LMP–1 OVEREXPRESSION IN INTERVERTEBRAL DISC CELLS INCREASES BMP-2 GENE EXPRESSION AND UPREGULATES PROTEOGLYCAN PRODUCTION

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Introduction: One of the hallmarks of intervertebral disc degeneration is a decrease in proteoglycan in the disc. Various molecules have been evaluated for their ability to stimulate intervertebral disc cell synthesis of proteoglycans in the hope of developing a way of retarding or reversing disc degeneration. LMP-1 is a LIM domain intracellular protein that has potential as a stimulator of intervertebral disc cells. LMP-1 is best known for its ability to induce bone formation in vitro and in vivo. Its mechanism of action in bone formation is thought to involve the induction of differentiation in immature osteoblasts. We speculated that this mechanism might be effective in stimulating intervertebral disc cells as well. We tested the hypothesis that LMP-1 can upregulate BMP-2 gene expression and increase the production of proteoglycan in intervertebral disc cells.

Methods: After Institutional Animal Care and Use Committee approval was obtained for usage of the rats, lumbar intervertebral disc cells were harvested from Sprague-Dawley rats and cultured in monolayer. Institutional Animal Care and Use Committee approval was obtained for usage of the rats. The cells were infected with adenovirus containing the human LMP-1 cDNA (AdLMP-1) (MOI 0, 5, 10, 25, or 50) or with adenovirus containing the LacZ marker gene (AdLacZ MOI 25) (negative control). Real-time PCR was used to determine mRNA levels of overexpressed LMP-1, aggrecan, and BMP-2 at 12 hours, 1 day and 3 day after infection. Proteoglycan production was estimated by measuring the sulfated-glycosaminoglycans (sGAG) present in the culture media (at day 3, and 6) using the DMMB assay. Standard error of the mean is shown in Figure 2. All experiments were performed in triplicate and repeated at least twice to insure reproducibility.

Results: There was a dose dependent increase in overexpressed LMP-1 in the disc cells 12 hours after infection (Figure 1), with the peak LMP-1 level attained at an MOI of 25. The media sGAG at day 3 and day 6 after infection with Ad-LMP-1 at different MOIs are shown in Figure 2. There was a time dependent increase in sGAG production, with higher levels noted at day 6 than day 3. The optimal dose of AdLMP-1 was at MOI of 25, resulting in 326% enhancement of sGAG production over the untreated controls at day 6. Treatment with AdLacZ did not significantly change the sGAG production. The regulation of BMP-2 and aggrecan mRNA with MOI of 25 is shown in Figure 3. BMP-2 was upregulated as early as 12 hours after infection with AdLMP-1. Aggrecan was not upregulated until 24 hours after AdLMP-1 infection. When the time to upregulation of gene expression to 200% of baseline is compared between BMP-2 and aggrecan (Figure 3), there is approximately a 2 day time difference.

Discussion: This study demonstrates that intervertebral disc cells infected with AdLMP-1 can overexpress LMP-1, upregulate BMP-2 and aggrecan gene expression, and increase sGAG production. A close correlation between LMP-1 expression and sGAG synthesis was noted. BMP-2 gene expression was upregulated very soon after overexpression of LMP-1 could be detected. However there was significant delay before aggrecan upregulation could be detected. This suggests that LMP-1 is an indirect regulator of aggrecan. Since we have recently established that BMP-2 can upregulate aggrecan gene expression, we propose the following model of LMP-1 action. LMP-1 upregulates gene expression of BMP-2 (and perhaps other BMPs) which leads to increased synthesis and secretion of BMP-2 which then upregulates the gene expression of aggrecan and the production of sGAGs.