THE EFFECT OF OSTEOGENIC PROTEIN-1 ON INTERVERTEBRAL DISC CELLS PRE-EXPOSED TO INTERLEUKIN-1

*Takegami, K; *Masuda, K (A-NIH, Stryker Biotech); **An, H (A-Stryker Biotech); *Kamada, H; ***Pietryla, D; ****Thonar, E (A-NIH, Stryker Biotech)

INTRODUCTION: Intervertebral disc (IVD) degeneration is a major cause of low back pain. The proteoglycan (PG) content of the disc decreases with aging and degeneration. While it is not clear what triggers this change, the detection of interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α in degenerated and herniated discs suggests that such cytokines may be involved in IVD degeneration. As IL-1 is most effective in stimulating the production of some matrix metalloproteinases, nitric oxide and prostaglandin E2 by normal IVD cells [1], it is likely an inducer of the degradation of the extracellular matrix. We recently reported that osteogenic protein-1 (OP-1) stimulated PG synthesis by IVD cells in vitro [2]. That result raised the possibility that one may indirectly counteract the deleterious effects of a cytokine such as IL-1 and thus slow down or prevent the degeneration of an IVD by administering such a growth factor locally.

The purpose of this study was to investigate if OP-1 could promote replenishment of PGs in the extracellular matrix of nucleus pulposus (NP) and annulus fibrosus (AF) cells that had been previously exposed in vitro to IL-1 for several days.

MATERIALS AND METHODS: Culture Protocol: Intervertebral discs were aseptically removed from New Zealand white rabbits weighing 3-4 kg (IACUC approval #99-002). The NP and AF tissues were bluntly separated and cells were isolated from each tissue by sequential enzyme digestion [3]. The cells were suspended in alginate beads at 2x10^6 cells/ml and the latter were maintained in DMEM/F-12 medium containing 10% FBS, 25 µg/ml ascorbate and 50 µg/ml gentamicin. This complete medium was replaced with 4 M guanidine HCl and quantified by a rapid filtration assay after alcian blue precipitation [4].

PG Synthesis: At 1 and 2 weeks after IL-1 treatment, the rate of PG synthesis was assessed by incubating the beads for 4 hours in medium containing 35S-sulfate (20 µCi/ml). The 35S-PGs in the beads were extracted with 4 M guanidine HCl and quantified by a rapid filtration assay after alcian blue precipitation [4].

Statistical Analysis: For each time point, all analyses of NP and AF beads were performed on 3 sets of 9 beads each. The standard deviations are shown in the Figure. Statistical analyses were performed by one-way ANOVA with Fisher’s PLSD test as a post hoc test.

RESULTS: DNA Content: The 3 days of IL-1 treatment caused a significant inhibition of proliferation by both NP and AF cells (NP: NT = 4.5 µg / 9 beads, IL-1 = 3.0 µg / 9 beads; AF: NT = 4.6 µg / 9 beads, IL-1 = 2.7 µg / 9 beads, p < 0.01). During subsequent culture in the absence of IL-1, the DNA content increased moderately in all groups until day 14 when it reached a plateau. The DNA content of the IL-1 + OP-1 group reached that of the NT group on day 10 (NP) and day 7 (AF) respectively. For both NP and AF, the beads from the 3 groups had a similar DNA content on day 21.

PG Content: The IL-1 treated beads had significantly lower PG contents than NT beads at the end of the 3 days of treatment with this cytokine (NP: 48% of NT group; AF: 58% of NT group) (see Fig.). During the next 21 days, the PG content of NP beads in the NT group increased during the first 7 days before reaching a plateau, whereas that of AF beads continued to increase throughout the culture period. In the IL-1 group, the PG content of NP and AF beads showed similar changes with time but never reached the levels in beads not treated with IL-1. Importantly, in the IL-1 + OP-1 group, the PG content of both NP and AF cells rapidly returned to the levels seen in beads not treated with IL-1 and then continued to show a pronounced progressive rise (see Fig.).

DISCUSSION: We present evidence that IVD cells that have been exposed for several days to a relatively high dose of IL-1 have lost none of their potential to upregulate PG synthesis in response to stimulation with OP-1. It is worth noting that upon stimulation with OP-1, the IL-1-treated IVD cells not only replenished the matrix of PGs that had been lost during the IL-1 treatment but proceeded to form a matrix that was richer in these resilient molecules than that formed by cells never exposed to IL-1. This strongly suggests that this growth factor may have a place as a treatment modality for IVD herniation and/or degeneration.


ACKNOWLEDGMENTS: This work was supported in part by NIH grants AG-04736 and 2-P50-AR 39239 and by a Research Grant from Stryker Biotech.

*p<0.05 (vs. IL-1)

*Dept. of Orthop. Surg., Rush Medical College, Chicago, IL.

***Dept. of Biochem., Rush Medical College, Chicago, IL.