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Reveiw

# Receptor activator of nuclear factor-κB ligand in T cells and dendritic cells communication

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**ABSTRACT**

The receptor activator of NF-κB ligand (RANKL), a member of the tumor necrosis factor ligand family, has extensive functions beyond osteoclast development. RANKL is expressed in many immune cells such as osteoblasts, osteocytes, marrow stromal cells, activated T cells, synovial cells, keratinocytes, and mammary gland epithelial cells as well as in various tissues. The ligation of RANK by RANKL promotes dendritic cells (DCs) survival through prosurvival signals and the up-regulation of the anti-apoptotic proteins Bcl-2 and Bcl-$x_L$ and plays a crucial role in DCs-mediated Th1 differentiation. Therefore, RANKL plays an important role in the regulation of DCs/T cells-mediated specific immunity. This review will briefly inform our current understanding of the role of RANKL signaling in T cells-DCs communication in the immune system.

**Keywords** RANKL, dendritic cells, T cells, differentiation, immunity

## INTRODUCTION

### Receptor activator of nuclear factor-κB ligand (RANKL) history

In 1981, Rodan and Martin proposed a new hypothesis that bone resorbing hormones-induced osteoblast products directly activate osteoclast formation (Rodan and Martin, 1981). The study of the osteoclast formation *in vitro* co-culture system of embryonic bone rudiments and hematopoietic cells strongly supported this hypothesis (Bruger et al., 1984). However, how osteoblasts/stromal cells activated osteoclast development was not fully understood until the osteoclast differentiation factors (ODF) were determined. Simonet et al. (1997) identified a novel member of the tumor necrosis factor receptor (TNFR) superfamily, termed osteoprotegerin (OPG). OPG was encoded by the *Tnfrsf11b* gene and a secreted protein unlike other members of the TNFR superfamily. This protein arrested osteoclast maturation in vitro and protected bones from normal osteoclast remodeling and ovariectomy-associated bone loss (Simon et al., 1997). At around the same time, Eisuke and coworkers at Snow Brand Milk Products Co. also purified an identical molecule, termed the ostogenesis inhibitory factor, from human embryonic lung fibroblasts (Tsuda et al., 1997). Soon, they molecularly cloned an osteoprotegerin ligand (OPGL) using OPG as a probe in 1998 (Lacey et al., 1998; Yasuda et al., 1998). OPGL was a long-sought ODF and found to be identical to the TNF-related activation-induced cytokine/RANKL, which exerts a dendritic-cell (DCs) function (Anderson et al., 1997).

### RANKL

RANKL is expressed on a wide variety of different cell lineages and tissues, including osteoblasts, osteocytes, marrow stromal cells, activated T cells, synovial cells, keratinocytes, mammary gland epithelial cells, thymus, spleen, bone marrow, heart, skeletal muscle, lung, stomach, placenta, thyroid gland, and brain (Anderson et al., 1997; Fata et al., 2000; Kartsogiannis et al., 1999; Lacey et al., 1998; Loser et al., 2000; Schett et al., 2005: Wada et al., 2006; Wong et al., 1997; Yasuda et al., 1998). Structurally, RANKL (encoded by the *Tnfsf11* gene located in human chromosome 13q14) is a type II transmembrane molecule which contains a membrane anchoring domain, a long extracellular stalk, and a receptor-binding ectodomain (Ikeda et al., 2001; Lam et al., 2001; Wong et al., 1997). The extracellular domain of RANKL self-assembles into stable homotrimers with four unique surface loops that are necessary for the engagement and activation of its signaling receptor (Ito et al., 2002; Lam et al., 2001). Intriguingly, there are three isoforms of RANKL, which is named RANKL1, RANKL2, and RANKL3. Both RANKL1 and RANKL2 are membrane-bound proteins of ~40 - 45 kDa, but RANKL2 has a shorter intracellular domain. This fact may cause the different distribution of these proteins and the functional differences. In contrast, RANKL3 lacks the intracellular or transmembrane domain and is secreted into extracellular environments as a soluble form of 31 kDa (Table 1) (Ikeda et al., 2001). Initially, RANKL is released as a membrane-bound form and sheds from the plasma membrane by the proteolytic action of TNF-α convertase which is a member of a distintegrin and metalloprotease domain family or a related metalloprotease (Lum et al., 1999). Nakashima et al. (2000) proposed that cytokines mediating osteoclastogenesis such as IL-1β, IL-6, IL-11, IL-17, and TNF-α seem to regulate the ectodomain shedding of RANKL by the activation of metalloprotease. In osteclastgoenesis, the membrane-anchored RANKL is more effective than soluble RANKL (Nakashima et al., 2000).

