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Inflammatory Cytokine and Catabolic Enzyme Expression in a Goat Model of Intervertebral Disc Degeneration

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Abstract

Intervertebral disc degeneration is implicated as a leading cause of low back pain. Persistent, local inflammation within the disc nucleus pulposus (NP) and annulus fibrosus (AF) is an important mediator of disc degeneration and negatively impacts the performance of therapeutic stem cells. There is a lack of validated large animal models of disc degeneration that recapitulate clinically-relevant local inflammation. We recently described a goat model of disc degeneration in which increasing doses of chondroitinase ABC (ChABC) were used to reproducibly induce a spectrum of degenerative changes. The objective of this study was to extend the clinical relevance of this model by establishing whether these degenerative changes are associated with local expression of inflammatory cytokines and catabolic enzymes. Degeneration was induced in goat lumbar discs using ChABC at different doses. After 12 weeks, degeneration severity was determined histologically and using quantitative MRI. Expression levels of inflammatory cytokines (TNF- α , IL-1 β and IL-6) and catabolic enzymes (MMPs-1 and 13, and ADAMTS-4) were assessed as the percentage of immunopositive cells in the NP and AF. With the exception of MMP-1, cytokine and enzyme expression levels were significantly elevated in ChABC-treated discs in the NP and AF. Expression levels of TNF- α , IL1- β , and ADAMTS-4 were positively correlated with histological

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grade, while all cytokines and ADAMTS-4 were negatively correlated with MRI T2 and T1p scores. These results demonstrate that degenerate goat discs exhibit elevated expression of clinically-relevant inflammatory mediators, and further validate this animal model as a platform for evaluating new therapeutic approaches for disc degeneration.

Keywords

Intervertebral disc degeneration; caprine; large animal model; inflammation; preclinical

Introduction

Low back pain is the number one cause of disability globally, with associated socio-economic costs in the United States alone estimated to be in excess of \$80 billion annually.^{1; 2} Intervertebral disc degeneration, a cascade of cellular, structural and biomechanical changes closely linked to aging, is considered to be a primary cause of low back pain.^{3; 4} The intervertebral disc is comprised of a central, proteoglycan-rich and highly-hydrated nucleus pulposus (NP), a peripheral, fibrocartilaginous annulus fibrosus (AF), and two cartilaginous endplates that interface superiorly and inferiorly with the adjacent vertebrae.⁵ These sub-structures interact to support the distribution and transfer of compressive loads, and facilitate complex mobility of the intervertebral joint. Degenerative changes typically manifest first in the NP, where loss of proteoglycans and associated hydration compromises the ability of this tissue to pressurize and support compressive loads, leading to progressive structural and biomechanical deterioration of the entire disc.^{6; 7} The initiating factors for disc degeneration are multifactorial: aging, trauma, genetics and lifestyle may all contribute to earlier onset and accelerated progression.^{8–10}

Current therapeutic approaches for painful disc degeneration, whether they be conservative or surgical in nature, have limited long term efficacy as they seek only to manage symptoms without restoring healthy disc structure and mechanical function.⁴ Next generation therapies that incorporate stem cells, structural implants and bioactive molecules hold significant promise but have failed to see widespread clinical adoption.¹¹ With respect to stem cells, specifically, therapeutic efficacy is impeded by the *in vivo* microenvironment of the degenerate disc, which is characterized by limited nutrient and oxygen availability, low pH and persistent local inflammation.^{11–13}

Local inflammation within the NP and AF is defining feature of disc degeneration, and is characterized by persistent upregulation of a wide array of pro-inflammatory molecules by endogenous cells.¹² These include cytokines such interleukin-1 β (IL-1 β) and tumor necrosis factor-alpha (TNF- α), which in turn induce upregulation of downstream catabolic enzymes that accelerate destruction of critical extracellular matrix components including collagens and proteoglycans.^{14; 15} Increased inflammatory cytokine expression may stimulate neurotrophin production and potentiate nerve ingrowth into the otherwise aneural disc.¹⁶ In addition to accelerating the degenerative cascade, inflammation also negatively impacts the performance of therapeutic stem cells.^{17–20}

