EFFECT OF INTERLEUKIN-1 ALPHA ON THE TURNOVER OF PROTEOGLYCAN PRODUCED BY ANNULUS FIBROSUS AND NUCLEUS PULPOSUS CELLS IN ALGINATE BEADS

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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* microculture 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 occurrs mainly in the thin rim of the cell-associated matrix (2). There is only 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 (IACUC approval # 94-052). 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 II for 16 h). Isolated cells were suspended in 1.2% sodium alginate and the cell suspension expressed into 102mM $CaCl_2$ 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 (20μCi/ml). After washing the unincorporated radioisotope, the beads were cultured for 3 additional weeks in isotope-free medium in the absence or presence of human recombinant IL-1α at a concentration of 1ng/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 compartment further removed from the cells surface (further removed 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 precipitation of glycosaminoglycans with alcian 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. Those results are consistent with the view that, as we have shown in the case of articular chondrocytes (2), the IL-1-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).

Table Calculated half life of newly-synthesized PGs (days)

	NP		AF	
	Cont	IL-1	Cont	IL-1
Cell-associated matrix	12.6	4.5	13.7	4.2
Whole Beads	27.7	14.7	32.2	19.8

The half life was calculated using a single exponential decay curve fit.

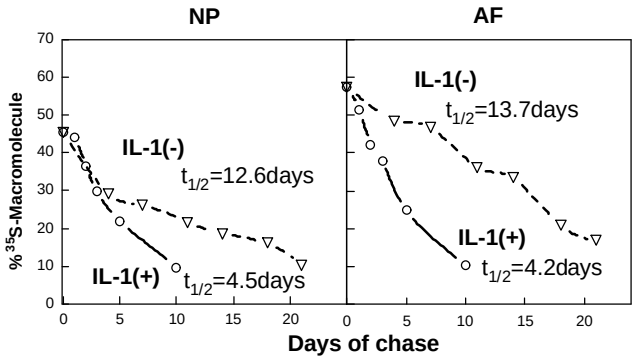

Figure: Turnover of Newly-synthesized PGs in the CM Compartment

DISCUSSION: The results of our studies demonstrate, as far as we know for the first time, that IL-1 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 rim 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-1 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-1-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 countereact those deleterious effects.

REFERENCES: 1. Chiba, K., et al., Spine 22: 2885-93, 1997; 2. Masuda, K., et al., Orthop. Trans. 19: 1066-7, 1995. 3. Shinmei, M., Spine 13: 1284-1290, 1988. 4. Masuda, K., et al., Anal. Biochem. 217: 167-75, 1994. 5. Chiba, K., et al., Orthop. Trans. 20: 792-3, 1996.

ACKNOWLEDGMENTS : This work was supported in part by NIH grants 2-P50-AR39239 and AG04736, by a research grant from the Rush University Committee on Research and by the Rush Arthritis and Orthopedics Institute.

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