Introduction

Herniated discs are a common problem that is responsible for symptoms in up to 40% of all patients with low back pain. We previously conducted consecutive sequential observations on lumbar HD patients with conservative treatment by magnetic resonance imaging (MRI) examination. HD patients showed a progressive decrease in size. Based on our previous studies, MCP-1 (Monocyte Chemoattractant Protein-1) derived from intervertebral disc cells may play a critical role in natural HD resorption by triggering macrophage infiltration into HD tissue. Generation of soluble TNF-α was essential for the induction of MMP-3 (matrix metalloproteinase-3) in disc cultures, which in turn was required for the generation of a macrophage chemoattractant, subsequent macrophage infiltration and disc matrix degradation (J Clin Invest 2000). This study was undertaken to investigate the age-related differences of MCP-1 and MMP-3 expression in murine intervertebral disc (IVD) and to determine whether MMP-3 plays a role in disc degeneration.

Methods

Mice

Homozygous wild type C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). MMP-3-deficient mice were generated by homologous recombination as described previously and maintained on a 129/SvEv background. Experimental protocols were approved by the Institutional Animal Care and Use Committee of our University (No 19-105).

Disc organ culture

Murine coccyeal intervertebral disc tissue specimens were obtained from the tail bone using a dissecting microscope after the skin and soft tissue were removed. For organ culture assays, microcopy was used to harvest intervertebral disc tissue completely from the end plates. Whole intervertebral disc tissue specimens were cultured in a 24-well plate in 1 ml of DMEM containing 0.1% FBS and 50 microg/ml penicillin and streptomycin in the presence or absence of 10 ng/ml rm TNF-α or 100 ng/ml rm TWEAK for 12 or 72 h in a humidified environment of 5% CO2 at 37°C.

MCP-1 and MMP-3 expression

Expression of MCP-1 and MMP-3 messenger RNA (mRNA) in IVD derived from 4- to 64-week-old mice was assessed by quantitative PCR. The ability of MCP-1 and MMP-3 expression in IVD to respond to TNF-α or TWEAK stimulation was examined by quantitative PCR, Western blot analysis, ELISA, and immunohistochemistry.

Immunopositive cells were evaluated by independent pathologists examined the number of cells in 5 high power fields in a blinded fashion. IVD derived from 6- or 64-week-old MMP-3-deficient and wild-type mice were compared using Safranin-O staining.

Magnetic Resonance Imaging

In vivo MRI was performed on a 3T Sina scanner (GE Medical, UK Ltd). The mice were initially anesthetized with 2,2,2-Tribromoethanol (Avertin, Sigma-Aldrich, St. Louis, MO) (i.p. 125 mg/kg) and then placed on a imaging stage and analyzed by the MRI system. T2-weighted sections in the sagittal plane were obtained as a series of multiple two-dimensional slices.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). The significance was then determined using a Student’s or Welch’s t-test after an F-test was performed, unless otherwise stated. If the raw data did not fit a normal distribution, the Mann-Whitney U-test was used. P values less than 0.05 were considered significant.

Results

1) MCP-1 and MMP-3 mRNA expression in IVD

MCP-1 and MMP-3 was expressed in murine IVD. MCP-1 or MMP-3 generation was significantly increased in the presence of rm TNF-α or rm TWEAK after 72 hours treatment in culture. Both MCP-1 and MMP-3 mRNA levels in mouse IVD significantly diminished as age increased from 4 to 64 weeks. The ability of MCP-1 or MMP-3 expression to respond to TNF-α or TWEAK stimulation was significantly reduced as age increased from 4 weeks to 6 weeks.

2) MCP-1 and MMP-3 protein expression in IVD

MCP-1 and MMP-3 protein expression was revealed to diminish as age increased from 4 weeks to 32 weeks by western blotting of culture disc tissues. These protein expressions in supernatants of IVD cultures gradually decreased as age increased from 4 weeks to 64 weeks assessed by ELISA.

3) MCP-1 and MMP-3 immunoreactivity in IVD

MCP-1 and MMP-3 immunoreactivity was detectable predominantly in annulus fibrosus cells (AF) in young with nucleus pulposus (NP) cells. MCP-1 and MMP-3-positive cells were abundant in rm TNF-α or TWEAK-treated IVD compared with untreated IVD. However, MCP-1 and MMP-3-positive cells were decreased with increasing age from 4 weeks to 64 weeks in AF (p<0.05).

4) Aging or MMP-3 suppression of proteoglycan synthesis in IVD

IVD from 64-week-old wild mice showed a marked effect on proteoglycan (PG) loss compared with 4-week-old mice, whereas IVD derived from 64-week-old MMP-3-deficient mice was spared for PG loss by measuring alterations in PG content by Safranin-O staining.

T2 MRI images show water content of IVD. Although high intensity T2 images in IVD from 6-week-old wild mice was observed, a marked suppression of high signal intensity was found in IVD from 64-week-old wild mice. In contrast, IVD derived from 64-week-old MMP-3-deficient mice maintained high signal intensity.

Discussion

Current observations demonstrated that aging is associated with a reduction of cell numbers and protein production of MCP-1 and MMP-3. Our histological analyses showed that the process of disc degeneration progressed with age. This result suggests that MMP-3 plays a key role in IVD degradation. In vivo MRI result confirmed the ex vivo results and suggests that down-regulation of MMP-3 may be a possible therapeutic strategy for IVD degeneration. Our data support that MMP-3 is one of the target molecules involved with both disc herniation and disc degeneration. In addition, the natural resorption process of HD and disc degeneration may vary with the patients’ age. In young human IVD, down-regulation or inhibition of MMP-s may prevent induction of disc degeneration and disc herniation. In the case of old patients, artificial MMP-3 up-regulation may contribute to the natural resorption of HD by inducing degeneration of herniated disc. Administration of MCP-1 may also contribute to the natural resorption process through the induction of macrophages and release of TNF-α from activated macrophages, resulting in the induction of MMP-3 from disc chondrocytes. This method might be very useful for patients with HD because patients undergoing conservative treatment have a chance to receive curative treatment that induces the natural resorption process and patients may be spared invasive surgical treatment.

Conclusion

MCP-1 and MMP-3 expression in IVD derived from 4 to 64-week-old mice demonstrated age-related decreases. The response to inflammation in IVD also displayed age-related changes. Therefore, the natural resorption process of herniated disc and disc degeneration may vary with the patient’s age. Targeting MMP-3 may be a possible future therapeutic strategy for disc degeneration.

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