Anti-inflammatory drugs such as cytokine inhibitors have the potential to both arrest native tissue destruction and provide an environment more conducive to stem cell-based regeneration, and may thus represent an important component of biological regenerative therapies. To progress such therapies towards clinical application, it is important to undertake *in vivo* efficacy studies in large animal models. It is essential that such models effectively recapitulate the cellular microenvironment of the degenerate human disc, including local inflammation. Recently, we described a goat model of lumbar disc degeneration in which locally-delivered chondroitinase ABC (ChABC) across a range of doses was used to produce a spectrum of clinically-relevant compositional, structural and biomechanical changes.²¹ The objective of this study was to further establish the appropriateness of this animal model as a platform for evaluating stem cell and anti-inflammatory therapies by quantifying the expression of physiologically-relevant pro-inflammatory cytokines and catabolic enzymes in the NP and AF. We hypothesized that expression levels of inflammatory molecules in goat discs would correlate positively with disc degeneration severity assessed both histologically and using quantitative magnetic resonance imaging (MRI).

Methods

Animals and Surgical Procedures

The study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania, and the Animal Ethics Subcommittee of the Corporal Michael J. Crescenzi Philadelphia VA Medical Center. Nine male large frame goats, aged approximately 3 years, underwent a surgical procedure to induce degeneration of the lumbar intervertebral discs.²¹ Male goats were used to maximize disc size. Data from this same cohort of animals, together with the detailed surgical procedure were published previously.²¹ Briefly, under general anesthesia and using an open, lateral, retroperitoneal transposoatic approach, the lumbar spine was exposed and L1–2, L2–3, L3–4 and L4–5 lumbar discs randomized to receive either subtotal nucleotomy (n=4, 0.43 ± 0.17 g of NP tissue removed using 2mm pituitary rongeurs), saline sham injection (n=4), or 0.1U, 1U or 5U of ChABC (n=4 per dose). ChABC was suspended in 200µl of normal saline and injected into the NP using a 22G spinal needle under fluoroscopic guidance. Sham injections consisted of 200µl of normal saline. The T13–L1 or L5–L6 discs served as intact controls. The incision was closed in layers, and the animal recovered and returned to housing. Animals were administered per-operative transdermal fentanyl (2.5mcg/kg/hr) and intravenous flunixin meglumine (Banamine, 1.1mg/kg) for analgesia. Florfenicol (40 mg/kg) was administered for antimicrobial prophylaxis. Animals were housed in a 25 × 25m outdoor barn in groups of 2 or 3 with access to full exercise, and evaluated daily by a veterinarian for signs of pain, behavior changes or gait abnormalities for the duration of the study. Animal diet consisted of grass hay provided ad libitum, plus up to 1lb of goat chow per day calculated based on body score. Animals were euthanized 12 weeks after surgery, and lumbar spines harvested for the postmortem analyses described below.

Magnetic Resonance Imaging

Quantitative MRI was used to non-invasively assess disc degeneration severity as described previously.²¹ Excised lumbar spines were imaged in the sagittal plane on a 3T clinical MRI scanner (Siemens Magnetom TrioTim; Munich, Germany) with a voxel size of 0.6 mm × 0.6 mm × 5 mm. Series for T2 (Echo Time = 13 * i, i = 1, 2 ... 6) and T1ρ (Spin Lock Time = 12 * i, i = 1, 2 ... 5) mapping were also obtained, and the T2 and T1ρ relaxation times were quantified in a manually segmented circular region of interest in the NP using ImageJ (NIH; Bethesda, USA) as described previously.²¹ Shorter T2 and T1ρ times correspond to lower NP water and proteoglycan contents.^{22; 23} Standard T2-weighted images were also obtained for qualitative assessment of disc condition.

