Oncostatin M modifies the mechanism of proteoglycan aggregate degradation induced by IL-1 and TNFα

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Introduction

The ability of articular cartilage to withstand compressive loads is in large part due to its high content of the proteoglycan aggrecan. Aggrecan is present in the cartilage extracellular matrix (ECM) as proteoglycan aggregates, in which many aggrecan molecules associate non-covalently with a filament of hyaluronan with each interaction being stabilized by a link protein. The catabolism of aggrecan in the proteoglycan aggregates is thought to be an early event in cartilage degeneration in arthritis. It is now well accepted that degradation of aggrecan is mediated by pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα), via their ability to stimulate the production of metalloproteinases. Aggrecan degradation was initially ascribed to members of the matrix metalloproteinase (MMP) family, though more recent studies indicate a major role for more specific aggrecanases (ADAMTS 4 and 5). Proteolysis of the aggrecan core protein at any site results in one component that is no longer bound to hyaluronan, and this component is rapidly lost from the ECM by diffusion. In contrast, the aggrecan component bearing the hyaluronan-binding region (G1 region) will remain in the ECM. In theory aggrecan depletion from the ECM may also occur via hyaluronan degradation. Such a mechanism would be distinguished from proteolysis of the aggrecan core protein by the concomitant release of aggrecan fragments bearing the G1 region and link protein from the cartilage ECM.

The purpose of this study was to investigate whether the sites of proteolytic cleavage within the aggrecan core protein vary with the type of cytokine to which the cartilage is exposed and whether hyaluronan degradation is a cytokine-inducible event.

Methods

Bovine articular cartilage was obtained from the metacarpophalangeal joints of skeletally mature animals. Cartilage explants were maintained in DMEM at 100 ng tissue per 2 ml medium, with the medium being changed every 2 days. Cultures were maintained for 8 days, at which time both the medium and the tissue were analysed for aggrecan degradation. Degradation was induced by treatment with a variety of cytokines, used either alone or in combination: human IL-1α and IL-1β (5 ng/ml), TNFα (10 ng/ml), IL-6 (10 ng/ml), IL-17 (50 ng/ml), and oncostatin M (Osm, 10 ng/ml). The culture media were analysed for proteoglycan release by a colorimetric glycosaminoglycan (GAG)-binding assay using dimethylmethane blue (DMMB). The culture media and tissue were analyzed for aggrecan degradation products and link protein by SDS-PAGE and immunoblotting. For this purpose the cartilage was extracted with 4M guanidinium chloride in the presence of protease inhibitors, and the guanidine subsequently removed by dialysis. Samples were routinely treated with chondroitinase ABC and keratanase prior to electrophoretic analysis. SDS/PAGE was carried out under reducing conditions in 4-12% polyacrylamide gels, and proteins were then electroblotted onto nitrocellulose for immunodetection. Aggrecan and its degradation products were detected using rabbit polyclonal antibodies recognizing the amino terminal G1 region and the carboxy terminal G3 region. Link protein was detected using a mouse monoclonal antibody (8A4).

Results

By day 2 of culture, IL-1β treatment alone induced a two-fold increase in GAG release compared to the controls with no cytokine, although maximal GAG release was not observed until day 4. Treatment with IL-1β in combination with IL-6 or IL-17 showed no major additive influence. In contrast, samples treated with IL-1β plus Osm resulted in a striking 8-fold increase in GAG release by day 2. A similar effect was observed when Osm was used together with IL-1α or TNFα.

Immunoblot analysis of the culture media and tissue extracts indicated that IL-1β treatment alone or in combination with IL-6 or IL-17 results in the accumulation of G1-containing products in the tissue. Many of these products represent free G1 regions derived by proteolytic cleavage within the adjacent interglobular domain. The sizes of the G1 regions are compatible with aggrecanase action but not that of MMPs. As expected for aggrecanase-mediated degradation, the size of the link protein was unaffected. In contrast, two major differences were observed in the presence of OsM. First, the level of G1 products in the medium was much higher and the level in the tissue was proportionately reduced. The release of the aggrecan G1 products was accompanied by the simultaneous release of intact link proteins, again without any evidence of proteolytic modification. Second, an additional component bearing the G3 region of aggrecan was detected in the culture medium. This product required chondroitinase treatment for detection, and following removal of its GAG chains had a molecular size of about 45 kD. A similar product was also observed when OsM was used together with IL-1α or TNFα.

Discussion

The degradation of aggrecan by aggrecanases occurs via cleavage at distinct sites on the aggrecan core protein. Four such sites have been described in the CS2 region of the core protein and one in the interglobular domain. Generation of the additional degradation product seen in the presence of OsM must involve a unique cleavage site in the carboxy terminal portion of the aggrecan core protein to produce a product possessing the G3 region and at least one chondroitin sulfate attachment site from the adjacent CS2 region. This is likely to involve the participation of a proteinase in addition to aggrecanase. This proteinase could either act directly on the aggrecan core protein or could act on aggrecanase to alter its specificity. For example, carboxy terminal truncation of aggrecanase has been reported to influence its action on aggrecan.

The accumulation of aggrecan G1-containing products in the tissue is the result of their continued interaction with hyaluronan in a link protein mediated process. Release of these components via a proteolytic mechanism would require degradation within the G1 regions and link protein, which are extremely resistant to proteolytic cleavage when interacting in a proteoglycan aggregate. However, hyaluronan cleavage would result in rapid release of both the aggrecan G1 regions and link protein, and this is the most likely explanation for the action of OsM observed in this work. It is possible that hyaluronan degradation could be due to either an enzymic or non-enzymic mechanism. Enzyme-mediated release would involve the participation of hyaluronidases, whereas non-enzyme-mediated release would involve the action of free radicals, and it is conceivable that cytokines could be involved in either pathway.

Irrespective of the mechanism involved, the results imply that aggrecan degradation is modulated and loss from articular cartilage is accelerated under conditions of joint inflammation where OsM is produced in conjunction with IL-1α, IL-1β or TNFα.

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