

Interaction of Human Recombinant Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand and Osteoprotegerin Could Contribute to Enhancement of the Erosive Processes Induced by Human Synovial Cells

To the Editor:

Rheumatoid arthritis (RA) is a chronic inflammatory disease for which the etiology is unknown. Patients with RA are known to have lower bone mineral density and are at risk of pathological fracture caused by cytokines produced by RA synovial fibroblasts, which have the potential to promote osteoclast formation and bone resorption. It is now clear that osteoclast formation and activation at the cartilage-pannus junction is an essential step in the destruction of bone matrix in RA^{1,2}.

Our aim was to investigate key factors that regulate focal bone erosion in RA, and their relationships with human recombinant tumor necrosis factor (TNF)-related apoptosis-inducing ligand (hr-TRAIL), in order to propose a strategy to block bone destruction in RA.

A number of inflammatory cytokines found in the RA synovial tissue [interleukin 1 (IL-1), IL-1 β , IL-6, TNF- α , and macrophage colony-stimulating factor] have the potential to promote osteoclast formation and bone resorption^{3,4}. Cells within RA synovial fibroblasts also are substantial sources of soluble receptor activator of nuclear factor- κ B (sRANKL) and osteoprotegerin (OPG), establishing a contribution of these cytokines to the process of erosion⁵.

Bone erosion depends mainly on the synergistic action of these cytokines, where RANKL, produced by osteoblasts, fibroblasts, and T cells, and receptor activator of nuclear factor B (RANK) are mainly expressed on pre-osteoclasts, possibly of the macrophage lineage⁶⁻⁹. TRAIL, a member of the TNF protein family along with RANK and RANKL¹⁰, with which it shares homology, could be a key factor in this process. TRAIL is able to induce cell death¹¹ through association with the death-domain-containing receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5)^{12,13}. In addition to the 2 decoy receptors already discussed, a third decoy receptor and a fifth receptor for Apo2L/TRAIL has also been described, namely OPG¹⁰. OPG is a soluble member of the TNF receptor family, for which the best-described action is the inhibition of RANKL-stimulated osteoclast formation. OPG can bind to RANKL and prevent interaction with its cognate receptor, RANK; however, OPG can also interact with Apo2L/TRAIL¹⁰. Since the prevalence of OPG expression is a critical determinant of the degree to which RANKL can stimulate osteoclast generation and activation, we examined the expression of OPG and RANKL proteins in RA synovial fibroblasts. Interestingly, Colucci, *et al*¹⁴ recently demonstrated that osteoclastogenesis can still occur *in vitro* in the presence of high OPG concentrations due to the formation of OPG/TRAIL complexes, possibly leading to the sequestration of OPG by TRAIL, which subsequently prevents its binding to RANKL. Therefore, the localization of OPG in the RA bone microenvironment could be contributing to the erosive process, rather than being inhibitory. To elucidate the important relationship between RANKL, TRAIL, and OPG in human RA synovial fibroblasts we analyzed RANKL and OPG expression after administration of 100 ng/ml of human recombinant TRAIL. RA synovial fibroblasts also produce a basal level of RANKL (Figure 1, right side, C1, D1 green fluorescence) and OPG (Figure 2), making them capable of influencing the erosion process; this is illustrated in Figure 1 by the use of a TRAP immunoenzymatic assay in which cells without TRAIL (Figure 1B) are less TRAP-positive versus the cells treated with TRAIL (Figure 1B.1). Moreover, cells treated without recombinant human-TRAIL show results less differentiated toward the osteoclast phenotype compared to the TRAIL-treated cells, as indicated by the formation of multinucleated cells. As shown in Figure 2 the addition of 100 ng/ml recombinant human-TRAIL induced a significant upregulation of RANKL and simultaneously a downregulation of OPG. In addition, OPG and RANKL were produced in physiological amounts that corresponded to 668 pg/ml OPG and 345 pg/ml RANKL, as determined by ELISA. After the addition of TRAIL

(100 ng/ml) to the medium we obtained 328 pg/ml of OPG compared to 1243 pg/ml of RANKL.

