ACL TRANSECTION LEADS TO INCREASED EXPRESSION OF TYPE X AND XII COLLAGENS IN RABBIT MEDIAL MENISCI

*Hellio Le Graverand, M (A-The Arthritis Society); *Eggerer, J; **Vignon, E; *Barclay, L (A-CHHR); ***Mazzorana, M; +*Hart, D (A-CHHR, The Arthritis Society) +*McCaig Center for Joint Injury and Arthritis Research, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1. McCaig Center for Joint Injury and Arthritis Research, Faculty of Medicine, University of Calgary, HSC, 3330 Hospital Drive, NW, Calgary, Alberta, Canada T2N 4N1, 403 220-6885, Fax: 403 283-7742, hartd@ucalgary.ca

Introduction: The menisci of the knee joint are fibrocartilaginous structures that can resist both compressive and tensile loads, distributing them across the joint. The major collagen of the meniscus is type I collagen, but type II, III, V and VI collagens are also present (1). Increases in type I, II, III collagen deposition has been previously reported in torn menisci from ACL deficient rabbit knees (2). The present study was undertaken to develop a more complete understanding of changes in the extracellular matrix of the meniscus during the early stages of osteoarthritis (OA). Using the ACL transection model of OA in the rabbit, we investigated type X and XII collagen expression in both the medial and the lateral meniscus.

Methods: Skeletally mature (12 months of age) female New Zealand White rabbits were used in this study approved by the institutional animal care committee. All rabbits received a transection of the ACL of the right hind limb. Three rabbit groups were defined: 3 weeks post-ACL transection, 8 weeks post-ACL transection and non-operated age-matched rabbits as controls. (N=2)

Immunohistochemistry: After sacrifice, the menisci from the ACL transection rabbits and their age-matched controls were resected and immediately fixed in 10% formalin buffer, and processed for paraffin embedding. 6 um sections were immunostained with a human type X monoclonal antibody (gift from Von der Mark, K, Erlangen University, Erlangen, Germany) and with a type XII monoclonal antibody prepared in the laboratory of Dr. Mazzorana (IBCP, Lyon, France) (3). Sections were incubated with the primary antibody at a 1/100 dilution overnight at 4°C. The sections were washed twice in PBS and incubated in the appropriate secondary antibody solution for one hour followed by three washes in PBS.

Results: Figure 1 illustrates the strong immunostaining for type X collagen in the MM following ACL transection. The strongest deposition of type X collagen was observed in the numerous fibrochondrocyte clusters located on the outer edge of the bucket-handle tear of the MM which develops at 8 weeks post-ACL transection. In contrast, in the controls as well as in the LM at any time points following ACL transection, type X collagen protein was not detected. Type X collagen immunostaining co-localized with calcium deposits stained with alizarin red and birefringent in polarized light (Figure 2).

The deposition of type XII collagen was increased in the MM at both 3 and 8 weeks post-ACL transection. Figure 3 shows the increased immunostaining for type XII collagen within the degenerated region of the MM at 8 weeks post-ACL transection. In contrast, in the LM, type XII collagen deposition was not increased following ACL transection compared to controls.

Discussion: These results demonstrated that deposition of both Type X and XII collagen protein depositions are elevated during the early phases of OA in the medial meniscus of rabbit ACL deficient joint. The immunostaining for type X collagen demonstrated that only a sub-population of fibrochondrocytes organized in clusters in the MM expressed type X collagen compared to the remainder of the tissues. The pattern of expression for both Type X and XII collagens correlated with the observation of tissue degeneration (remodelling of the matrix around bucket-handle tears in the MM).

Our data demonstrated a strong correlation between calcium deposition and the type X collagen findings in the medial meniscus. Type X collagen is a non fibrillar collagen, classically expressed by hypertrophic chondrocytes in fetal growth plates and suggested to be involved in mineralization and endochondral ossification (4). In adults, type X collagen expression is usually restricted to the calcified cartilage region below the tide-mark (5). However, type X collagen expression has also been reported in chondrocyte clusters from osteoarthritic cartilage (6).

The present findings also demonstrated an increase in type XII collagen deposition in the torn meniscal meniscus during OA development. Type XII collagen belongs to the fibril-associated collagen with interrupted triple helix (FACIT), molecules that could act as links between the fibrillar network and other molecules of the extracellular matrix (7). Interestingly, the expression of type XII collagen has been shown to be regulated by mechanical stress, in particular by changes in tensile loads (8).

Taken together, the present data suggest that in the ACL deficient joint, the medial meniscus undergoes extensive matrix remodelling, as well as mineralization. Thus, increased expression of type X and XII collagen in the medial meniscus of the ACL transected knee could be an attempt to respond to the altered mechanical environment in such knees.

Acknowledgments: This work was supported by CIHR and the Arthritis Society.

References:
1: Cheung, 1987, Connect Tissue Res, 16, 343-356
2: Hellio Le Graverand et al., 2000, Osteoarthritis Cart, in press
3: Berthod et al., 1997, J Invest Derm, 108, 737-742
4: Schmid and Linsenmayer, 1985, Dev Biol, 107, 373-381
6: Von der Mark et al., 1992, Arthritis Rheum, 35, 806-811
7: Font et al., 1996, Matrix Biol, 15, 341-348
8: Trachslin et al., 1999, Exp Cell Res, 247, 320-328

**Claude Bernard University, Lyon, France.
***Institut de Biologie et Chimie des Proteines (IBCP), CNRS UPR 412, Passage du Vercors, 69367 Lyon Cedex 07- France.