Interleukin-1 induces aggrecanase-mediated cleavage in human articular cartilage without up-regulating stromelysin or glycosaminoglycan release

Elizabeth C Arner, Theresa M DiMeo, Michael A Pratta and Micky D Tortorella

The DuPont Merck Pharmaceutical Company, Wilmington, DE 19880, USA. Tel +1-302 695 7078. Fax +1-302 695 7873

Normal turnover of cartilage proteoglycan as well as loss in arthritic conditions involves proteolytic cleavage of the aggrecan core protein within the interglobular domain (IGD) releasing a large GAG-containing aggrecan fragment which diffuses out of the cartilage matrix. However, the proteinases responsible for the normal turnover and pathological loss of aggrecan have not been identified. Two major sites of proteolytic cleavage have been identified within the IGD between amino acid residues Asn341-Phe342 and Glu373-Ala374. Many matrix metalloproteinases (MMP-1, -2, -3, -7, -8 and -9) have been shown to cleave in vitro at the Asn341-Phe342 site [1–3]. However, attempts to generate cleavage at the Glu373-Ala374 site with a number of purified proteinases [1–4] have been unsuccessful, indicating that this cleavage is the result of a novel, as yet unidentified, proteinase, given the name "aggrecanase" based on its ability to cleave the aggrecan core protein. Analysis of aggrecan fragments from several in vitro systems [5–9] and from human arthritic synovial fluid [10–11] have identified fragments with the Ala374 N-terminus indicating that cartilage degradation involves cleavage at the Glu373-Ala374 "aggrecanase" site. To investigate the relationship between proteoglycan degradation, matrix metalloproteinase production and cleavage of aggrecan at the "aggrecanase" clip site, human articular cartilage was incubated in the absence or presence of interleukin -1 (IL-1).

Methods

Normal human articular cartilage slices were maintained in explant culture with Dulbecco's modified Eagle's medium containing 5% heat-inactivated fetal bovine serum for at least 3 days at 37 °C. After this equilibration period, media were replaced with serum-free media with or without IL-1. At the end of the incubation, media were removed and stored at –70 °C for analysis. Sulfated glycosaminoglycan (GAG) levels in the media were determined by the dimethylmethylene blue assay [12]. Stromelysin-1 (MMP-3) protein was evaluated by Western analysis using a polyclonal rabbit anti-human MMP-3 sera. For analysis of aggrecan fragments generated by cleavage at the Glu373-Ala374 site, proteoglycans were enzymatically deglycosylated with chondroitinase ABC, keratanase and keratanase II. Equivalent amounts of GAG from each sample were loaded and then separated by SDS-PAGE (4–15% gradient gels), transferred to nitrocellulose and immmunolocated with antibody BC-3 recognizing the new N-terminus ARGSVIL... on aggrecan degradation products generated by the action of "aggrecanase" [13].

Results

In normal adult human articular cartilage stimulated with IL-1 over a broad range of concentrations (0.1–10,000 ng/ml), GAG released into the culture media was not significantly elevated above control levels and MMP-3 protein was not detected in media from control or IL-1 stimulated cultures. However, IL-1 caused a concentration-related up-regulation of

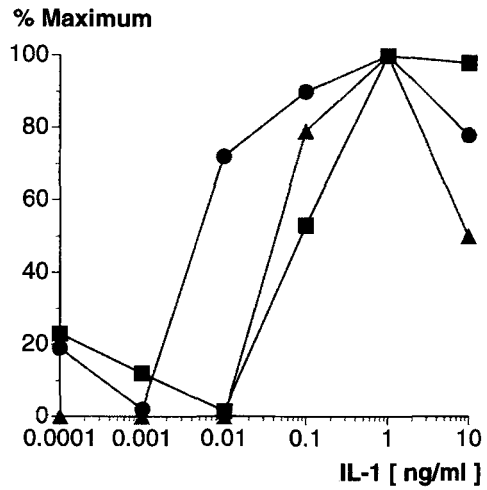

Figure. Concentration–response for interleukin-1 stimulation of newborn human articular cartilage. Following a 48 h incubation, the media from control or IL-1-stimulated cultures were analyzed for GAG (■) by DMMB assay and data quantitated as μg GAG / mg wet weight cartilage. MMP-3 protein (●) and BC-3 reactive aggrecan fragments (▲) in the media were evaluated by Western analysis and quantitated by scanning densitometry. Results are plotted as percent of maximal response.

