Abstract

We investigated the expression and localization of the receptor activator nuclear factor κB ligand (RANKL) in cartilage from patients with rheumatoid arthritis (RA) of relevance to cartilage degeneration. We also examined the role of exogenous lymphotoxin (LT)-α on RANKL expression in human chondrocytes and its effect on *in vitro* osteoclast differentiation.

Cartilage and synovial fluid samples were obtained from 45 patients undergoing total joint replacement surgery or joint puncture, including 24 patients with osteoarthritis (OA) and 21 patients with RA. RANKL expression in articular cartilage was examined by immunohistochemistry. LT-α concentrations in synovial fluid were measured using an enzyme-linked immunosorbent assay (ELISA). Normal human chondrocytes were stimulated with LT-α, and the relative mRNA levels of RANKL, osteoprotegerin (OPG), matrix metalloproteinase-9, and vascular endothelial growth factor were examined by real-time polymerase chain reaction. Soluble RANKL protein in culture media was measured using ELISA, and membrane-bound RANKL protein in cells was examined by western blotting. Co-cultures of human chondrocytes with peripheral blood mononuclear cells (PBMCs) were stimulated with macrophage-colony stimulating factor and LT-α, and osteoclast differentiation was evaluated by staining for tartrate-resistant acid phosphatase.

LT-α concentrations were higher in RA synovial fluid than in OA samples. The population of RANKL-positive chondrocytes of RA cartilage was higher than that of OA cartilage, and correlated with cartilage degeneration. Stimulation of cultured human chondrocytes by LT-α increased RANKL expression, the RANKL/OPG ratio, and

angiogenic factors. Membrane-bound RANKL in chondrocytes was up-regulated after stimulation of LT-α, whereas soluble RANKL in culture medium did not increase. Co-cultures of human chondrocytes and PBMCs demonstrated that LT-α stimulated human chondrocytes to produce RANKL and induced osteoclastic differentiation of PBMCs.

RANKL produced by chondrocytes may contribute to cartilage destruction during RA and LT-α could promote the expression of RANKL in human chondrocytes.