Histological Grading

Following MRI, posterior bony elements were removed and spines divided into bone-disc-bone segments by cutting through intervening vertebral bodies. Spine segments were then fixed in 10% buffered formalin for 1 week, completely decalcified (Formical-2000; StatLab Medical Products, McKinney, USA) and processed into paraffin. Mid-sagittal, 8μm-thick sections were cut and double stained with either Alcian blue (glycosaminoglycans) and picrosirius red (collagen), or hematoxylin and eosin (cellularity). Semi-quantitative histological grading was performed by 3 blinded assessors (averaged) as described previously.²¹ Parameters assessed included NP cellularity, NP extracellular matrix, endplate structure, NP-AF boundary, and AF organization, each on a continuous scale from 0 (best) to 100 (worst). An overall grade (ranging from 0 to 500) was calculated as the sum of these individual scores.

Immunohistochemical Assessment of Cytokine and Enzyme Expression

Protein expression levels of inflammatory cytokines (TNF-α, IL-1β and IL-6) and catabolic cytokines (MMPs -1 and -13, and ADAMTS-4) in the NP and AF were examined using immunohistochemistry. Positive reactivity of all antibodies was validated using primary goat NP cells cultured in six well plates and pre-stimulated with lipopolysaccharide (LPS) for three days (Supplementary Figure 1). Negative controls were produced by replacing primary antibodies with an isotype control at the equivalent protein concentration (Supplementary Figure 1). The optimal antigen retrieval method and working dilution were determined for each antibody (Table 1). For each antibody, sections from all study groups were stained in parallel to ensure consistent results. Antigen retrieval was carried out on rehydrated sections using either a heat-mediated technique in a bath of Tris-EDTA pH 8.0 buffer at 60°C, overnight, or enzymatically using proteinase K (10mg/ml; Roche Diagnostics; Basel, Switzerland) for 4 minutes at 37°C. Sections were treated with 3% hydrogen peroxide for 12 minutes to block endogenous peroxidase activity, followed by Background Buster (Innovex Biosciences; Richmond, USA) for 10 minutes at room temperature to block non-specific protein binding. Sections were then incubated with each primary antibody (Supplementary Table 1) overnight at 4°C. Antibody staining was visualized using the Vectastain Elite ABC-Peroxidase Kit (Vector Laboratories; Burlingame, USA) and diaminobenzidine chromogen (ThermoFisher Scientific; Waltham, USA) according to the manufacturer's protocol. Finally, sections were counterstained with hematoxylin QS (Vector Laboratories; Burlingame, USA)

and cover-slipped with aqueous mounting medium (Agilent; Santa Clara, USA). For analysis, all slides were imaged under bright field light microscopy (Eclipse 90i; Nikon; Tokyo, Japan). One slide from each disc was analyzed for each molecule. Three randomly-selected regions of interest within both NP and AF were analyzed. For each region, the number of immunopositive cells was counted and normalized as a percentage of the total number of cells present. Cell counting for each region was performed by 3 individuals who were blinded to the study groups and averaged. The average result for all three regions (in the NP or AF) was then determined and taken as a single biological replicate for statistical comparisons.

Statistical Analyses

Differences in cytokine and enzyme expression levels between each intervention group and controls were established using Kruskal-Wallis tests with post-hoc Dunn's tests. Spearman's rank-order tests were used to examine direct correlations between cytokine and enzyme expression levels, as well as between expression levels of each molecule and disc degenerative condition assessed as overall histology grade, or NP T2 or T1 ρ relaxation times. Correlations were defined as weak ($r < 0.3$), moderate ($0.3 < r < 0.7$) or strong ($r > 0.7$). A value of $p < 0.05$ was considered statistically significant for all tests, while $p < 0.1$ was considered a trend.

Results

No adverse events occurred as a consequence of the surgical procedure or during the subsequent 12-week study. As described previously, the different interventions used to initiate degeneration in goat lumbar discs produced a spectrum of degeneration severities, ranging from mild (nucleotomy and 0.1U ChABC) to moderate (1U ChABC) to severe (5U ChABC), as visualized histologically and by T2-weighted MRI (Figure 1). Saline sham discs showed no obvious signs of degeneration and were indistinguishable from intact, healthy control discs.

