INTRODUCTION:
Intervertebral disc degeneration of the cervical and lumbar spine is associated with axial pain and other degenerative spinal conditions such as facet arthropathy and stenosis. The pathobiology of intervertebral disc degeneration is characterized by a loss of water and proteoglycan content in the disc. Transfer of genes encoding for growth factors that might inhibit or reverse these biologic processes may afford an opportunity to prevent or retard disc degeneration. The LMP-1 cDNA has been shown to upregulate proteoglycan synthesis though a BMP mediated pathway in disc cells harvested from normal lumbar rat discs. While these rat studies were compelling, the effect on degenerated human cervical disc cells was not known. Furthermore, the rat experiments were conducted with the type 5 adenovirus, which is a strain that a significant number of humans have a preimmunity against. Therefore we chose to ask the following three questions: 1) Can the chimeric type 5 adenovirus with serotype 35 fiber (type 5/F35 adenovirus), which has a much lower level of human preimmunity, be used to overexpress LMP-1 in disc cells? 2) Can annulus fibrosus and nucleus pulposus cells from degenerated human cervical discs upregulate BMP-2 mRNA? 3) Can these cells upregulate proteoglycan synthesis in response to LMP-1 stimulation?

METHODS:
The human LMP-1 cDNA, driven by the CMV promoter, was incorporated into the type 5/35F adenovirus, a replication deficient recombinant adenovirus, to produce our working adenoviral construct (AdLMP-1). This chimeric adenovirus is capable of infecting human cells through a mechanism independent of the CAR receptor and is thought to have higher infectivity. IRB approval was obtained to use disc material that would ordinarily be discarded. Degenerative intervertebral disc tissue was collected from 2 patients undergoing ACDF for disc herniation and cervical radiculopathy. The discs used in this experiment were clearly degenerated on T2 weighted sagittal MRI views. Human annulus fibrosus (AF) and nucleus pulposus (NP) tissues were harvested at the time of surgery. The cells were extracted from tissues using Pronase (0.02%) for one hour then Collagenase P (0.0025%) over night. Cells were cultured at 37°C and 5% CO2 in standard DMEM/F12 with 10 % FBS, L-glutamine, L-ascorbic acid, Penicillin, Streptomycin and Amphotericin for 14 to 25 days with media exchange every 2-3 days. Cell viability was determined by trypan blue exclusion. Cells were then transfected with AdLMP-1. Cultures treated with an identical type 5/35F virus containing the green fluorescent protein gene (AdGFP) instead of the LMP-1 gene served as a negative control. Cultures treated without any virus served as the no-treatment (NT) control. A viral dose of multiplicity of infection (MOI) 10 was used for transfections, as this was established as the optimal dose in previous experiments (data not shown). At Day 6 after viral exposure, the cells were harvested and RNA was isolated. Real time PCR was used to quantify the expression level of specific mRNA (LMP-1 and BMP-2). Media were evaluated at day 3 and 6 for proteoglycan levels by DMMB assay. The proteoglycan level was normalized to the cell number (DNA content) of the culture e as measured by the Hoechst dye assay. All experiments were performed in triplicate and repeated at least twice to insure reproducibility. Two-tailed student’s t-test was used to calculate p value. P < 0.01 was used as criteria for statistical significance.

RESULTS:
Viable cells were isolated and cultured from human degenerative cervical disc from both annulus fibrosus and nucleus pulposus tissues. Cell viability remained high throughout the incubation and transfection periods (>95%). Basal low level of LMP-1 mRNA expression was found in the non-treatment (NT) and control virus (AdGFP) treated groups. The LMP-1 mRNA level was significantly increased by 40 fold (p<0.01) in AdLMP-1 infected NP cells and by 29 fold (p<0.01) in AdLMP-1 infected AF cells as compared to controls (Figure 1). The BMP-2 mRNA levels were increased by approximately 20 fold (p<0.01) in nucleus pulposus (Figure 2A) and 12.5 fold (p<0.01) in annulus fibrosus (Figure 2B) cells treated with AdLMP-1. The proteoglycan levels in cell cultures treated with AdLMP-1 were increased by 35% six days after transfection as compared to controls for both NP cells (p<0.01) (Figure 3A) and AF cells (p<0.01) (Figure 3B). There was a minimal rise in proteoglycan levels at day 3.

DISCUSSION:
This study confirms that disc cells from degenerated human cervical intervertebral discs can be transfected with the type 5/35F adenovirus to induce expression of potentially therapeutic genes. The upregulation of BMP-2 mRNA and proteoglycan production in response to overexpression of LMP-1 indicate that even cells from degenerated discs can upregulate stimulatory cytokines and increase their anabolic activity. This represents an important step in the development of a clinically useful gene therapy for disc degeneration.

REFERENCES:

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