SPECIES AND AGE-RELATED DIFFERENCES IN THE TURNOVER OF AGGRECAN AND LINK PROTEIN

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Introduction: The maintenance of an organized extracellular matrix in articular cartilage is a prerequisite for the continued mechanical stability of the tissue. Aggrecan is one of the major macromolecules in the matrix and is normally anchored in the tissue by its interaction with hyaluronan and link protein. The turnover of aggrecan is facilitated by metalloproteinases (MMPs) and a putative proteinase, 'aggrecanase', which cleave aggrecan within its interglobular domain to generate fragments that have neo-carboxy terminals with the sequences FVDIPEN and NITEGE respectively. Recent studies have shown, i) that the half-life of these fragments is very much longer than the complete molecule and ii) that there is an age-related increase in the non-enzymatic cross links in cartilage matrix (1,2). Both of these events indicate that there may be an age-related change in the organization of aggrecan in cartilage and that this would influence the tissue compartment selected for turnover and the rate at which this happens. **Our hypothesis is, that the composition and organization of aggrecan in the extracellular matrix of cartilage reflects species, age and disease related changes in the tissue.**

Methods: Fresh porcine (6 months) and equine (4 years) articular cartilage was obtained from metacarpophalangeal joints. Fresh 'normal' human articular cartilage (16 years & 65 years) was obtained from knee joints surgically resected for bone tumors not involving the joint and osteoarthritic cartilage (70 years & 74 years) was removed from knee joints at the time of joint replacement. Cartilage explants were cultured for 10 days in DMEM in the presence and absence of IL-1β or retinoic acid (RA). Aggrecan degradation was assessed by analyzing fragments released into the culture medium and in 4M guanidine HCl extracts of cartilage, in 4-16% SDS-PAGE. The functional properties of the G1-fragments were assessed by agarose/PAGE and also by gel exclusion chromatography in Sepharose CL-6B and Sepharose CL-2B. Proteins were transferred to Immobilon or Hybond N+ membrane by Western blotting and their structure was investigated by immunoreaction with rabbit polyclonal antibodies recognizing the neoepitope generated by 'aggrecanase' (anti-NITEGE) or MMPs (anti-DIPEN).

Results: Chondroitinase ABC/keratanase II treatment of culture media and extracts significantly improved the localization of the neo-eoitopes NITEGE and VDIPEN on Western blots. In so doing the enzyme treatment identified a number of interesting characteristics of the G1-fragments which appeared to be species specific and also varied with age and pathology. For example, the single glycosylated-NITEGE product released into the culture medium of RA-treated explants of equine cartilage, had the highest molecular weight (110-120kD) compared to that of porcine and human cartilage. In the latter case three glycosylated-NITEGE products were released and three NITEGE-reactive proteins with mol. wts. of (80-90kD, 160-170kD & 220-230kD) were observed after enzyme treatment (Fig1): these could not be disrupted with mercaptoethanol. A similar observation was made for the VDIPEN epitope except that, as expected, all molecular weights were reduced. The only anomalous result obtained was with the porcine glycosylated-VDIPEN fragment which had the same molecular weight as the glycosidase-treated sample (approx.,55kD), suggesting that there are significant glycosaminoglycan additions within the proteinase cleavage sites of the IGD (Fig1). The G1-fragments generated by RA and IL-1 that were retained in the tissues at the end of culture, also reflected the species diversity. There were also significant differences in the molecular weights of the fragments in the tissue and in the culture medium. These were partly dependent on the proportions of the NITEGE and VDIPEN fragments present in each of the cartilages prior to culture.

The turnover of link protein in these cultures was also investigated by using the Mab 8A4 to identify the proteins released into the culture medium and to compare them with those retained in the tissue.. The kinetics of degradation and release of link protein mirrored that of the G1-domain of aggrecan and showed the same species and age-related changes.

The proteinase inhibitor, Batimastat, was effective at blocking aggrecan turnover. However, its inhibition of 'aggrecanase' and MMPs was selective and depended mainly on the maturity of the cartilage, but also on the pool of aggrecan fragment (medium or tissue) that was investigated.

Discussion: It is well known that cytokines accelerate the turnover of aggrecan by inhibiting its synthesis and promoting its degradation and loss from the tissue. However, even though it is accepted that there is considerable heterogeneity in aggrecan composition and structure most experimental studies of turnover assume that the substrate is a homogeneous one. Our investigations have identified species and age-related differences that can only be explained using a model for the organization of aggrecan, and the complex that it forms with hyaluronan and link protein, which relies on a varying molecular pool. This finding is supported by the kinetics of release of NITEGE and FVDIPEN fragments from immature cartilage which suggest that the proteinases involved in the turnover of aggrecan in this developing tissue are expressed at different times. Our studies of human cartilage also support this general hypothesis and highlight the dramatic effect that age can have on the chondrocyte's response to cytokine treatment. It is also clear that there must be a biological mechanism for removing the G1-domain from hyaluronan when the tissue is exposed to adverse conditions, but that this mechanism is not activated during normal turnover of aggrecan. Finally, the unusual fragmentation of the G1-domain in human cartilage suggests that some of the aggrecan molecules in adult cartilage may exist in higher ordered forms than are usually found in immature cartilage. **These results, therefore, are consistent with our recent identification of an age-related increase in pentosidine cross-links in purified aggrecan (2) and the values of turnover of the 'free' G1-domain vs. G1-aggrecan (t1/2 of 25yrs and 3.5yrs respectively) (1).**

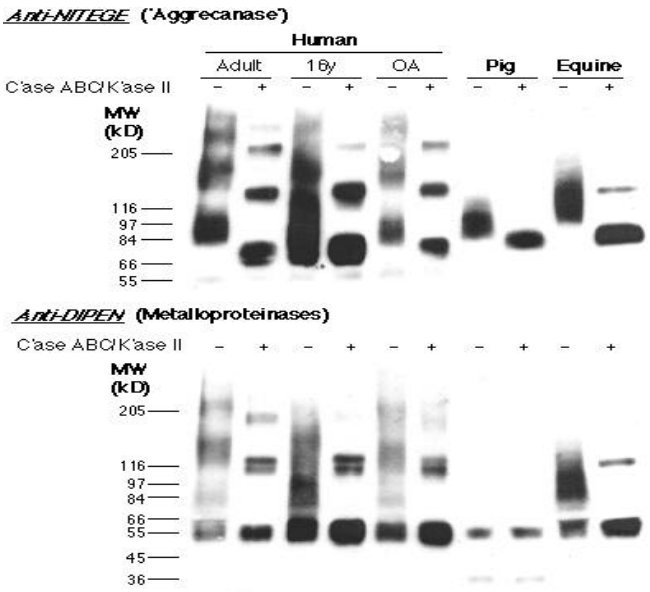

Figure 1. SDS-PAGE of culture medium from RA-treated cartilage. Western blot of the gels were probed with the polyclonal antisera recognizing the neo-epitopes in the IGD of aggrecan generated by 'aggrecanase' and MMPs.

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References: 1) Bank et al (1998) Biochem.J. 330, 345-351; 2) Maroudas et al (1998) Arch. Biochem. Biophys. 350, 61-71.

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