INTRODUCTION. Recent in vitro studies of human adult articular chondrocytes have shown that interleukin-1 (IL-1) is much more effective in shutting down aggrecan synthesis than in promoting its degradation by proteolytic enzymes such as aggrecanase [1]. Indeed, at the low concentrations (< 100 picogram/ml) found in OA joint fluid, IL-1 inhibits aggrecan synthesis without inducing chondrocytic chondrolysis [1]. We have postulated, therefore, that it is much more likely that loss of matrix homeostasis in the late stages of OA is the direct result of a downregulation of the enhanced biosynthetic “repair” processes rather than of a further increase in the rate of catabolic activities by the chondrocytes [1]. To shed more light on the response of chondrocytes to IL-1, we examined if adult human articular chondrocytes, which synthesize aggrecan at a fast rate, are more or less susceptible to IL-1-induced downregulation of aggrecan synthesis.

MATERIALS AND METHODS. Chondrocyte cultures - Ankle joints from 30 human donors (ages: 16-74 years, mean = 46.67; 23 males and 7 females) were obtained within 24 hours of death from the Regional Organ Bank of Illinois. Normal articular cartilage (full depth; Collins score = 0 in all cases) was removed and digested (pronase 0.2%, collagenase 0.025%). The chondrocytes thus released were suspended in 1.2% low viscosity alginate (Keltone LV) beads as previously described [2]. The beads were cultured at 37°C in daily changes of medium (DMEM/F-12: 1/1) containing 10% fetal bovine serum and supplements [2]. The cells from six of the donors (ages:16-74 years, mean = 48) also were cultured in the presence of osteogenic protein-1 (OP-1) at 100 ng/ml to stimulate aggrecan synthesis [1]. All media were replaced daily.

Effect of IL-1β treatment upon proteoglycan (PG) synthesis - On days 7, 8 and 9 of culture, IL-1β was incorporated in some of the media at 0.01, 0.1 or 0.5 ng/ml. During the last 4 hours of culture on day 9, 35S-sulfate was added to the medium at 20 µCi/ml to measure PG synthesis [2]. The radiolabeling medium was then collected and the 35S-PGs in the beads solubilized by the addition of 4 M guanidine HCl with protease inhibitors [2]. In each case, 35S-PGs in the medium + corresponding extract were separated from unincorporated 35S-sulfate by a rapid filtration assay following 35S-PG precipitation by albuin blue [3]. Analysis of 35S-PGs by column chromatography on Sepharose CL-2B confirmed that 35S-aggreccan made up greater than 90% of the 35S-PGs synthesized by the cells from all donors (data not shown). The values for 35S-incorporation reported here represent in each case the mean of the data obtained for the analysis of 3 separate cultures. Correlation coefficients were calculated using Statview.

RESULTS. Treatment of the cells with IL-1β for 3 days was the most effective in causing an inhibition of 35S-PG synthesis. For each dose of IL-1β, the percent inhibition of PG synthesis was directly proportional to the rate of 35S-PG synthesis in the absence of IL-1β (35S-incorporation as % of control cultures: 0.01 ng/ml: R = 0.297, P = 0.132; 0.1 ng/ml: R = 0.367, P = 0.046; 0.5 ng/ml: R = 0.346, P = 0.069). This relationship became even more statistically significant after inclusion of the data obtained for cells stimulated by OP-1 (35S-incorporation as % of control cultures with the OP-1 data added: 0.01

DISCUSSION. The findings presented here provide strong evidence that the faster an adult human articular chondrocyte synthesizes aggrecan in culture, the more susceptible it becomes to the deleterious effects of IL-1β upon the production of this important matrix macromolecule. If this relationship is operative in vivo, then it follows that OA chondrocytes that have become hypermetabolic in their attempt to repair the matrix are likely to rapidly lose the ability to maintain matrix metabolism at steady state when they encounter IL-1β (and most likely, also IL-1α). In this regard, it is worth noting that the lowest dose of IL-1 shown to have an effect in this study was well within the range of concentrations known to be present in OA joint fluids [4]. The observation that the relationship held true over wide ranges of (i) cytokine concentrations and (ii) rates of aggrecan synthesis clearly suggests that the metabolic processes responsible for the correlation are highly regulated. Chondrocyte hypermetabolism is a prominent feature of early metabolic changes after traumatic joint injury predisposing to OA as well as in early primary OA. The fact that a low dose of IL-1 is most effective in blocking the upregulation of biosynthetic processes by chondrocytes attempting to compensate for the increase in matrix degradation may help explain why the attempt at repair in OA usually cannot be sustained once inflammation sets in.


ACKNOWLEDGMENTS. This work was supported in part by NIH grant AG04736 and 2-P50-AR39239, and the Rush Arthritis and Orthopedics Institute.

**Depts of Biochemistry and Orthopedic Surgery at Rush Medical College, Rush-Presbyterian-St. Luke’s Medical Center**