Introduction: Although herniated disc (HD) is rarely a fatal disorder, HD related symptoms are pervasive. The granulation tissues of HD are composed of a prominent infiltration of macrophages, neovascularization, and disc cells. Previously, we have determined that infiltrated macrophages and disc cells express the matrix metalloproteinases (MMP) MMP-3 (stromelysin-1) and MMP-7 (matrilysin), which are both potent proteoglycanases. The co-localization of these MMPs, together with previous magnetic resonance imaging (MRI) findings that epidurally displaced HD are more commonly resorbed, suggests the involvement of these MMPs in the resorption process. We speculate that these MMPs may be upregulated by chondrocytes and/or macrophages in the process of disc herniation and contribute to the resorption process. We have developed a coculture system in which chondrocytes or disc tissues were incubated with macrophages to investigate the role of MMPs and chondrocyte/macrophage interaction in disc matrix degradation.

Materials and Methods: Chondrocytes for coculture with macrophages were obtained by one of two methods: murine coccygeal intervertebral discs from homozygous MMP-3-null, MMP-7-null, and wild type (wt) 129/Sv mice were cultured directly, or rat RCJ 3.1CS.18 (C5.18) chondrocyte cells (generously provided by Dr. Jane Aubin, Univ. of Toronto) were suspended in 1.2% low-viscosity alginate. Murine macrophages were obtained by intraperitoneal administration of thioglycollate. Chondrocytes were placed in cell culture inserts in 6-well dishes, while macrophages were placed on the bottom of each well. Cells were cultured in DMEM+10%FBS, 25µg/ml ascorbic acid, and 50µg/ml gentamycin for histological analysis or OPTI-MEM for RT-PCR and western blotting. Following 2-6 days of co-culture, cells or medium were processed for determination of chondrocyte differentiation state and MMP mRNA, protein, and activity. Paraformaldehyde-fixed, paraffin-embedded sections of alginate beads were stained with 0.25% Safranin O to detect proteoglycan synthesis, and with anti-proteoglycan and anti-type II collagen antibodies by the streptavidin-biotin method. Serum-free cell culture medium was collected and total protein quantitated using the Bio-Rad assay. Medium was subjected to SDS-PAGE, proteins were transferred onto nitrocellulose membranes, and MMP-3 and MMP-7 levels analysed with specific antibodies and chemiluminescent detection. Total RNA was extracted using a modified guanidinium isothiocyanate procedure. RT-PCR analysis was performed with specific MMP-3, MMP-7, and GAPDH primers. To analyze MMP activity in situ, C5.18 cells in alginate beads or murine disc tissue was cultured directly with macrophages that had been fluorescently labeled with PKH26 red fluorescent cell linkers. Samples after 2 days of coculture were embedded in OCT compound and 6µm cryo sections cut directly onto slides containing a 2% gelatin/2% sucrose film with fluorescent DQ-casein dissolved to a final concentration of 25 µg/ml in the presence and absence of proteinase inhibitors. Sections were incubated in a darkened, humid box at 37°C for 24h. Red fluorescence identified macrophages and green fluorescence identified cleaved casein.

Results: Rat C5.18 cells differentiated into chondrocytes in alginate beads as determined by the production of proteoglycan and type II collagen. Chondrocytes alone produced very low levels of MMP-3, and peritoneal macrophages produced sporadic, low levels of MMP-7 mRNA. In contrast, co-cultures of chondrocytes and macrophages produced elevated levels of both MMP-3 and MMP-7 protein by Western analysis. RT-PCR revealed that the majority of the MMP-3 was produced by the chondrocytes, and the majority of the MMP-7 by the macrophages under coculture conditions, but both cell types produced detectable levels of both enzymes. In addition, an assay which detects enzyme activity in situ through the use of a quenched substrate which fluoresces upon cleavage revealed that the coculture samples generated high levels of caseinolytic activity in chondrocytes which was inhibited by the MMP inhibitor batimastat (BB-94) but not by inhibitors of serine, cysteine, or aspartyl proteases. Macrophages derived from MMP-7-null mice and discs derived from MMP-3-null mice had significantly reduced macrophage infiltration and lower levels of in situ caseinolytic activity compared to WT controls or MMP-7-null discs and MMP-3-null macrophages.

Discussion: The metalloproteinases MMP-3 and MMP-7 are strongly upregulated when macrophages are added to chondrocyte cultures. The in situ fluorescent substrate assay revealed that metal-dependent caseinolytic activity produced by chondrocytes was significantly elevated by co-culture, strongly suggesting that co-culture produces active MMP-3 and MMP-7 that would be expected to degrade the surrounding proteoglycan. Interestingly, the absence of MMP-7 from the macrophage and MMP-3 from the chondrocyte resulted in decreased macrophage infiltration and decreased degradative activity. We speculate that MMP-7 may be required for macrophage invasion, and that MMP-3 may play a role in the release of macrophage chemotractants from chondrocytes. We conclude that the invasion of HD tissue with macrophages results in the induction of elevated levels of MMP-3 and MMP-7 in the chondrocytes and induces resorption of the disc tissue. The administration of exogenous sources of these enzymes may physiologically facilitate the natural course of HD resorption.

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