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 pathologic 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-18, IL-6, TNF-α, and macrophage colony-stimulating factor] have the potential to promote osteoclast formation and bone resorption3,4. Cells within RA synovial fibroblasts also are substantial sources of soluble receptor activator of nuclear factor-xB (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 family along with RANK and RANKL,10 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 RANK 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 al14 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). 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.

PAOLA NARDUCCI, Degree in Biological Science, Professor; RENATO BAREGGI, Degree in Pharmaceutical Science, Professor; VANESSA NICOLIN, nat. Assistant Professor, Department of Biomedicine, University of Trieste, via Manzoni 16, 34138 Trieste, Italy. Address reprint requests to Dr. V. Nicolin; E-mail: nicolin@units.it

REFERENCES


J Rheumatol 2009;36:8; doi:10.3899/jrheum.081089

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2009. All rights reserved.
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 µm.
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.