In summary, we demonstrated the expression pattern of RANKL, and OPG protein in RA synovial fibroblasts, after administration of human recombinant TRAIL (100 ng/ml). We have described the relationship of the TRAIL-RANKL-OPG axis, suggesting that RA synovial fibroblasts produce a basal level of OPG that interferes with TRAIL, inducing an undisturbed production of RANKL. From these results we suggest that TRAIL is not suitable for a therapeutic role in the treatment of RA.

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REFERENCES

1. Gravalles EM, Manning C, Tsay A, et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. *Arthritis Rheum* 2000;43:250-8.
2. Haynes DR, Crotti TN, Capone M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappa B ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. *Rheumatology* 2001;40:623-30.
3. Chu CQ, Field M, Allard S, Abney E, Feldmann M, Maini RN. Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implications for the role of cytokines in cartilage destruction and repair. *Br J Rheumatol* 1992;31:653-61.
4. Chu CQ, Field M, Feldman M, Maini RN. Localization of tumor necrosis factor in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum* 1991;34:1125-32.
5. Vengunst CE, Gerlag DM, Lopatinskaya L, et al. Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial. *Arthritis Rheum* 2008;58:1889-91.
6. Tunyogi-Csapó M, Kis-Toth K, Vermes C, et al. The role of synovial fibroblasts in pathologic bone resorption: RANKL and OPG expression by human and mouse fibroblasts in arthritis. *Bone* 2008;43 Suppl: S40.
7. Takayanagi H, Iizuka H, Juji T, et al. Involvement of receptor activator of nuclear factor B ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. *Arthritis Rheum* 2000;43:259-69.
8. Yasuda H, Shima N, Nakagawa N, 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.
9. Gravalles EM, Galson D, Goldring SR, Auran PE. The role of TNF-receptor family members and other TRAF-dependent receptors in bone resorption. *Arthritis Res* 2001;3:6-12.
10. Emery JG, McDonnell P, Burke MB, et al. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem* 1998;273:14363-7.
11. Walczak H, Miller RE, Ariail K, et al. Tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. *Nat Med* 1999;5:157-63.
12. Walczak H, Degli-Esposti MA, Johnson RS, et al. TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *EMBO J* 1997;16:5386-97.
13. Macfarlane MM, Ahmad SM, Srinivassula T, Fernandes-Alnemri GM, Cohen GM, Alnemri ES. Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. *J Biol Chem* 1997;272:25417-20.
14. Colucci S, Brunetti G, Rizzi R, et al. T cells support osteoclastogenesis in an *in vitro* model derived from human multiple myeloma bone disease: the role of the OPG/TRAIL interaction. *Blood* 2004;104:3722-30.

