INTRODUCTION: Most studies on the metabolism of intervertebral disc (ID) cells, including those investigating the effects of various cytokines and growth factors, have been performed using experimental animal models (in vivo) or discs from cadavers or patients (in vitro). Experimental animals are expensive and require access to large animal facilities, while specimens from cadavers or patients are not readily available. The developments of reproducible and cost-effective in vitro microuniverse systems that can be used to identify subtle but important changes taking place in the metabolism of ID cells are thus worthwhile goals. We have cultured cells populating the nucleus pulposus (NP) and the annulus fibrosus (AF) in alginate gel, a medium which consists of highly negatively-charged polysaccharides and mimics the extracellular environment rich in anionic proteoglycans (PGs), and have begun to compile fundamental information on the metabolism of these cells (1).

Interleukin-1 (IL-1) has deleterious effects on cartilage matrix metabolism. For example, using a unique alginate gel cell culture system, we have shown that the IL-1-induced degradation of aggrecan by proteolytic enzymes released by articular chondrocytes occurs mainly in the thin rim of the cell-associated matrix (2). There is also scant information about the effects of IL-1 upon the metabolism of intervertebral disc PGs (3). The present study was thus undertaken to study the effects of IL-1α upon the metabolism of the PGs synthesized and turned over by ID cells cultured in alginate gel.

MATERIALS AND METHODS: General cell culture protocol - Lumbar intervertebral discs were dissected aseptically from the spine of New Zealand white rabbits after euthanasia with Euthanasin (ECCU approval #94-035). The NP and AF were separated by blunt dissection and separately pooled. Cells were released from each tissue by sequential enzyme digestion (Pronase 1 h, and Collagenase P and DNAAse B for 16 h). Isolated cells were suspended in 1.2% sodium alginate, and the cell suspension expressed into 102 mM CaCl₂ solution to effect gelation of the alginate (1). The cultures were maintained as a batch culture in DMEM/F12+10% FBS (complete medium). The medium was changed daily.

Changes in the rate of turnover of PGs by IL-1α - On day 7 of culture, i.e. after PGs in the CM have reached near steady state metabolism, beads were incubated for 16 h in complete medium containing 35 S-sulfate (20 µCi/ml). After washing the unincorporated radioisotope, the beads were cultured for 3 additional weeks in isotope-free medium in the presence of human recombinant IL-1α at a concentration of 1 ng/ml. The medium in all cases was changed daily.

At various times, beads were solubilized with Ca ++ chelating agents followed by mild centrifugation to separate the cells and their associated matrix (cell-associated matrix: CM) in the pellet from molecules derived from the further degradation of the PGs (cell-associated matrix: FRM) in the supernatant (1). The content of 35 S-PGs in the CM, FRM and the spent medium fraction was measured by a rapid filtration assay following development with alkaline blue (4). For each set of conditions (i.e. with or without IL-1α), the amount of radiolabeled PGs remaining in each matrix pool was plotted against time of chase to measure the average half-life of 35 S-PGs in each compartment.

RESULTS: Treatment with IL-1α caused a significant increase in the rate of loss (following degradation) of PGs from the beads (Figure). After 10 days in culture with IL-1α, 24% and 40% of the 35 S-PGs were still present in alginate beads of the NP and AF, respectively. In contrast, less than 10% of these newly-synthesized PGs remained in the CM of the NP and AF cells. These results are consistent with the view that, as we have shown in the case of articular chondrocytes (2), the IL-1α-induced acceleration of PG degradation by ID cells is more pronounced in the CM than in the FRM. Indeed, comparison of the calculated half lives of radiolabeled PGs (see Table) in NP beads showed that the addition of IL-1α resulted in a greater increase in the rate of loss of 35 S-PGs from the CM than from the beads as a whole (approximately 2-fold vs 3-fold). The same conclusions were drawn in the case of AF beads (see Table).

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Table Calculated halflife of newly-synthesized PGs (days)

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>AF</th>
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<tbody>
<tr>
<td>Cont</td>
<td>B.1</td>
<td>Cont</td>
</tr>
<tr>
<td>Cell-associated</td>
<td>12.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Whole Beads</td>
<td>27.7</td>
<td>14.7</td>
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The half life was calculated using a single exponential decay curve fit.

DISCUSSION: The results of our studies demonstrate, as far as we know for the first time, that IL-1α is most effective in causing an acceleration in the rate of degradation and loss of PGs from the matrix formed by NP and AF cells in alginate beads. The data also showed that this acceleration was more pronounced in the CM of metabolically active CM than in the further removed, normally less active, but more abundant FRM. Further experiments are in progress to determine if IL-1α at even lower concentrations limits its effects to the metabolism exclusively upon the CM, as has been shown to be true in the case of articular chondrocytes (1).

The alginate bead system should prove as useful to study matrix formation and turnover by AF and NP cells as it has been for articular chondrocytes. It is interesting, for example, that the IL-1α-induced increase in the rate of degradation of PGs mimics the age-related increase in the rate of turnover of aggrecan (5). By helping identify metabolic changes occurring in distinct compartments of the extracellular matrix, the alginate bead culture system appears ideally suited to study the effects of other cytokines upon the metabolism of ID cells. It also should help shed light upon differences in the metabolism of normal and diseased NP and AF cells. Such studies should provide useful information that may help improve our understanding of metabolic changes that contribute to disc degeneration as well as help develop therapeutic approaches to counteract these deleterious effects.
