Results: Coculture of the U937 cells with synovial fibroblasts resulted in differentiation of U937 into monocytes, as evidenced by FACS indicating that they were CD14+. Over 7 days, the cytokine and chemokine levels in the media of these cocultured cells with cartilage explants approached levels similar to those reported for OA synovial fluid, including low levels of II-1β and TNF-α. GAG release was observed in co-cultures stimulated with II-1β, uPA, or Fn-f, with the most severe effects in the II-1β-treated group (Figure). However, the concentration of II-1β used in the control samples, typical of concentrations used by others for release of GAGs from cartilage disks in vitro, is far greater than the concentration reported in OA synovial fluid. Pretreatment with II-1ra was able to inhibit the loss of GAG from the cartilage in the II-1β-stimulated samples, but did not block Fn-f-stimulated GAG release in the co-culture system, and had a small effect on uPA stimulated GAG release.

Conclusions: An in vitro model of cartilage degradation has been developed utilizing a co-culture of human synovial fibroblasts with differentiated U937 cells, in the presence of bovine cartilage explants. The interaction of these cell types generated a cytokine profile similar to OA synovial fluid, and stimulation of this system with uPA or Fn-f led to cartilage degradation (GAG loss). Our ongoing work will continue to explore the roles of various cytokines and the molecular mechanisms through which they influence cartilage degeneration in this in vitro joint model system, and their potential relation to OA disease progression.

Matrix Biochemistry

444 OSTEOARTHRITIS AND AGE RELATED CHANGES IN AGGREGAN AND COLLAGEN IN HUMAN HIP CARTILAGE

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Purpose: To confirm that previously reported changes in collagen and GAG content from knee OA cartilage also occur in hip OA and that these changes are different from those in reference cartilages. We also examined the relationships between total collagen, % denatured collagen and GAG content and with age.

Methods: 18 OA (aged 45 to 81, 11 women) and 26 reference (aged 55 to 99 years, 20 women) femoral heads were collected at hip replacement surgery, due to OA or femoral neck fracture. OA cartilages were taken from sites where it was possible to sample full-depth cartilages whereas reference cartilages were taken from the superior most loaded areas. Cartilages from the same site were examined for routine histology and immunohistochemistry. Each sample was divided into two parts that were either, digested with 1mg/ml α-Chymotrypsin in 50 mM tris containing the proteinase inhibitors to extract denatured collagen, or with papain at 80C for 18 h (1–2 μl of papain/10 mg wet weight tissue) in 0.1 M Tris-HCl, pH 7.2, containing 0.01 M disodium EDTA and 0.005 cysteine-HCl to enable GAG analysis. The Chymotrypsin supernatants (containing cleaved collagen) and residues (containing intact collagen) were hydrolyzed in 12N and 6N HCl, respectively, at 110°C overnight and then dried. Subsequently the samples were dissolved in 500 μl of distilled water and clarified using an equal amount of charcoal and AG-1 X8 anion exchange resin. The amount of hydroxyproline (Hyp, μg/mg wet weight) was measured colorimetrically at 550 nm. The values from the assay were compared to those from a standard curve prepared with L-4-Hydroxyproline. Amounts of hydroxyproline were expressed either as total collagen or % degradation. The papain-digested samples were assayed for GAG by a commercially available kit using dye-precipitation of sulphated GAGs with Alcian blue (www.wieslab.com).

Results: The reference group was older than the OA group (p < 0.001). Collagen content was 23% lower in OA than reference cartilage (p < 0.002). % Denatured collagen content was 2.5 times higher in OA than that of reference cartilages (p < 0.001). % Denaturation was inversely related to collagen content in OA cartilage (r = −0.78, p = 0.000) but not in the reference cartilage (p = 0.5).

Conclusions: The present study provides new perspectives of the relationships between the two main molecules, collagen and aggrecan, in OA and reference articular cartilage. Similar collagen damage in hip OA cartilage as previously shown in knee OA cartilage suggests similar OA degenerative mechanisms of collagen in different joints. With respect to age, our study shows similarities between OA and reference cartilage. The lack of relationships between molecule contents and age may suggest that subjects age is not related to disease activity or susceptibility. Rather we believe that exposure time to a risk factor is of importance. The increased degree of denatured collagen and the inverse relationship between % denatured collagen and collagen content in OA compared to reference cartilage indicates mechanisms of progressiveness in disease that are not activated in normal ageing. Therapeutic interventions in progressive hip and knee OA may include means to control collagen cartilage content using inhibitors that decrease collagen degradation as well as treatments that increase cartilage GAG content in order to improve cartilage quality.

445 P38 MAPK IS AN ESSENTIAL UPSTREAM SIGNALLING PATHWAY IN PROTEOLYTIC CARTILAGE DEGRADATION

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Purpose: Matrix Metalloproteases (MMP) and aggrecanases are essential players in cartilage degradation. However, the signaling pathways involved that results in MMP and aggrecanase synthesis and activation are not well understood. We investigated whether the p38 MAPK was involved in processes leading to collagen type II degradation in catabolic stimulated articular cartilage.

Methods: Bovine articular cartilage explants were isolated from stifle joints from slaughtered cows 1.5–2 years of age. The explants were cultured serum-free in Dulbecco’s Modified Eagle’s Medium in the presence of 10 ng/ml Oncostatin M (OSM) and 20 ng/ml Tumor Necrosis Factor-α (TNF) with or without the specific inhibitors of p38 MAPK p38 MAPK was involved in processes leading to collagen type II degradation in catabolic stimulated articular cartilage.

Results: p38 MAPK inhibition abrogated collagen type II degradation in cartilage explants treated and metabolic inactive controls. The total levels of proteoglycan in the explants decreased by 50%, p < 0.001, compared to vehicle control, but the p38 inhibitor SB-203580 was able to retain the levels of proteoglycans by 35%, p < 0.01, compared to vehicle control. Exposure of the articular cartilage explants to OSM + TNF, and the specific p38 MAPK inhibitors SB-203580 or SB-202190, resulted in a dose-dependent complete abrogation of MMP mediated collagen type II degradation (CTX-II). This was in alignment with the quantification of the fluorescence.

Conclusions: We found that the inhibition of p38 MAPK abrogated proteolytic cartilage degradation by blocking MMP synthesis and activity. These results suggest that pathways resulting in less p38 signaling may be potential options for indirect modulation of MMP expression and activity. This may be important for the discovery of new potential OA treatments.

446 DETECTION OF THE EARLY CHANGE OF THE CARTILAGE DEGENERATION WITH FTIR AND ULTRASOUND EVALUATION

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Purpose: Early diagnosis of osteoarthritis is important, although the diagnostic method is not fully established yet. It is well known that