Inflammatory Cytokine Expression Levels

Positive expression of TNF- α , IL-1 β and IL-6 was found in both the NP and AF of degenerate goat discs (Figures 2A, D & G, and Supplementary Figure 2), with the percentage of immunopositive cells varying significantly as a function of intervention type. For TNF- α , expression in the NP was greatest in for 1U ChABC discs ($p = 0.013$ vs control, Figure 2B), followed by 5U ChABC discs ($p = 0.062$ vs control). Similarly, in the AF, TNF- α expression was greatest for 1U ChABC discs ($p = 0.053$ vs control, Figure 2C). For IL-1 β , expression in the NP was greatest for 5U ChABC discs ($p = 0.008$ vs control, Figure 2E), followed by 1U ChABC discs ($p = 0.049$ vs control). In the AF, IL-1 β expression was greatest for 5U ChABC discs ($p = 0.015$ vs control, Figure 2F), followed by 0.1U ChABC discs ($p = 0.038$ vs control). Finally, for IL-6, expression in both the NP and AF was greatest for 5U ChABC discs ($p = 0.006$ and 0.046 vs control, respectively, Figures 2H and I). Cytokine expression levels were not significantly elevated for saline sham and nucleotomy discs compared to controls.

Catabolic Enzyme Expression Levels

Similar to inflammatory cytokines, positive expression of MMPs -1 and -13, and ADAMTS-4 was found in both the NP and AF of degenerate goat discs (Figures 3A, D & G, and Supplementary Figure 3), with the percentage of immunopositive cells varying as a function of intervention type. For MMP-1, expression was not significantly elevated for any intervention type compared to controls in either the NP or AF (Figures 3B and C). For MMP-13, expression in the NP was greatest for 0.1U ChABC discs ($p=0.046$ vs control, Figure 3E). In the AF, MMP-13 expression was greatest for 5U ChABC discs ($p=0.068$ vs control, Figure 3F). Finally, for ADAMTS-4, expression was greatest for 0.1U ChABC discs ($p=0.016$ vs control, Figure 3H), followed by 5U and 1U ChABC discs ($p=0.023$ and $p=0.064$ vs control, respectively). In the AF, ADAMTS-4 expression was greatest for 5U ChABC discs ($p<0.016$ vs control, Figure 3I), followed by 0.1U and 1U ChABC discs ($p=0.029$ and $p=0.091$ vs control, respectively). Similar to cytokines, enzyme expression levels were not significantly elevated for saline sham and nucleotomy discs compared to controls. Examining direct correlations between cytokine and enzyme expression levels, significant, moderate positive correlations were found between TNF- α , and all three enzymes in both the NP and AF (Table 1). Significant, moderate positive correlations were found between IL-1 β and all three enzymes in the NP, and ADAMTS-4 in the AF. IL-6 expression was not significantly correlated with expression of any enzyme in either the NP or AF.

Correlations between Cytokine and Enzyme Expression Levels, and Histological Grade

Correlations between cytokine and enzyme expression in the NP and AF, and disc degeneration severity assessed histologically, were determined using Spearman's rank-order tests. In general, cytokine and enzyme expression levels increased with increasing (worse) histology grade. For cytokines (Figure 4), in the NP, significant, moderate, positive correlations were found between overall histology grade and TNF- α ($r=0.585$, $p=0.005$) and IL-1 β ($r=0.584$, $p=0.006$). In the AF, a significant, moderate, positive correlation was found between overall histology grade and IL-1 β expression ($r=0.521$, $p=0.016$). For enzymes (Figure 5), there were significant, positive correlations between overall histology grade and ADAMTS-4 expression in the NP (moderate correlation: $r=0.545$, $p=0.013$) and in the AF (strong correlation: $r=0.727$, $p<0.001$).

