The Role of IL-6 in Osteoarthritis and Cartilage Regeneration

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INTRODUCTION

One of the cytokines shown to play a role in cartilage destruction during rheumatoid arthritis is interleukin 6 (IL-6). However, the role of IL-6 in osteoarthritis (OA) remains controversial. IL-6 is elevated in the synovial fluid (SF) of OA compared to healthy donors, but reports regarding its effects on cartilage matrix metabolism are conflicting. Even less is known about the role of IL-6 in focal cartilage lesions, a condition postulated to predispose to early OA. This may be due to trauma-induced upregulation of inflammatory cytokines such as IL-6. The purpose of this study was to determine the levels of IL-6 in knee synovial fluid of donors with symptomatic cartilage defects, OA and healthy donors, and evaluate IL-6 production during regeneration of chondrocytes obtained from these three groups of donors. Subsequently, the role of Il-6 in OA and regeneration of cartilage defects was evaluated in OA explants and during the 3D redifferentiation of the cells.

METHODS

Il-6 levels were determined by ELISA in SF from healthy and OA donors and donors with symptomatic cartilage defects, and in conditioned media of OA, healthy and defect p2-expanded chondrocytes redifferentiated on filters for 28 days. To study the role of IL-6 in SF, OA cartilage explants were cultured for 14 days with 25% OA SF in the medium and either with or without blockage of IL-6 using an activity inhibiting antibody. Similarly the role of IL-6 during redifferentiation in filter culture was investigated by culturing either with or without IL-6 blockage. Readout parameters were glycosaminoglycan (GAG) and DNA content. Differences in IL-6 concentration between healthy, OA and cartilage defect were determined by Kruskal-Wallis test with posthoc Mann-Whitney U test with Bonferroni correction for SF and by nested ANOVA with posthoc t-test with Bonferroni correction for conditioned media. Differences between IL-6 blocked samples and controls were determined by univariate analysis of variance with randomized block design.

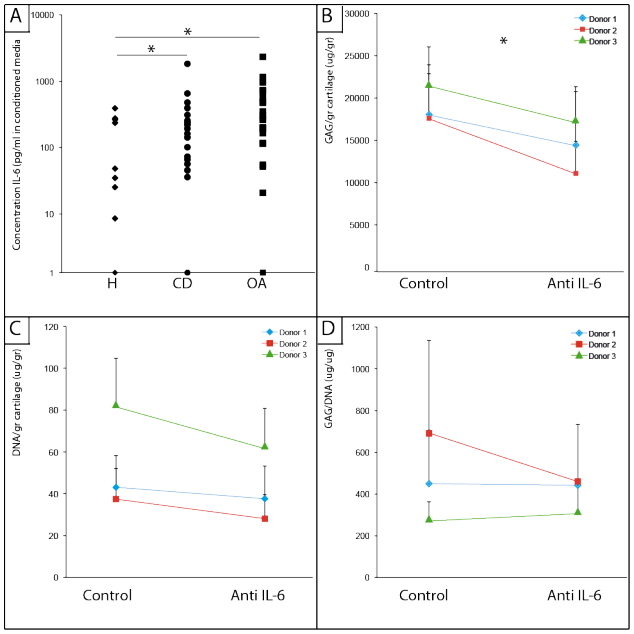

Figure 1A: Concentration IL-6 in synovial fluid of healthy (H), cartilage defect (CD) and osteoarthritic (OA) donors; *p<0.001, **p=0.001. **B-D**: GAG content, DNA content and GAG/DNA in explants from 3 OA donors (mean ± SD); †p=0.018.

RESULTS

The IL-6 concentration in healthy SF was significantly lower compared to defect and OA SF (p<0.001 and p=0.001 respectively, fig. 1A). Blocking IL-6 in the SF resulted in a significant decrease in GAG content per gram cartilage, but not when normalized to DNA (figs. 1B-D). Cells in redifferentiation culture were found to produce high levels of IL-6, which were significantly higher in OA cells compared to defect and healthy cells (p<0.001, fig. 2A). During redifferentiation culture, inhibition of IL-6 resulted in increased DNA and subsequently decreased GAG/DNA in OA chondrocytes, but not in defect chondrocytes (figs. 2B-D).

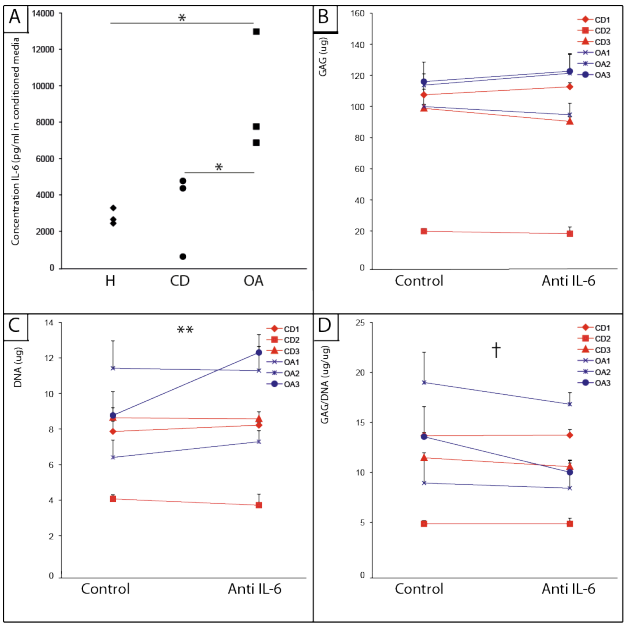

Figure 2A: Concentration IL-6 in conditioned media of healthy (H), cartilage defect (CD) and osteoarthritic (OA) chondrocytes; *p<0.001. **B-D**: GAG content, DNA content and GAG/DNA in redifferentiated chondrocytes from 3 CD and 3 OA donors (mean ± SD); **p=0.008 for OA donors, n.s. for CD donors, †p=0.013 for OA donors, n.s. for CD donors.

DISCUSSION

This study demonstrates the increased presence of IL-6 in the synovial fluid of patients with symptomatic cartilage lesions and OA donors when compared to healthy donors. Furthermore, we demonstrated for the first time that chondrocytes produce high concentrations of IL-6 during regeneration, especially osteoarthritic chondrocytes. However, IL-6 does not seem to play a direct role in cartilage matrix turnover. Furthermore, only in OA chondrocytes, IL-6 does seem to play a role in proliferation, although the effect was different in explants in the presence of synovial fluid compared to 3D differentiation by expanded chondrocytes.

SIGNIFICANCE

Soluble mediators, including IL-6, in the synovial fluid of knees with symptomatic cartilage defects are thought to cause progression to osteoarthritis and also hamper cartilage regeneration after cartilage surgeries such as autologous chondrocyte implantation. Identifying and targeting those soluble mediators will increase the success of cartilage repair surgery and prevent the progression to early osteoarthritis.