### Receptor activator of nuclear factor-κB (RANK), a cognate receptor of RANKL

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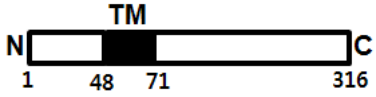
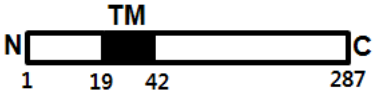
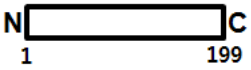
**Table 1.** Isoforms of RANKL

| Isoforms | Intracellular distribution | Structure | |
|---|---|---|---|
| **RANKL1** | EM, Golgi networks, Cytoplasm, Membrane |   | 316 amino acids |
| **RANKL2** | EM, Golgi networks |   | 287 amino acids |
| **RANKL3** | EM, Golgi networks, Cytoplasm |   | 199 amino acids |

EM; endoplasmic reticulum, TM; transmembrane. (Ikeda et al., 2001).

The RANK was identified as a signaling receptor for RANKL by two groups independently in the mid to late 1990s (Anderson et al., 1997; Nakagawa et al., 1998). RANK is encoded by the *Tnfrsf11a* gene located in the human chromosome 18q22.1 and a type I transmembrane protein of 616 amino acids with a extracellular domain of 184 amino acids and a cytoplasmic domain of 383 amino acids (Anderson et al., 1997). Moreover, it belongs to the TNFRs, so RANK should form trimeric complexes on cell surface prior to RANKL binding owing to signaling transduction (Francis et al., 2000). RANK is strongly expressed in skeletal muscles, bone, thymus, liver, colon, small intestine and adrenal gland and also detected in the spleen, bone marrow and several human cell lines such as K-562 (chronic myelogenous leukaemia), HFF (human foreskin fibroblasts), MP-1 (B lymphoblastoid line), A-172 (glioblastoma), and WI-26 (SV40-transformed lung fibroblast) (Anderson et al., 1997, Nakagawa et al., 1998). In addition, RANK is found to be expressed in mammary epithelial cells and several cancer cells, such as breast and prostate cancers (Chen et al., 2006; Kim et al., 2006).

**RANKL in T cell–DCs communication**

DCs play an important role in the regulation of specific immunities by presenting antigens to T cells. In particular, CD40, a member of TNFR family, is widely known to increase the functional activities of DCs (Caux et al., 1994). Initially, Anderson et al. isolated RANK, which has a 40% conserved extracellular domain with CD40, as a new TNFR family in DCs and also found its ligand, RANKL, by direct expression screening (Anderson et al., 1997).

RANKL is expressed on the activated $CD4^+$ and $CD8^+$ T cells by the T cell receptor/CD3 complex, but not on resting T cells, whereas high levels of RANK are expressed on mature DC (Wong et al., 1997). Although T and B cells slightly express RANK on their surfaces, RANKL was not able to activate T and B cells (Josien et al, 1999). The induction of RANKL in T cells is dependent on the protein kinase C, phosphoinositide 3-kinase and calcineurin-mediated signaling pathways (Kong et al., 1999). When RANKL derived from activated T cells binds to RANK on DCs, it activates intracellular tumor necrosis factor receptor-associated factor family proteins, which mediates NF-κB and MAPK signaling pathways correlated with cell proliferation, survival and differentiation (Akiyama et al., 2012; Wong et al., 1998). Actually, Wong et al reported that RANKL was a DCs-specific survival factor, because RANKL/RANK signaling via TRAF2 increased DCs survival by upregulating Bcl-$x_L$ expression known as an anti-apoptotic molecule and the co-stimulatory molecule CD40 on DCs (Anderson et al., 1997; Wong et al., 1997). Moreover, RANKL rearranged CD40L/CD40-independent $CD4^+$ T cell priming against to viral infections (Bachmann et al., 1999). Activated T cells express CD40L, whereas DCs express their receptors, CD40, on their surfaces. CD40L/CD40 signaling in DCs plays a crucial role in the inducing of the expressions of co-stimulatory molecules and IL-12 in DCs, which potentiate $CD4^+$ T cell responses. However, it is observed that $CD4^+$ T cells produced normal levels of IFN-γ in CD40L- or CD40-deficient mice upon viral infection, suggesting a CD40L/CD40-independent mechanism of $CD4^+$ T cell priming. Bachmann et al. found that RANKL and RANK interaction was an additional mechanism of $CD4^+$ T cell stimulation during viral infection and it was required for $CD4^+$ T cell stimulation in the absence of CD40L/CD40 (Bachmann et al., 1999). This result suggests the role of RANKL in memory T cell responses. Interestingly, RANKL also regulates Th1 differentiation through the up-regulation of IL-12 in DCs. Activated DCs by RANKL produce various cytokines, including IL-12, IL-15, IL-1, and IL-6. Mice lacks CD40L, which is a critical factor involved in IL-12 production in DCs, also produced IL-2 and exerted Th1 differentiation. This result suggested that RANKL plays a crucial role in DCs-mediated Th1 differentiation. In contrast, IL-4, which is a key cytokine involved in Th2 differentiation, inhibited RANKL expression on T cells (Josien et al., 1999).

## CONCLUSION

RANKL signaling in the function of immune regulatory cells, such as DCs and helper T cells might open the possibility to provide a novel molecular paradigm for disorders of the immune system.

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## CONFLICT OF INTEREST

The author has no conflicting financial interests.

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