Correlations between Cytokine and Enzyme Expression Levels, and MRI Grade

Correlations between cytokine and enzyme expression in the NP and disc degeneration severity assessed using quantitative MRI were determined using Spearman's rank-order tests. For cytokines (Figure 6), there were significant, moderate, negative correlations between NP T2 relaxation time, and TNF- α ($r=-0.569$, $p=0.006$), IL-1 β ($r=-0.570$, $p=0.006$) and IL-6 ($r=-0.462$, $p=0.031$) expression. Similarly, there were significant, moderate, negative correlations between NP T1 ρ relaxation time and TNF- α ($r=-0.42$, $p=0.049$) and IL-1 β ($r=-0.445$, $p=0.038$) expression, while IL-6 ($r=-0.411$, $p=0.057$) trended towards significance. For enzymes (Figure 7), there was a significant, moderate, negative correlation between T2 relaxation time and ADAMTS-4 expression ($r=-0.634$, $p=0.002$), while MMP-1 trended towards significance ($r=-0.401$, $p=0.072$). There was a

significant, moderate, negative correlation between T1 ρ relaxation time and ADAMTS-4 expression ($r=-0.525$, $p=0.014$).

Discussion

Persistent, local inflammation is a defining characteristic of intervertebral disc degeneration and increases concomitantly with increasing degeneration severity.^{14; 15} Inflammation potentiates disc extracellular matrix degradation, nociception and innervation, and limits the performance of therapeutic cells.^{12; 17} Attenuating inflammation therefore represents a promising therapeutic strategy for disc degeneration and by extension, low back pain. The objective of this study was to characterize inflammatory cytokine and catabolic enzyme expression in a goat model of lumbar disc degeneration, in order to better validate this model as a clinically-relevant platform for evaluating cell-based and anti-inflammatory disc therapies. The inflammatory molecules we selected for investigation each have well-defined expression patterns in human discs.¹² Our results showed that, similar to human discs, goat discs exhibited increasing levels of expression of inflammatory cytokines and catabolic enzymes concomitantly with increasing degeneration severity.

Prior studies assessing the local inflammatory milieu of degenerate discs using *in vivo* animal models have primarily used rodents.^{24–28} There have been relatively few such studies in large animal models of disc degeneration, despite their importance for conducting preclinical therapy studies and potential for yielding more clinically relevant findings. In one study, disc cells were found to exhibit increased immunopositivity for TNF- α and IL-10, but not IL-6, following trans endplate injury in a porcine model of end stage lumbar disc degeneration.²⁹ In another study, NP cells isolated from canine lumbar discs 6 weeks following AF puncture injury were found to secrete elevated levels of IL-1 β and TNF- α .³⁰ To our knowledge, the current study is the first to describe elevated expression (measured as immunopositivity of both NP and AF cells) of inflammatory molecules in a caprine model of lumbar disc degeneration, and the first to demonstrate a correlation with degeneration severity in any large animal model. We found that expression of cytokines and enzymes was most consistently upregulated when degeneration was induced with higher doses (1 and 5U) of ChABC, which in general correspond to moderate and severe degeneration, respectively.²¹ The lack of significant inflammation in saline and nucleotomy-treated discs is unsurprising given the absent and mild degenerate responses reported previously for these discs, respectively.²¹ The upstream mechanisms driving increased tissue-level inflammation in our model remain to be fully elucidated, but are likely multifactorial and a consequence in part of pathological changes to the local biochemical and biophysical cellular microenvironments. For example, we showed previously that degenerate goat discs exhibit altered compressive biomechanical properties,²¹ and abnormal loading has been shown to upregulate inflammatory gene expression in disc cells in a range of *in vivo* and *ex vivo* model systems.^{25; 28; 31–34} Alternatively, accumulation of extracellular matrix degradation products in the degenerate NP may induce an inflammatory response via toll-like receptor signaling.³⁵ Further, the nutrition supply to disc cells may be altered as a consequence of endplate structural changes, potentially increasing cell stress and promoting a more catabolic phenotype.²¹ Finally, it is also possible that elevated local inflammation is in part due to a residual immunogenic response to exogenous ChABC protein.