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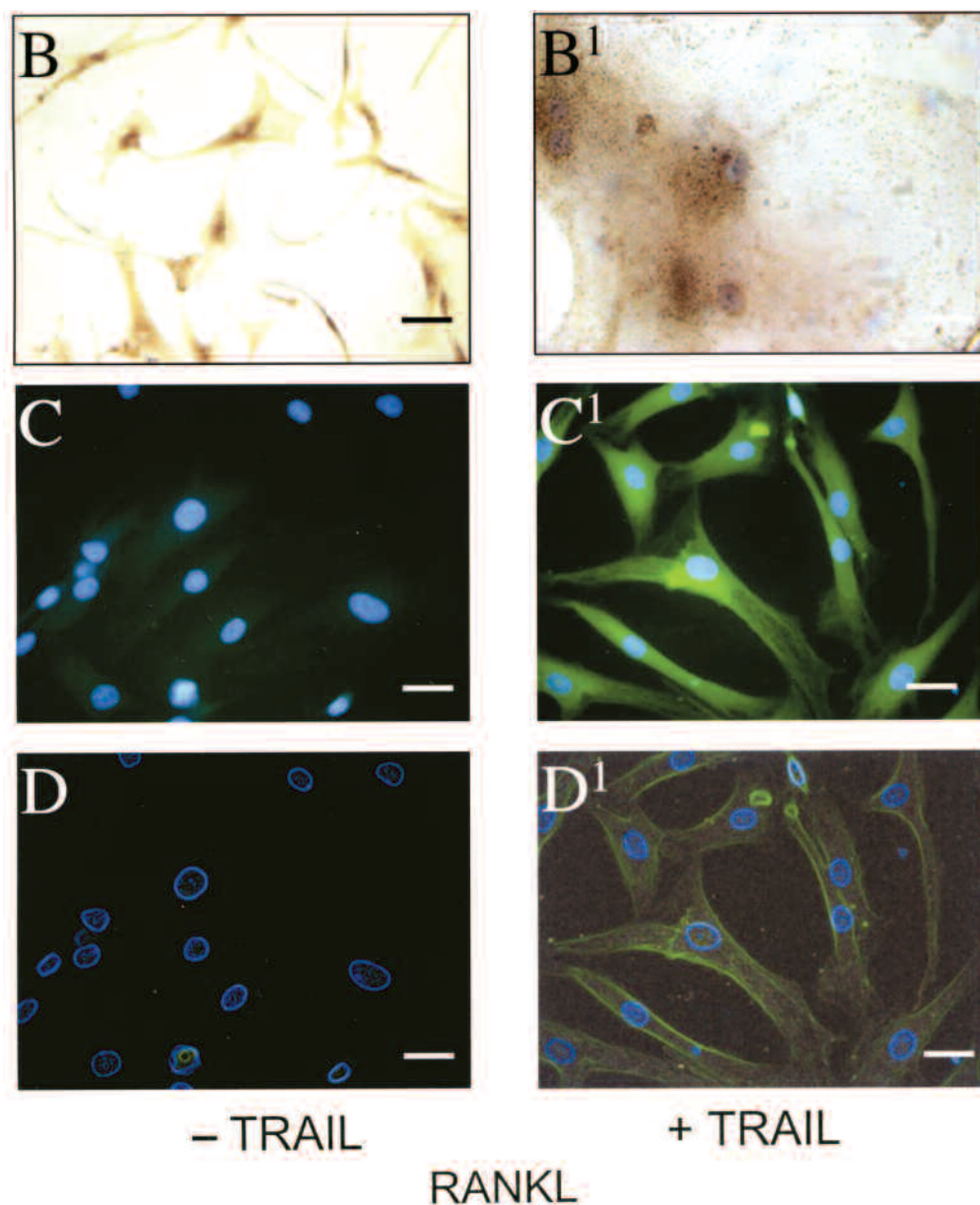


Figure 1. Effect of human recombinant TRAIL on RANKL expression in RA synovial fibroblasts cultured with human recombinant TRAIL (100 ng/ml) for 4 days. B-B1. Immunoenzymatic assay for TRAP positivity in the presence or absence of human recombinant TRAIL. C-C1. Immunocytochemical expression of RANKL antibody FITC-conjugated and DAPI nuclei staining in the presence (C1) or absence (C) of treatment with human recombinant TRAIL (100 ng/ml). D-D1. Image analysis of immunocytochemical expression of RANKL in the presence or absence of human recombinant TRAIL (100 ng/ml). All the fluorescence images show that synovial cells treated with 100 ng/ml TRAIL expressed upregulation of RANKL compared to control (–TRAIL). All experiments were done in triplicate. Scale bar = 25 μ m.

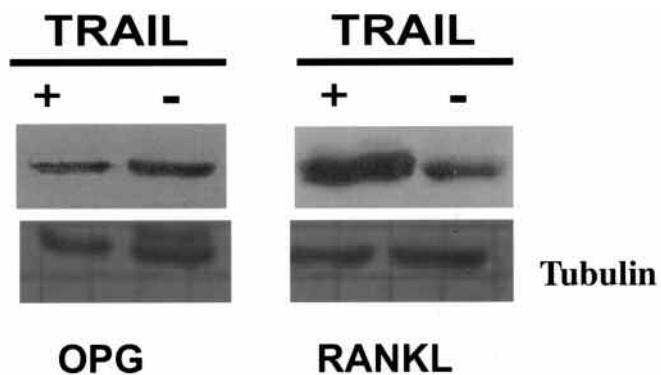


Figure 2. Effect of human recombinant TRAIL (100 ng/ml) on RANKL and OPG expression in RA synovial fibroblasts. RA synovial fibroblasts were cultured with human recombinant TRAIL (100 ng/ml) for 4 days. Culture was analyzed, measuring the levels of RANKL and OPG expression in contrast with the control protein tubulin.