Mann-Whitney t-test. Correlations between cytokines, proteases, and cell-lineage markers were detected using Spearman’s correlation test.

**Results:** SM II-15 mRNA and SF protein levels were detectable in all early OA patients (18 SM and 11 SF), and in most end-stage patients as well (11/13 SM and 9/10 SF); furthermore protein levels were elevated in the early knee OA patients when compared to end-stage \( (p=0.0004) \). In contrast, SM II-21 mRNA levels were detectable in 11/18 early OA patients and in all end-stage patients, while SF protein levels were measured in 7/10 end-stage and 5/9 early OA patients. II-21 mRNA and protein levels did not differ in the two patient groups. In early OA patients, II-15 transcript levels within the tissue were associated with CD8 transcript levels \( (r=0.508, p=0.04) \), and II-21 mRNA levels correlated with CD19 \( (r=0.746, p=0.0009) \) and CD56 \( (r=0.742, p=0.0015) \); its expression was consistently higher in the early stage compared to the fibrotic stage. Moreover, the number of positive cells of HAS-1 and HAS-2 was lower in RA advanced stage although that of HAS-3 positive cells reached maximum level in the active inflammatory stage. Moreover, the number of HAS-3 positive cells was negatively correlated with the HA molecular weight in synovial fluid \( (r=-0.51, p<0.005) \) and positively correlated with histologically defined local inflammatory activity in RA \( (r=0.563, p<0.005) \).

**Conclusions:** The results suggest that HA may be actively synthesized by HAS-1 or HAS-2 in early stage of RA and become to smaller size by HAS-3 products and degradation by Hyals in inflammatory stage of RA. The number and distribution of Hyals and HASs can be affected by synovial inflammation.

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**443 GENERATION OF OSTEARTHROTIS-LIKE CYTOKINE PROFILES USING AN IN VITRO CO-CULTURE MODEL**

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**Purpose:** There are many in vitro systems for studying cartilage degeneration, but most do not model interactions between the various cell types in a joint. We developed an in vitro model of cartilage degradation using cells that are representative of those found in osteoarthritic joints. Our goal was to generate a compositional profile similar to that found in OA synovial fluid (as distinct from that in inflammatory conditions, such as rheumatoid arthritis) that would lead to cartilage degeneration.

**Methods:** Synovial fibroblasts (Type B cells), isolated from a 68-year-old normal donor, were cultured overnight at 2.5 x 10⁵/cm² in 24-well cell culture inserts in medium containing DMEM with 10% FBS and 50 µg/ml Gentamicin. The next day, premonocytic U937 cells, representing synovial Type A cells, were added to the insert at 1.0 x 10⁶/cm². The two cell types were cultured together for 3 days, promoting differentiation of the U937 cells into monocytes. After 3 days, medium was changed to serum-free and 6-mm full-thickness cartilage discs isolated from 6-month-old calves were added to the wells containing the cell-laden inserts. To stimulate the system with potential mediators of OA, cultures were treated with the N-terminal 30 KDa fibronectin fragment (Fn-f, 0.8 µM) or urokinase-type plasminogen activator (uPa, 15 nM). IL-1β treatment (5 ng/mL) was used as a positive control. To study the specific effects of IL-1 on stimulated co-cultures, some samples were preincubated with 1.4 nM IL-1α for 4 hours before stimulation. After 7 days, medium was assessed for sGAG content (DDMMB), and for II-1, II-1α, II-6, II-8, RANTES, TNFa and MMP-1, -3, and -13 (Luminex xMAP). GAG was also determined in cartilage discs (DDMMB, and Safranin O histology).