The roles of TNF- α and IL-1 β in the pathophysiology of human disc degeneration are particularly well characterized, with mRNA expression and the percentages of TNF- α and IL-1 β immunopositive cells in both the NP and AF significantly higher in degenerate versus healthy human discs.^{15; 36; 37} The role of IL-6 in disc degeneration is less-well studied, but has been shown to be elevated in NP and AF tissues from patients with symptomatic lumbar disc herniations.³⁸ Here we show that degenerate goat discs exhibit elevated expression of TNF- α , IL-1 β and IL-6, with expression levels positively correlated with degeneration severity as measured using histology and/or MRI. We also examined expression of three extracellular matrix degrading enzymes (MMPs -1 and -13, and ADAMTS-4) elevated in human disc degeneration.¹⁴ Expression of these enzymes may be induced downstream of TNF- α , IL-1 β and IL-6, which is broadly supported by our correlation analyses (Table 1).¹² MMP-1 breaks down interstitial collagens types I, II and III, MMP-13 breaks down collagen II, while ADAMTS-4 degrades aggrecan, which is the major aggregating proteoglycan in the disc.^{5; 39} Interesting, of the three enzymes examined, quantitative MRI parameters were only found to be significantly correlated with ADAMTS-4 expression. This may reflect the fact that MRI T2 and T1 ρ relaxation times are surrogates for disc proteoglycan content,^{22; 23} and ADAMTS-4 specifically degrades proteoglycans.

From a therapeutic standpoint, the rationale for inhibiting local inflammatory cytokine expression is three-fold. First, the associated decrease in downstream catabolic enzyme activity and extracellular matrix destruction may slow structural deterioration of the intervertebral joint. Second, the decrease in downstream neutrotrophin production may inhibit symptomatic nerve ingrowth. Finally, inhibiting inflammatory cytokine expression may enhance the viability and performance of therapeutic cells such as mesenchymal stem cells (MSCs). Both IL-1 β and TNF- α negatively impact the viability and differentiation potential of MSCs^{17-19; 40} and may promote aberrant mineral deposition.⁴¹ Further, IL-1 β and TNF- α were recently demonstrated to induce rapid GAG loss from MSC-seeded hydrogel constructs in a dose-dependent manner.²⁰ Notably, MSCs themselves, provided they remain viable upon implantation, may impart immunomodulatory effects on native disc cells to reduce inflammation.¹⁷

Both IL-1 β and TNF- α have been explored as potential therapeutic targets for treating disc degeneration,^{12; 42-48} and inhibitors of both these cytokines have been used clinically for a number of years for other chronic inflammatory disorders such as rheumatoid arthritis.⁴⁹ IL-6 inhibitors are also approved for various conditions.⁵⁰ Experimentally, interleukin-1 receptor antagonist (IL-1ra) has been shown to effectively inhibit the downstream catabolic effects of IL-1 β at the molecular, compositional and functional levels *in vitro* and *in vivo*.^{42; 43; 45; 47} While both IL-1 β and TNF- α can induce downstream catabolic enzyme activity,¹² it has been suggested that IL-1 β may be a more effective therapeutic target, as degenerate human discs were reported not to exhibit elevated TNF receptor expression levels¹⁵ and IL-1 β was found to be more potent at inducing a catabolic response in disc cells.⁴⁴

This study had a number of limitations. The inclusion of male animals only precluded an analysis of potential sex-dependent effects on cytokine and enzyme expression levels. Additionally, there were insufficient sections available for multiple sections to be quantified for each molecule, potentially adding to the variability of results. There is the possibility that

immune cells infiltrate degenerate goat discs, where they could participate in the local inflammatory microenvironment as they do in degenerate human discs.¹² Identifying and characterizing the potential roles of such infiltrating cells will be the subject of future investigations. An additional limitation was the fact that we did not assess potential adjacent level effects of degeneration due to biomechanical and biological crosstalk between adjacent discs. Finally, the molecules we selected for analysis in this study have well-defined roles in human disc degeneration; however, in the future a more comprehensive examination of the inflammatory milieu of degenerate goat discs in comparison to human discs is warranted.

