-protein of 401 amino acids and undergoes an intracellular cleavage resulting in a final 380 amino acids mature protein (Khosla et al., 2001). OPG functions as an endogenous antagonist receptor that prevents the biological effects of RANKL, both membranous and soluble, and thus acts as an inhibitor of bone remodelling/resorption. OPG is highly expressed in the adult lung, heart, kidney, liver, thymus, lymph nodes and bone marrow, osteoblasts, vascular smooth muscle cells, B lymphocytes, and articular chondrocytes (Simonet et al., 1997). OPG can directly inhibit osteoclast activity, independently of RANKL, through interactions with receptors present on osteoclasts. The biological effects of OPG on bone cells include inhibition of the terminal stages of osteoclast differentiation, suppression of mature osteoclast activation and induction of apoptosis of these cells.

### **Role of the RANK/RANKL/OPG pathway in bone resorption**

The RANK/RANKL/OPG signaling pathway is essential for osteoclastogenesis, as previously discussed: the binding of RANKL (produced by osteoblasts) to RANK, located on the surface of osteoclast precursors, recruits the cytoplasmic adapter protein tumour necrosis factor receptor-associated factor 6 (TRAF6), leading to NF- $\kappa$ B activation and translocation to the nucleus; in the nucleus, the translocated NF- $\kappa$ B increases the expression of c-Fos; the interaction of c-Fos with nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), leads to increased expression of various osteoclastogenic genes; consequently, osteoclast formation is increased resulting in increased bone resorption (Fig.1).

### **RANK/RANKL/OPG key triad of osteoimmunology**

The generation of thymus microenvironment which will allow self-tolerance and avoidance of auto-immunity to be established by the deletion of self-reactive T cells. Moreover, RANKL has been shown to play a role in a pathological model of inflammatory bowel disease by stimulating dendritic cells, suggesting that RANKL is distinctly involved in the activation of dendritic cells under certain autoimmune conditions. It has been shown that some members of the tumor necrosis factor (TNF) superfamily play major roles in regulating bone metabolism. The molecular triad composed of OPG/RANK/RANKL, members of this superfamily, has been described as a key cytokine system for controlling the differentiation and function of osteoclast biology. Moreover, independent of the bone system, studies have also revealed new and important functions of this triad in other pathologies and tissues including the immune system. For example, RANKL produced by B cells also contributes to bone resorption during periodontal infection and to the increase in osteoclasts and in trabecular bone loss upon estrogen withdrawal. Based on these interconnections, the RANKL/RANK axis has been defined an essential regulator of both immune responses and bone physiology. Several reports have highlighted the cross-talk



**Figure 1** – Interplay between RANK and RANKL during bone resorption.

between immune and bone cells through this molecule, and the importance of the contribution of immune cells becomes particularly evident in pathological conditions. The RANKL cytokine has been a pioneering discovery in the field of osteoimmunology; the elucidation of its signalling pathway has shown the first of the many, and continuously increasing, interconnections between bone and immune systems.

Advances in the understanding of bone biology and the role of the RANK/RANKL/OPG pathway have opened new treatment avenues for osteoporosis.

### Conflict of interests

All authors state that they have no conflict of interests to declare. The authors thank Fondazione Casali and Fondazione CRTrieste for financial support to their research.

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