INTRODUCTION

Growth factors have been tested in animal models and are widely reported to enhance tissue repair. Thompson and colleagues showed that addition of TGF-β1 to canine intervertebral disc tissue in culture stimulated in-vitro proteoglycan synthesis—pointing to the possibility of disc regeneration using growth factors (1). To overcome difficulties related to sustained in-vivo delivery of exogenous growth factors to an intervertebral disc, our group has explored genetic modification of disc cells through gene therapy to effect continuous endogenous production of growth factors (2,3). Previously, we successfully transferred human TGF-β1 cDNA to rabbit intervertebral disc cells using a direct, in-vivo adenovirus-mediated gene transfer technique (3). One week after injection of Ad/CMV-hTGFβ1, the discs exhibited an approximately five-fold increase in total TGF-β1 production over that of control discs, and a 100% increase in proteoglycan synthesis (3).

Because successful gene therapy requires sufficient levels and duration of transgene expression, we undertook the present study to examine adenovirus-mediated human TGF-β1 transgene expression in rabbit intervertebral discs for an extended period of up to 6 weeks. Effect on proteoglycan synthesis activity was also quantified.

MATERIALS AND METHODS

Surgical procedures and in-vivo transduction.

The anterior aspects of the lumbar intervertebral discs of fifteen skeletally mature New Zealand white rabbits were exposed using a retroperitoneal approach. 15 μl of saline containing Ad/CMV-hTGFβ1 (6x10^6 plaque forming unit [PFU]) were injected through a 28-gauge hypodermic needle into the nucleus pulposus of the L2-3, L3-4, and L4-5 intervertebral discs in six rabbits (therapeutic gene group). In four rabbits (viral control group), 15 μl of saline with Ad/CMV-luciferase (6x10^6 PFU) were injected. In the remaining five rabbits (saline control group), 15 μl of saline were injected. After injection, all wounds were closed routinely. The rabbits were sacrificed 6 weeks after surgery. For each rabbit, the L1-2 intervertebral disc was used as its own intact control.

Bioassays for determination of TGF-β1 production and proteoglycan synthesis.

The nucleus pulposus tissues were harvested, weighed, and cultured in Neuman-Tytell serumless medium for 48 hours, after which the medium was extracted for enzyme-linked immunosorbent assay (ELISA) to detect TGF-β1 production. New medium containing 35S-sulfate (10 μCi/ml) was then added to the cultures, and 48 hours later, each specimen with medium was digested at 60°C for 24 hrs in 0.1M phosphate buffer pH 5.7 containing 0.005M EDTA, 0.005M cysteine hydrochloride and 28mg ml^-1 papain (Sigma, St Louis, MO). To separate 35S incorporated into macromolecules from unincorporated 35S, 200μl aliquots of each sample were applied to PD-10 columns equilibrated and eluted with 4M GuHCl containing 0.05M Tris and 0.05M Na2SO4. 1 ml fractions were collected in scintillation vials, mixed with 6 ml of scintillation mixture, and counted in a liquid scintillation counter.

Data analysis. For each rabbit, TGF-β1 production and proteoglycan synthesis by the nucleus pulposus tissues from each of the injected discs (L2-3, L3-4, and L4-5) were normalized to wet tissue weight. TGF-β1 production and proteoglycan synthesis by the nucleus pulposus tissue from the intact control disc (Figures 1 and 2).

RESULTS

No rabbits developed systemic illness secondary to virus injection. At sacrifice, almost all discs appeared macroscopically normal, but some demonstrated spur formation at the site of injection.

At 6 weeks, the nucleus pulposus tissue from the discs injected with Ad/CMV-hTGFβ1 exhibited an approximately three-fold increase (p<0.05) in total TGF-β1 production and 85% increase in proteoglycan synthesis (p<0.05) over that of nucleus pulposus tissue from the intact control discs (Figures 1 and 2). There were no significant differences in TGF-β1 production or proteoglycan synthesis between one and six weeks. No significant increase in either TGF-β1 production or proteoglycan synthesis was observed in nucleus pulposus tissue from discs injected with Ad/CMV-luciferase or saline.

DISCUSSION

The current study has demonstrated that in-vivo adenovirus-mediated human TGF-β1 transgene expression persists in the rabbit intervertebral disc up to at least 6 weeks after transduction. Moreover, the biological effects—measured in terms of increased proteoglycan synthesis—remained strong over this period.

For gene therapy to be successful in any clinical application, sufficient levels and duration of transgene expression are necessary. Adenoviral vectors can efficiently deliver genes to a wide variety of dividing and non-dividing cell types, but in most in-vivo systems, the current adenoviral vectors are able to facilitate only short-term gene expression (~1-2 weeks) because they elicit strong immune responses by the host (4). This is regarded as a major limitation of adenoviral gene therapy, especially for chronic types of diseases. The interior of the intervertebral disc, however, appears to be protected from the immune system and clearly permits long-term gene expression, as demonstrated in this study. These results are remarkably encouraging, and suggest that adenovirus-mediated gene transfer may be applicable to chronic conditions of the intervertebral disc such as degenerative disc disease and associated spinal disorders.

REFERENCES


ACKNOWLEDGMENTS

The authors thank Dr. Savio L-Y. Woo, Dr. Freddie H. Fu, and Dr. Kosaku Mizano for their generous guidance and support of this study.