Conclusions

In this study we have demonstrated that ChABC-induced degeneration in goat intervertebral discs is associated with persistently elevated expression levels of clinically-relevant inflammatory cytokines and catabolic enzymes after 12 weeks. Expression levels were found to be positively correlated with degeneration severity assessed both histologically, and non-invasively using quantitative MRI. Our results serve to further validate this goat model as an effective preclinical platform for evaluating new therapeutic approaches for disc degeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative histology (Alcian blue and picrosirius red staining) and corresponding T2-weighted magnetic resonance imaging of goat lumbar discs showing a spectrum of degeneration severities across intervention types. Mid-sagittal sections. Histology scale = 1mm; MRI scale = 5mm.

Figure 2.

Representative immunostaining and quantification of cells immunopositive for inflammatory cytokines in the NP and AF of healthy and degenerate goat discs. **A-D.** TNF- α ; **E-H.** IL-1 β ; and **I-L.** IL-6 immunopositive cells. Scales = 100 μ m (20x) and 20 μ m (100x). Median and interquartile range; * $p < 0.05$ and + $p < 0.1$ versus control. One section from each disc was analyzed for each cytokine.

Figure 3.

Representative immunostaining and quantification of cells immunopositive for catabolic enzymes in the NP and AF of healthy and degenerate goat discs. **A-D.** MMP-1; **E-H.** MMP-13; and **I-L.** ADAMTS-4 immunopositive cells. Scales = 100 μ m (20x) and 20 μ m (100x). Median and interquartile range; *p<0.05 and +p<0.1 versus control. One section from each disc was analyzed for each enzyme.

Figure 4.

Correlations between inflammatory cytokine expression levels (percent immunopositive cells) in the NP and AF, and disc degeneration severity (histological grade) after 12 weeks.

A. TNF- α ; **B.** IL-1 β ; and **C.** IL-6. Dotted lines indicate 95% confidence intervals.

Figure 5.

Correlations between catabolic enzyme expression levels (percent immunopositive cells) in the NP and AF, and disc degeneration severity (histological grade) after 12 weeks. **A.** MMP-1; **B.** MMP-13; and **C.** ADAMTS-4. Dotted lines indicate 95% confidence intervals.

Figure 6.

Correlations between inflammatory cytokine expression levels (percent immunopositive cells) in the NP, and disc degeneration severity (quantitative MRI) after 12 weeks. **A.**

Cytokine expression vs NP T2 relaxation time. **B.** Cytokine expression vs T1 ρ relaxation time. Dotted lines indicate 95% confidence intervals.

Figure 7.

Correlations between catabolic enzyme expression levels (percent immunopositive cells) in the NP, and disc degeneration severity (quantitative MRI) after 12 weeks. **A.** Enzyme expression vs NP T2 relaxation time. **B.** Enzyme expression vs T1 ρ relaxation time. Dotted lines indicate 95% confidence intervals.

Table 1.

Correlations between inflammatory cytokine and catabolic enzyme expression levels in the nucleus pulposus and annulus fibrosus.

			Nucleus Pulposus		Annulus Fibrosus	
			r	p-value	r	p-value
TNF-α	vs	MMP-1	0.522 *	0.013	0.474 *	0.026
		MMP-13	0.617 *	0.002	0.545 *	0.009
		ADAMTS-4	0.689 *	<0.001	0.471 *	0.027
IL-1β	vs	MMP-1	0.501 *	0.018	0.404	0.062
		MMP-13	0.489 *	0.021	0.395	0.071
		ADAMTS-4	0.677 *	0.001	0.609 *	0.003
IL-6	vs	MMP-1	0.233	0.296	0.088	0.694
		MMP-13	0.366	0.094	0.411	0.057
		ADAMTS-4	0.357	0.075	0.002	0.934

* Significant correlation; spearman rank tests