INTRODUCTION: Intervertebral disc (IVD) degeneration is a common cause of low back pain (LBP) and immune cells such as mast cells have been identified in painful human IVD tissue and in vivo discogenic pain models1,2. However, their role in the pathogenesis of IVD disease is unclear. Mast cells are the “first responders” during tissue injury, releasing pre-formed granules containing cytokines, growth factors, and enzymes (TNF-α, IL-6, NGF, VEGF, substance P, ADAMTS5 and Tryptase) to augment angiogenesis, healing and repair3. Additionally, mast cells are responsible for recruitment of other cell types (e.g., macrophages) that promote matrix remodeling in similar degenerative diseases such as osteoarthritis4. Our central hypothesis is that mast cells are present in the painful intervertebral disc and that upon activation augment catabolic, inflammatory and angiogenic processes as well as initiate further immune cell recruitment perpetuating a chronic inflammatory painful microenvironment within the IVD. The aims of this study were to: 1) confirm the presence of mast cells in painful IVD tissue, 2) identify whether mast cells are able to survive in the harsh IVD niche and 3) investigate the effects of soluble factors from mast cells on inflammatory, angiogenic and catabolic gene expression in IVD cells and conversely how soluble factors from healthy and degenerate IVD cells influence mast cells.

METHODS: Aim 1: Mast cells in IVD tissue - Waste canine IVD tissue (N=3) discarded from microdiscectomy procedures (exempt from IACUC approval) was fixed, paraffin embedded and sectioned. Histological staining was performed using Giemsa and mast cell specific marker tryptase. Bright-field images were captured at x20 magnification. Aim 2: Mast cells in an IVD niche - Human leukemia mast cell line HMC-1 cells were cultured in both normoxic (20%) and hypoxic (5%) (N=4) conditions for 24 hours. Live/dead analysis was performed using calcine/ethidium respectively, imaged at x10 and quantified. Aim 3: Mast cell- IVD cell interactions - Bovine Nucleus Pulposus (NP), Annulus Fibrosus (AF) and Cartilage endplate (CEP) (N=6) cells were cultured in 2% agarose gels at a density of 1.6 x 10⁶ cells per gel for all experiments. Mast cell conditioned media (MCCM) was generated by stimulating HMC-1 cells, cultured at a density of 5.0 x 10⁵ cells/ml, with calcium ionophore A23187 for 2.5 hours, after which the media was added to disc cells in agarose gels and cultured for 24 hours. Disc cell conditioned media (CM) was generated in both healthy (non-TNF-a) and degenerate (+ 10 ng/ml TNF-a) conditions for 24 hours and was then added to 5.0 x10⁵ HMC-1 cells for 24 hours. Gene expression was evaluated in HMC-1 and IVD cells using qRT-PCR and statistics assessed using Kruskal Wallis & Mann-Whitney tests (p<0.05).

RESULTS: Mast cells identified by Giemsa and mast cell specific marker tryptase were present in painful canine IVD tissue in dense areas of granulation and in close proximity to blood cells (Fig. 1A-F). Live/dead analysis of mast cells cultured in a harsh IVD environment (Hypoxia 5% O₂ and low serum 1% FBS) demonstrated no significant differences in HMC-1 cell viability compared to their normal conditions (Normoxia 21% O₂ and 10% serum) (P>0.05). When examining the effects of MCCM on IVD cells, mast cells significantly increased the gene expression of IL-6 and a trend of increased expression in ADAMTS5 (p=0.08) in NP cells compared to controls (Fig. 2). In CEPs, exposure to MCCM significantly increased CCL2/MCP-1 and IL-6 expression (P<0.05) however no significant differences in AF cells were observed. When investigating the effects of healthy IVD CM on mast cells, we observed significant decreases in VEGF, TNF-α and IL-1β (P<0.05) and a trend of decreased expression in CCL2/MCP-1 (P>0.057) in mast cells treated with healthy AF CM compared to both untreated controls and degenerate AF CM (Fig.3). Interestingly, degenerate NP CM significantly decreased IL-1β expression in mast cells compared to control and healthy NP CM (P<0.05) and CEP CM had no significant effects on mast cell gene expression.

DISCUSSION: Results are consistent with the hypothesis that mast cells play a role in painful IVD degeneration. Mast cells were present in painful canine IVD tissue and were able to withstand the harsh IVD microenvironment in vitro culture. Mast cell conditioned media also caused CEPs to up-regulate the expression of IL-6 and a trend of increased expression in ADAMTS5 (p=0.08) in NP cells compared to controls (Fig. 2). In CEPs, exposure to MCCM significantly increased CCL2/MCP-1 and IL-6 expression (P<0.05) however no significant differences in AF cells were observed. When investigating the effects of healthy IVD CM on mast cells, we observed significant decreases in VEGF, TNF-α and IL-1β (P<0.05) and a trend of decreased expression in CCL2/MCP-1 (P>0.057) in mast cells treated with healthy AF CM compared to both untreated controls and degenerate AF CM (Fig.3). Interestingly, degenerate NP CM significantly decreased IL-1β expression in mast cells compared to control and healthy NP CM (P<0.05) and CEP CM had no significant effects on mast cell gene expression.

SIGNIFICANCE: This study is the first to investigate the role of mast cells in the painful intervertebral disc and suggests that immune modulation may hold promise as a potential therapeutic target for discogenic back pain.


ACKNOWLEDGEMENTS: The authors would like to thank Taylor Yeater, Pavel Sul and Katherine Lakstins for technical assistance.

ORS 2017 Annual Meeting Poster No.1816