the proportion of BC-3-reactive aggrecan fragments generated by "aggrecanase" cleavage, with maximal stimulation seen with IL-1 concentrations 1 ng/ml and above. In contrast, IL-1 stimulation of newborn human articular cartilage resulted in a concentration-related increase in MMP-3, which was present exclusively in the zymogen or pro form, and GAG release into the culture media as well as an induction of BC-3-reactive fragment generation. The IL-1 concentration-response curves corresponded for induction of cleavage at the "aggrecanase" site and GAG release, but not for induction of MMP-3 (Figure). The concentration of IL-1 causing half-maximal stimulation (EC50) of MMP-3 induction was approximately 20 fold lower than that for GAG release whereas EC50 values were similar for GAG release and BC-3 fragment generation.

Discussion

These data support the induction of "aggrecanase" cleavage occurring in the absence of detectable MMP-3 up-regulation in adult human articular cartilage and suggest that stimulation with IL-1 causes an alteration in aggrecan catabolism that is not readily detectable by measuring the total amount of GAG release into culture media. The detection of increased GAG levels in the media from newborn cartilage stimulated with IL-1 along with the generation of BC-3-reactive fragments opens the possibility that the increased collagen crosslinking present in adult cartilage may result in retention of clipped aggrecan fragments within the matrix. In addition, in newborn cartilage there was a correlation between the IL-1 concentration-response curves for induction of cleavage at the "aggrecanase" site and GAG release, suggesting that "aggrecanase" may be responsible for aggrecan breakdown in this system.

References

1. Flannery C R, Lark MW, Sandy JD. J Biol Chem 1992; 267: 1008–14.
2. Fosang A J, Neame P J, Last K, Hardhingham T E, Murphy G, Hamilton J A. (1992) J Biol Chem 1992; 267: 19470–4.
3. Fosang A J, Last K, Knauper V, Neame P J, Murphy G, Hardingham T E, Tschesche H, Hamilton J A. Biochem J 1993; 295: 273–6.
4. Flannery C R, Sandy J D. Orthop Trans 1993; 17: 677.
5. Sandy J D, Boynton, P L, Flannery C R. J Biol Chem 1991; 266: 8198–205.
6. Sandy J D, Neame P J, Boynton P L, Flannery C R. J Biol Chem 1991; 266: 8683–5.
7. Leulakis P, Shirkhanda A V, Davis G, Maniglia C A. Biochem J 1992; 264: 589–93.
8. Ilic M Z, Handley C J, Robinson H C, Mok M T. Arach Biochem Biophys 1992; 294: 115–22.
9. Lark M W, Gordy J T, Weidner J R, Ayaia J, Kimura J H, Williams H R, Mumford R A, Flannery C R, Carisoni S S, Iwatai M, Sandy J D. J Biol Chem 1995; 270: 2550–6.
10. Sandy J D, Flannery C R, Neame P J, Lohmander L S. J Clin Invest 1992; 69: 1512–6.
11. Lohmander L S, Neame P J, and Sandy J D. Arthritis Rheum 1993; 36: 1214–22.
12. Farndale R W, et al. Conn Tiss Res 1992; 9: 247.
13. Hughes C E, Caterson B, Fosang A J, Roughley P J, Mort J S. Biochem J 1995; 305: 799–804.

Immunogenetic profiles correlate with pro-MMP expression in rheumatoid arthritis

Helmut Fenner[1], David Taylor[2], Manfred Schattenkirchner[3], Gerd Folkers[1] and Stefan Zueger[1]

[1]Swiss Federal Institute of Technology, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland, [2]University Hospital of South Manchester, UK, and [3]Rheumatology Unit, University of Munich, Germany.
Correspondence: Dr H Fenner, Department of Pharmacy. Tel +41-61 981 5603. Fax +41-1 261 0691.

Distinct HLA-DRB1 haplotypes may predispose patients with rheumatoid arthritis (RA) to a more aggressive disease course. This was suggested from data generated by retrospective analysis of disease patterns and profiles (Weyand et al. 1992). It has not been studied so far whether the presence or absence of DRB1*0401, 0404, 0408 and DRB1*0101 is associated with a difference in expression / up-regulation of effector molecules involved in articular matrix degradation.

Our aim was to explore whether certain HLA-DR haplotypes determine the course of RA by inducing different pathophysiological profiles as defined by a difference in pro-MMP expression / up-regulation.

Patients and methods

118 patients diagnosed for RA (1987 revised ACR criteria) were enrolled in this cross-sectional study. Subpopulations defined by disease duration and subgroups of patients defined by immunogenetic profiles