INTRODUCTION: Thrombin, a serine protease, is a key enzyme in the coagulation system. Thrombin has also been shown to be a multifunctional protein involved in a variety of biological functions, including cell migration [1], mitogenesis [2], apoptosis [2], and the release of cytokines and proteases [3-4]. Recently, the involvement of thrombin in organ development, revascularization and cancer metastasis has attracted attention. Thrombin has also been shown to play a critical role in the matrix remodeling and degradation mediated by the matrix metalloproteinases (MMPs) in studies of thrombosis [4]. The expression of pro-thrombin in articular cartilage has recently been reported [5].

The regulatory mechanism for extracellular matrix catabolism in the intervertebral disc (IVD) is not well understood. Recent reports suggested that catabolic enzymes, which are stimulated by pro-inflammatory cytokines, may be involved in matrix degradation in IVD tissues [6]. An increased vascularity in degenerated discs is also well known [7]. This may suggest a potential involvement of thrombin in matrix degradation of IVD tissue. The purpose of this study was to identify the effect of thrombin on matrix degradation and the secretion of the matrix degrading enzyme, MMP-3 by bovine and human IVD cells.

METHODS: Human and bovine IVD tissues were obtained from human cadaver spines (grade 2-3, Thompson grade) and bovine steer 

PG content was observed in AF beads. (Fig. 1). MMP-3 secretion: MMP-3 secretion was increased by thrombin at 10 U/ml in the culture media of human NP and AF beads at 24 and 48 hrs (Fig. 5).

Expression of PAR-1: PAR-1 is constitutively expressed by human (Fig. 6) and bovine IVD cells (data not shown).

DISCUSSION: Thrombin accelerated the degradation of PGs (pulse-chase) in the CM of NP cells. It also decreased the amount of PGs in the CM; the specificity was confirmed by the inhibition study with thrombin inhibitors. The results of the MMP-3 secretion study in the NP cells and the identification of the thrombin receptor, PAR-1, mRNA expression suggested that the effect of thrombin may be mediated by IVD cells, rather than the direct effect of thrombin as a serine protease. However, the possibility exists that thrombin acts on other matrix components as a degrading enzyme and that the matrix fragments generated can act on the NP cells. An agonist/antagonist study using a specific peptide to PAR-1 is in progress to address this point. Our preliminary results also showed the presence of a tissue factor that activates pro-thrombin in IVD cells; the thrombin-related cascade may be a part of the degradation mechanism of IVD degeneration. The differences in the response to thrombin between NP and AF cells need to be clarified.

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