GENE EXPRESSION PROFILING OF NORMAL AND DEGENERATING INTERVERTEBRAL DISC TISSUE: ANALYSIS OF AN ANIMAL MODEL

INTRODUCTION
Although back pain related disability is a leading health problem, little is known regarding the molecular events that accompany intervertebral disc degeneration. In part, this is due to the difficulty of performing gene expression analysis of disc tissue. In addition, animal models of disc degeneration have not been validated at the mRNA level. In this study, we set out to determine the differences in expression profiles of normal and degenerating disc tissue using a common animal model of disc degeneration. To establish the validity of this model, we analyzed genes known to be highly expressed in human degenerating discs to determine if the mRNAs of these genes were up-regulated by induced disc degeneration from an annular laceration.

METHODS
After obtaining institutional animal committee approval, 18 young, male New Zealand white rabbits were subjected to a small annular laceration of the L1-2 and L2-3 discs. These animals were divided into three groups of six and sacrificed 1-, 3-, and 6-weeks after surgery. Two additional sham control animals received either surgery without annular laceration or medications but not surgery. Four additional animals underwent annular laceration (L1-2 and L2-3) and were subjected to sagittal T1 and T2 weighted MRI before surgery and 1-, 3-, and 6-weeks after surgery. At the time of sacrifice, the lacerated (lesioned) and unaltered (control) lumbar discs were harvested with the surrounding endplates. Annulus fibrosus and nucleus pulposus tissue was carefully removed with the assistance of a microscope and subjected to total RNA extraction. One lacerated and one control disc from each animal was examined histologically. The purified RNA was subjected to reverse transcription (RT) followed by polymerase chain reaction (PCR) amplification of a series of genes. The genes analyzed included collagen type I (Col I), collagen type II (Col II), decorin, fibronectin (FN), interleukin-1α (IL-1α), bone morphogenetic protein 2 (BMP-2), Fas, matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 9 (MMP-9), matrix metalloproteinase 13 (MMP-13), and tumor necrosis factor (TNF). The PCR products were detected electrophoretically and band intensity was determined densitometrically and normalized to the constitutively expressed gene glyceraldehyde-3-phosphate dehydrogenase (GADPH).

RESULTS
Before surgery all discs had a normal histologic appearance. Following annular laceration, the nucleus pulposus cells and matrix rapidly disappeared and the lamellar annulus progressively became replaced with a disorganized tissue resembling fibrocartilage. Similarly, the MRI appearance of discs before laceration and discs adjacent to the lacerated discs demonstrated a plump central region with uniformly high T2-weighted signal intensity. In contrast, lacerated discs demonstrated a decreased central T2 weighted signal intensity by 1-week and an absence of T2 weighted signal by 3-weeks following annular laceration. The lesioned discs demonstrated up-regulation of the expression of Col I, Col II, FN, MMP-1, MMP-9, MMP-13, and Fas at the 1- and 3-week lesioned discs. The lesioned discs had insufficient tissue to allow gene expression analysis.

DISCUSSION
Three aspects of this study are considered important. First, we have shown the feasibility of combining contemporary RNA isolation and purification techniques with RT/PCR, to provide a semi-quantitative evaluation of gene expression in disc tissue. Annular laceration followed by molecular expression analysis provides a simple, reproducible technique to compare normal and degenerating disc tissue in the same animal, making it an attractive method for the study of disc degeneration at the gene expression level. Second, our findings have demonstrated marked up-regulation of FN, MMP-1, MMP-9, MMP-13 and Fas, all suspected of playing a role in human disc degeneration due to the elevated levels of expression in degenerated human discs.

**Thomas Jefferson University, Philadelphia, PA.

Figure 1: Gene expression profiling of normal and degenerative disc using RT-PCR. Relative gene expression is demonstrated by band intensity of the PCR products on gel electrophoresis. 1L and 3L represents the PCR products from the 1- and 3-week lesioned discs respectively, while C represents the PCR products from control discs.

Figure 2: Relative change in gene expression normalized to control (For each gene the control is arbitrarily set at 100%). Note increased levels of expression of MMPs, collagen Iα, fibronectin and Fas in lesioned discs.

Although the exact role of these proteins in disc degeneration is unknown, the annular laceration induced up-regulation of these substances suggests that the rabbit annular laceration model is valid. Third, FN and Fas expression were not detected in control discs and the expression of MMP-9 and MMP-13 was only detected at trace levels. This suggests a minor or nonexistent role of these substances in normal disc metabolism. The marked up-regulation, coinciding with the onset of degenerative-like changes, suggests a possible causal relationship between these molecules and disc degeneration. More investigation into the function of these proteins in degenerating discs is needed. Our study did not allow for localization of the cells producing these proteins; this should be carried out using techniques such as in-situ hybridization. Also, although annular laceration has been widely used as a model of disc degeneration, our finding should be verified in a non-traumatic model of disc degeneration. Finally, although this study used a semi-quantitative technique for gene expression analysis (RT/PCR), the data remains compelling in that marked differences in gene expression were found between normal and degenerating discs as early as 1-week following the initiation of a degenerative process.

**Thomas Jefferson University, Philadelphia, PA.