INTRODUCTION. Proteoglycan depletion is a critical aspect of cartilage pathology in osteoarthritis (OA). Since the discovery in 1999 that an ADAMTS protease was the "aggrecanase" activity responsible for cartilage catabolism (1), a large body of work has been carried out delineating the specific enzymes responsible for cartilage degradation, and the regulatory mechanisms that control aggrecanase expression and activity. Two recent publications (2, 3) provide compelling evidence that ADAMTS 5 is the primary aggrecanase responsible for cartilage loss in murine arthritic models. The present study was carried out to develop an in vitro murine chondrocyte model to enable mechanistic studies on ADAMTS5-mediated aggrecanolysis, through gene deletion and transgenic strategies.

METHODS. Use of animals in these experiments was approved by the institutional review boards of the participating institutions. Distal femoral and proximal humeral epiphyses were collected from 4-day-old mice. In addition to wild-type mice, chondrocytes were also isolated from mice lacking functional expression of genes (CD44, MT4MMP, syndecan 1 and ADAMTS5) which were suggested from earlier studies (in bovine and human cells) to be involved in aggrecanolysis. Epiphyseal chondrocytes were isolated by sequential trypsin/EDTA and collagenase digestions, then cultured as non-adherent aggregates in serum-free medium supplemented with ascorbic acid. Five x 10^6 cells were seeded into 2cm-diameter wells of hydrogel-coated, low-attachment culture plates, in 1 ml of medium. Cultures were maintained for up to 12 days, with media changes and sample collections at 3-day intervals.

For collection purposes, the cellular aggregates were pelleted by low-speed centrifugation in the presence of protease inhibitors. Media were aspirated from the pellets and transferred to separate tubes. The pelleted cell aggregates were snap-frozen in liquid nitrogen, prior to further processing. The GAG content in the cellular and medium compartments was measured by DMMB assays. Aggrecan turnover was assessed by Western blot analyses of chondroitinase-treated lysates, using antibodies specific for the full-length protein and neoepitopes created by ADAMTS activity (4). Total RNA was isolated from aggregates using TRIzol reagent. Expression of the cartilage-associated ADAMTS genes (TSs 1, 4, 5, 8, 9 and 15) was determined by RT-PCR. Spatial abundance patterns were assessed in cell aggregates by immunohistochemistry with characterized IgGs to ADAMTS4 (JSCVMA), ADAMTS5 (JSCKNG), and two neoepitopes of degraded murine aggrecan, TSSELE (JSC3TAS) and NVTEGE (JSCNT).

RESULTS. Murine epiphyseal chondrocytes synthesized 60-80 ug GAG/million cells/3-days, with progressive accumulation of matrix throughout the culture period. Approximately 10% of total GAG was retained in the pericellular compartment while the remaining 90% was released into the medium. Aggrecan immunoblotting (Fig.1) demonstrated spontaneous degradation of the full-length protein with neoepitope formation characteristic of aggrecanase activity.

DISCUSSION. The IHC studies showed that the TSSELE and NVTEGE neoepitopes were present throughout the aggregates, with a predominantly pericellular distribution. ADAMTS5 was also detected throughout the aggregates, and most abundantly adjacent to cell surfaces. In contrast, ADAMTS 4 exhibited only low-level and discontinuous staining. These immunolocalization data provide strong corroboration of the kinetic and genetic analyses.

REFERENCES.

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