Introduction. Degradation of articular cartilage is a central feature of joint diseases. Proteolysis of the two major structural components of the extracellular matrix of articular cartilage, namely aggrecan and type II collagen, disrupts the mechanical integrity of the tissue leading eventually to erosion and exposure of subchondral bone. Depletion of aggrecan is one of the earliest events in cartilage degeneration while degradation of type II collagen occurs later in the disease process and may represent the point of irreversible cartilage damage[1]. The type II fibrils of cartilage have numerous associated molecules including members of the leucine rich repeat proteoglycan (LRR-PG) family, decorin, biglycan, fibromodulin and lumican. These molecules interact with fibrillar collagenas and may have important functions in stabilizing fibril interactions and modulating fibrillogenesis. In addition, it has been suggested that the localization of these molecules to the surface of type II collagen provides a form of protection to the fibrils[2] and thus, their degradation may be a prerequisite for subsequent collagenolysis. In the present study we have utilized in vitro models of progressive cartilage degradation to study the catabolism of aggrecan, typeII collagen, biglycan, decorin and fibromodulin. Understanding the pathophysiological processes that precede collagen degradation may provide areas for controlling the degeneration of cartilage in arthritic diseases.

Methods. Full-depth articular cartilage was harvested from the metacarpophalangeal joints of 3-6 month old pigs. Cartilage was pre-cultured in DMEM + 10% FBS, then washed and explanted cultured for 28 days in serum free DMEM ±10μg/ml LPS or 1ng/ml IL-1alpha + 50ng/ml oncostatin M (IL-1/OSM) with media changes every 7 days. Ten replicates of each treatment were harvested at days 7, 14, 21 and 28 and extracted with 4M GuHCl. The proteoglycan and collagen content of the medium, cartilage extract and cartilage residue were measured as sulphated glycosaminoglycan (GAG) and hydroxyproline (HyPro), respectively. The proteoglycan and collagen fragments in the medium and extracts were separated by SDS-PAGE, and Western blotted with antibodies recognizing; the aggrecanase generated aggrecan interglobular domain (IGD) neoepitopes ARG.. and ..EGE (BC-3 and BC-13, respectively); the matrix metalloproteinase (MMP) generated aggrecan IGD neoepitopes FFG.. and ..PGG (BC-14 and BC-4, respectively); the collagenase generated type II collagen neoepitopes AEGPPGPQG (9A4); decorin (70.6); the C-terminus of biglycan (PR-1); and the C-terminus of fibromodulin. Differences in GAG and HyPro release associated with joint region and treatment were analysed using ANOVA and Fisher’s post hoc analysis, with p<0.05 being considered significant.

Results and Discussion. LPS and IL-1/OSM stimulation significantly increased GAG release compared with control cultures (p < 0.0001), with 60-80% of the total GAG being released during the first week. There was no significant change in HyPro release in control cultures over the 4 week period, with less than 4% of the total HyPro being released during this time. In contrast, LPS increased HyPro release from week 2 onwards and this reached statistical significance during the fourth week of culture (p = 0.02), with some 20% of the total collagen being released by this time. The combination of IL-1/OSM significantly increased HyPro release during the second, third and fourth weeks of culture when compared with controls (p < 0.05 for all analyses). These results demonstrate that both LPS and IL-1/OSM induce progressive cartilage degeneration with the majority of the GAG being released early (week 1) followed by later collagen catabolism. IL-1/OSM induced a more rapid and marked collagenolysis with approximately 60% of the total collagen being released by the end of week 4.

The GAG release induced by both LPS and IL-1/OSM was associated with the release of BC-3 but not BC-14 positive aggrecan metabolites into the medium, indicating that both agents were increasing aggrecanase cleavage of the IGD. This was confirmed by the appearance of BC-13 positive metabolites in the cartilage extracts and medium during the first week of catabolic stimulation with both agents. Consistent with the lack of MMP cleavage of the IGD, no BC-4 positive metabolites were detected during the first week of culture. However, BC-4 positive aggrecan metabolites were found in both the cartilage extracts and medium during the third, fourth and fourth weeks of culture with LPS and IL-1/OSM but not controls. This increase in BC-4 was coincident with a decrease in BC-13 and a lack of BC-14 reactive metabolites throughout the culture period, suggesting that MMPs were secondarily cleaving aggrecanase-generated.EGE metabolites, but that primary IGD catabolism by MMPs was not induced. IL-1/OSM stimulation induced an increase in collagenase cleaved collagen metabolites (9A4 positive) in the medium and extracts from week 2 onwards, while this was only apparent in week 4 with LPS stimulation. This demonstrates that the HyPro release induced by both agents resulted from increased collagenase cleavage of type II collagen.

To determine whether there was a hierarchical and chronological catabolism of the collagen associated LRR-PGs and whether this was similar in the two models of degeneration, Western blot analysis of medium and cartilage extracts was performed. IL-1/OSM induced increasing release of biglycan into the medium from week one onwards (Fig 1). This was consistent with a decrease in biglycan in the comparable cartilage extracts during this time. In contrast, LPS decreased biglycan in the extracts from week 2 onwards but this was not accompanied by an increase in biglycan in the medium (Fig 1), suggesting that the catabolic mechanisms differed from those induced by IL-1/OSM. Nevertheless, the decrease in cartilage biglycan preceded type II collagenolysis induced by both agents. IL-1/OSM increased release of decorin into the medium from week 2 onwards (Fig 1), in conjunction with the increased HyPro release. In contrast LPS failed to increase decorin release into the medium (Fig 1) while still resulting in a decrease in decorin in the cartilage extracts, again demonstrating the difference between the two catabolic agents. Finally both agents induced a decrease in fibromodulin in the cartilage and this was coordinate with an increase in fibromodulin in the medium (Fig 1). There was little evidence of proteolytic fragmentation of the LRR-PGs in association with their release from the cartilage matrix with either catabolic agent.

Conclusions. These results have confirmed that aggrecan release precedes collagen catabolism in progressive articular cartilage degradation. Cleavage of the IGD by aggrecanases and not MMPs, was responsible for aggrecan release. Secondary catabolism of aggrecan metabolites by MMPs was evident in late stage degradation. Loss of biglycan, but not decorin or fibromodulin, from the cartilage preceded collagen catabolism. Nevertheless, all three LRR-PGs, were lost from cartilage only with progression to late stage degeneration associated with collagenolysis.


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