



# INTERLEUKIN-6 MEDIATES SUBCHONDRAL OSTEOBLASTS-INDUCED CARTILAGE DEGRADATION

Christelle Sanchez, Elisabeth Kaut, Michelle A. Deberg and Yves E. Henrotin

Bone and Cartilage Research Unit, University of Liège, Belgium

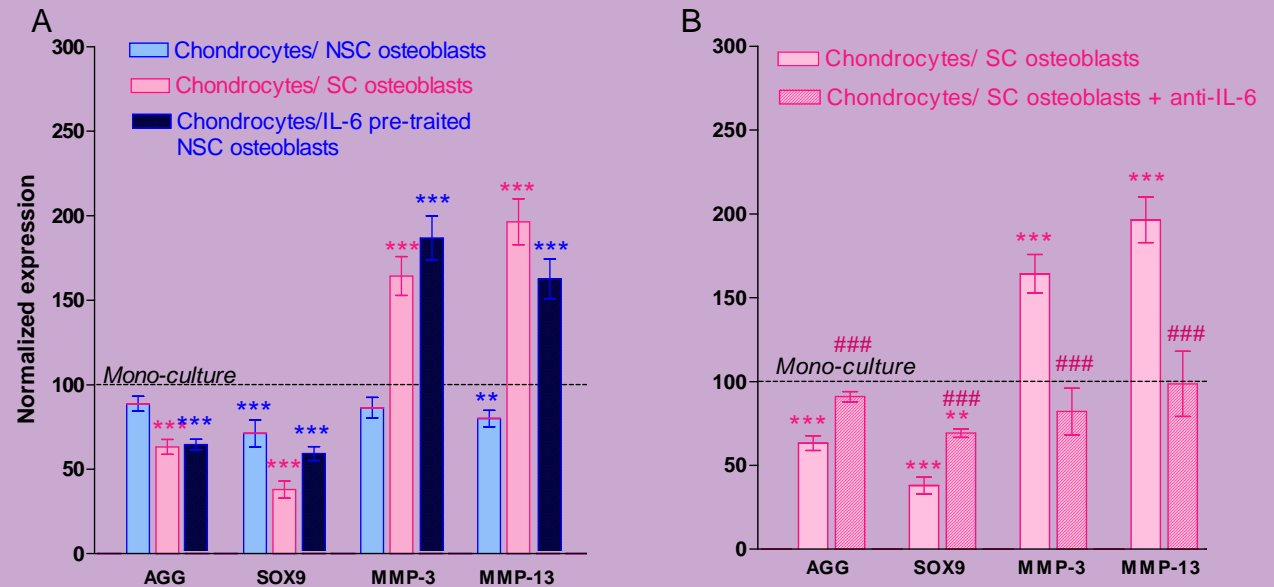


**Aim of study:** Previously, we have demonstrated that osteoblasts from the sclerotic subchondral bone express a particular phenotype characterized by an overproduction of interleukin (IL)-6, transforming growth factor (TGF)- $\beta$ 1, alkaline phosphatase and osteocalcin but similar amount of IL-1 $\beta$  than non sclerotic osteoblasts. Further, we have observed in a co-culture model that osteoblasts from the sclerotic zone of osteoarthritic (OA) subchondral bone dysregulated the metabolism of chondrocytes. This work was designed to identify the mediators involved in these osteoblasts-induced effects.

**Methods:** Human chondrocytes were isolated from OA cartilage and cultured in alginate beads for 4 days in the absence or in the presence of non sclerotic or sclerotic OA subchondral osteoblasts in monolayer (co-culture system). During co-culture, monoclonal antibodies (Mab) neutralizing IL-6 were added. Chondrocytes in monoculture and chondrocytes/fibroblasts co-culture were conducted in parallel as controls. Aggrecan, sox9 and matrix metalloproteases (MMP) -3 and -13 mRNA levels in chondrocytes were quantified by real time polymerase chain reaction. Aggrecan and MMP-3 production was assayed by a specific enzyme amplified sensitivity immunoassay.

**Results:** In co-culture, sclerotic, but not non sclerotic, osteoblasts significantly decreased (- 27 %,  $p < 0.001$ ) aggrecan production and aggrecan gene expression by human chondrocytes. In parallel, sox9 expression was decreased (- 52 %,  $p < 0.001$ ) whereas MMP-3 and MMP-13 gene expression were increased (+ 44 and + 76%, respectively,  $p < 0.001$ ). Anti-IL-6 Mab, prevents all these osteoblasts-induced effects.

**Conclusions.** OA subchondral osteoblasts could contribute to cartilage degradation by stimulating chondrocytes to produce more matrix metalloproteases and by inhibiting aggrecan synthesis. Herein, we have identified IL-6 as a key mediator of the osteochondral pathophysiological axis. To neutralize IL-6 biological activity prevents the negative effects of sclerotic osteoblasts on cartilage metabolism.



**Figure 1:**

AGG, SOX9, MMP-3 and MMP-13 genes expression by chondrocytes after 4 days of co-culture with non sclerotic (NSC) or sclerotic (SC) osteoblasts.

(A) NSC osteoblasts were or not pre-incubated during 72h with IL-6 (100 ng/ml + 50 ng/ml d'IL-6sR).

(B) Co-cultures were performed in the presence or not of monoclonal antibodies neutralizing IL-6 biological activity.

Results are exprimed in mono-culture percentage. Genes expressions are GADPH normalized and are represented by the mean of 3 independant cultures  $\pm$  SEM. Each experimental condition was performed in triplicate (n=9). Significant statistical differences are represented by \*\* =  $p < 0,01$  et \*\*\* =  $p < 0,001$  between co-culture and mono-culture, and ### =  $p < 0,001$  between co-culture with or without anti-IL-6 antibodies.