Fibronectin fragments, but not IL-1 and TNFα, induce aggrecanase-mediated catabolism of aggrecan in human articular cartilage

*Tortorella, M; **Anne-Marie Malfait, and *Elizabeth Arner
**Pharmacia Co, Skokie, IL.

Introduction
Degradation and loss of aggrecan from the cartilage matrix is an early characteristic of osteoarthritis. The breakdown of aggrecan is mediated by the cartilage aggrecanases, ADAMTS-4 and ADAMTS-5. Therefore, it is important to identify the factors that regulate the expression of these proteases in human cartilage. In the present studies, we evaluated the effect of IL-1, TNFα, and fibronectin fragments (Fnf) on aggrecanase expression and aggrecan degradation in human articular cartilage.

Materials and Methods
Fibronectin (Fn) was purified from human plasma, and digested with MMP-3. MMP-3 was then removed from the digest by adsorption to a hydroxamate-inhibitor-affinity resin. Intact human articular cartilage was taken post mortem from the knee. Bovine knee cartilage was taken from mature cows. Cartilage was cultured as explants in serum-free media and stimulated with IL-1, TNFα, fibronectin (Fn) or Fn fragments (FnF) for 48 hours, in the absence or presence of aggrecanase inhibitors. Culture media were analyzed for glycosaminoglycan (GAG) levels by DMMB assay, and for the release of aggrecanase-generated aggrecan fragments by neoepitope Western Blot analysis. The cartilage was analyzed for ADAMTS-4 and ADAMTS-5 protein and mRNA. Human osteoarthritic cartilage was analyzed for the presence of Fnf, and for the release of Fn-degrading activity by Fn zymography.

Results
IL-1 (1 – 100 ng/ml) and TNFα (10 ng/ml) were effective in inducing aggrecan degradation in bovine cartilage, but failed to induce the release of GAG from human articular cartilage. In contrast, FnF effectively induced digestion of both human and bovine cartilage aggrecan, whereas intact fibronectin had no effect. The degradative effect of FnF could not be abrogated by IL-1 receptor antagonist or neutralizing TNF antibody. GAG levels in the media were paralleled by the release of the aggrecanase-generated neoepitope 34ARGS (Fig.1).

FnF-induced aggrecan degradation was blocked by an ADAMTS-4/ADAMTS-5 selective inhibitor, but not by an MMP-inhibitor, indicating that the FnF-induced breakdown of aggrecan is mediated exclusively by aggrecanase. Moreover, normal human cartilage contained no ADAMTS-4 protein whereas FnF, but not intact Fn, induced the release of ADAMTS-4 protein from the matrix. Consistent with their failure to induce aggrecan catabolism, IL-1 and TNFα did not induce ADAMTS-4 protein. ADAMTS-5 protein was constitutively expressed in human cartilage, and FnF triggered the release of ADAMTS-5 into the culture medium.

In addition, ADAMTS-5 mRNA was constitutively expressed in bovine cartilage, whereas ADAMTS-4 was not present in unstimulated bovine cartilage, and was induced by FnF.

We found elevated levels of Fn in osteoarthritic compared with non-arthritic human cartilage. More importantly, the Fn in the osteoarthritic cartilage was present primarily as fragments, whereas the non-arthritis cartilage contained primarily intact Fn. Evaluation of culture media from osteoarthritic human cartilage as well as IL-1 stimulated bovine articular cartilage by Fn zymography, demonstrated the release of a Fn-degrading activity (Fig.2). In contrast, age-matched normal human or bovine cartilage did not release this activity.

Discussion
These findings suggest that cryptic epitopes, which are normally buried in the tertiary structure of the Fn molecule, can be revealed by enzymatic degradation and in turn signal the induction of aggrecanase activity by chondrocytes, leading to cartilage matrix degradation. They also suggest that in human articular cartilage, FnF rather than IL-1 and TNFα, may play a key role in the induction of proteolytic enzymes responsible for the degradation of the cartilage extracellular matrix in arthritic diseases. Also, our findings suggest that human OA cartilage releases an endogenous Fn-degrading activity that may mediate the disease process.