EVIDENCE FOR ZYMOCEN FORMS OF AGGREGANASE-1 (ADAMTS-4) IN CHONDROCYTE-AGAROSE CULTURES

Introduction:
A primary event in the destruction of cartilage in arthritic diseases is the loss of aggregan from the extracellular matrix of articular cartilage. During aggregan breakdown important cleavage sites are utilized which reside within the interglobular domain (IGD) of the aggregan core protein. The Asn341-Phe342 bond is cleaved by members of the Matrix Metalloproteinase (MMP) family and results in the N and C-terminal neoepitopes FFGV and DIPEN respectively. The second of the two cleavage sites, the Glu373-Ala374 bond, is cleaved by the aggrecanases which are all members of the A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS) family. The ADAMTS family members able to cleave at this so-called 'aggrecanase site' are ADAMTS-1, -4 and -5(1-3). Aggrecanase-1 (ADAMTS-4) is believed to be the specific aggrecanase induced by expression of cytokines (e.g. IL-1) in the pathogenesis of arthritic diseases. It is thought to be synthesized as an inactive zymogen which is activated in the Golgi via removal of its propeptide domain by furin. The secreted active protein has a molecular weight of 60-70KDa. However, little is known of its mechanisms of action in cartilage extracellular matrix. The objective of this study was to monitor Aggrecanase-1 (ADAMTS-4) secretion and sequestration in the extracellular matrix of chondrocyte-agarose cultures.

Methods:
Porcine articular chondrocytes were isolated using pronase and collagenase, embedded in 1% seaplaque agarose at 6 million cells per ml and plated onto a cell free agarose plug (4). The plates were precultured in DMEM + 50µg/ml gentamicin with 10% fetal bovine serum and 25µg/ml Phosphotan C for 7, 14 or 21 days to allow a matrix to be established. The plates were washed, then cultured in serum free DMEM + 50µg/ml gentamicin with or without 10ng/ml IL-1α for a maximum of 120 hours. Measurements for the release of sulfated GAG to the medium were obtained by using the Dimethylmethylene blue (DMMB) assay.

Media samples were analyzed by Western blotting for aggregan metabolites using monoclonal antibody BC-3 to recognize the aggrecanase generated neoepitope ARGSV, and BC-14 to recognize the MMP generated neoepitope FFGV... The presence of ADAMTS-4 in the media was analyzed using the newly characterized monoclonal antibody TS-4N, which recognizes the amino terminus of ADAMTS-4, activated at the Furin cleavage site (sequence FASLS...).

Agarose plugs were extracted either in detergent buffer containing Nonident P-40 or in 4M Guanidine HCl. The detergent extracts were analyzed by Western blotting for aggregan metabolites using monoclonal antibody BC-3 to recognize the aggrecanase generated neoepitope ARGSV, and BC-14 to recognize the MMP generated neoepitope FFGV... The presence of ADAMTS-4 in the media was analyzed using the newly characterized monoclonal antibody TS-4N, which recognizes the amino terminus of ADAMTS-4, activated at the Furin cleavage site (sequence FASLS...).

Essential Results:
In control cultures only 20-30% of the total GAG was released into the medium after 120 hours of culture. In contrast 80-90% of the total GAG was released in cultures exposed to IL-1.

Discussion:
This study indicates that several isoforms of ADAMTS-4 are synthesized by chondrocyte-agarose cultures in both the presence and absence of IL-1. However, in control cultures both the 75 and 60KDa isoforms are present without any detectable aggreganase activity. This indicates that these isoforms are either sequestered in the matrix as inactivezymogens (i.e. non furin-activated) or possibly inhibited by TIMPs. Since the 60KDa isoform corresponds to the full length ADAMTS-4 the 75KDa form is likely to have part if not all of its promatrix.

The presence of the 37KDa isoform in the IL-1 treated cultures suggests that this smaller isoform may be responsible for the 3-4 fold increase in aggregan release in the presence of IL-1. However since over 70% of the total GAG is released to the medium of the IL-1 treated cultures in 24 hours this fragment may alternatively be the result of ADAMTS-4 autocatalysis. Recombinant ADAMTS-4 has been shown to autokatolysate in the absence of a suitable substrate (5).

This study has identified multiple isoforms of putative aggreganase activity that could be responsible for increased aggregan catabolism that leads to cartilage degradation in arthritis.

References & Affiliations:
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3 Sandy et al (2000) J. Biol. Chem.: 1; 276; 13372-13378
5 Flannery et al unpublished findings

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