19 **RANKL PRODUCED BY ARTICULAR CHONDROCYTES CONTRIBUTES TO JUXTA-ARTICULAR BONE LOSS IN CHRONIC ARTHRITIS**

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Background and objectives Juxta-articular bone loss is an early lesion of rheumatoid arthritis (RA) whose molecular mechanisms are still unknown. Although the authors have previously demonstrated that RANKL diffuses from cartilage to subchondral bone, the role of the RANKL expressed by chondrocytes in the juxta-articular osteoporosis remains still unclear. This study was designed to test if RANKL produced by chondrocytes induces osteoclastogenesis and juxta-articular bone loss associated to RA.

Material and methods Osteoclast differentiation induced by chondrocytes was studied in co-culture of human chondrocytes with peripheral blood mononuclear cells (PBMC) in presence of Macrophage colony stimulating factor and PG-E₂, and further stained with TRAP. Chronic antigen-induced arthritis (AIA) was induced in seven New Zealand male rabbits of 3–3.5 kg by 4 weekly repeated intra-articular injections of ovalbumin in previously immunised animals. A control healthy group of eight age and sex-matched rabbits was simultaneously studied. Dual energy x-ray absorptiometry of subchondral knee bone was performed before sacrifice. Articular cartilage, synovium and subchondral bone of the tibia were isolated and frozen for

OPG and RANKL expression studies, while femurs were fixed, decalcified and embedded in paraffin for histological examination and RANKL immunohistochemistry.

Results Co-cultures demonstrated that PGE2-induced RANKL synthesis from human chondrocytes induced osteoclasts differentiation from PBMC. AIA rabbits had bone mineral density values of knee subchondral bone significantly lower than healthy ones (0.27 ± 0.04 vs 0.55 ± 0.05 gr/cm²; $p=0.029$). The expression of RANKL (876.8 ± 149.52 vs 64.15 ± 22.26 AU; $p<0.001$), OPG (153.79 ± 54.63 vs 35.63 ± 18.22 AU; $p<0.001$) and RANKL/OPG ratio (6.20 ± 2.13 vs 3.85 ± 0.90 , $p=0.009$) in cartilage were increased in AIA compared to healthy rabbits; however, this pattern was not seen in synovium. Furthermore, RANKL expression ($r=-0.89$, $p<0.001$) and RANKL/OPG ($r=-0.74$, $p=0.01$) were inversely related to subchondral mineral density. Peri-cellular RANKL expression was observed throughout all cartilage zones of rabbits and it was especially increased in the calcified cartilage of AIA animals. In this calcified cartilage, RANKL was also detected in the extracellular matrix, especially near the vessels.

Conclusions Our data show that the RANKL produced by chondrocytes is able to differentiate osteoclasts in vitro. Moreover, the authors have observed an increase in the RANKL/OPG ratio in cartilage of AIA rabbits, as measure of a resorptive signal. Our data suggest that the increased RANKL expression observed in the cartilage from arthritic rabbits could be responsible, at least partially, of the development of juxta-articular osteoporosis associated to chronic arthritis. These results point out a new mechanism of bone loss in patients with RA.