

### ***A New Method of Detecting Cartilage Damage in Horses***

**Summary:** The purpose of this study was to develop, using similar technology to that employed in the production of COL2-3/4C<sub>short</sub>, an antibody to identify the neopeptide that is the newly created end of the type II collagen fragment produced by collagenase digestion in horses. The researchers have generated an antibody that recognizes collagenase-cleaved, type II collagen fragments in the horse. This antibody can detect increases in type II collagen cleavage in diseased equine articular cartilage. The 234CEQ antibody has the potential of detecting cartilage degradation in the joints of horses for the early diagnosis of arthritis and to monitor response to treatment.

Type II collagen is the major collagen of articular cartilage and provides its tensile strength. It has recently been shown that during cartilage degradation induced by proinflammatory agents, as may occur in arthritis, there is an initial loss of proteoglycans that, upon reaching a critical level, is followed by the degradation of type II collagen. Due, in part, to its critical role in maintaining the integrity of articular cartilage, as well as its very slow rate of turnover, damage to the type II collagen fibrillar network is believed to initiate the irreversible stages of cartilage degradation. Increased degradation of type II collagen has been identified in human articular cartilage in osteoarthritis (OA). A method to detect and monitor this process would allow for intervention at an earlier point in time than is currently possible, before the irreversible changes of OA.

The cleavage of fibrillar collagens, such as type II collagen, by enzymes can occur in the triple helical region of these molecules. The intrahelical digestion occurs at a specific and well-identified site within each chain of the triple helical collagen molecule (Figure 1) through the action of "collagenases" belonging to a family of enzymes called matrix metalloproteinases (MMPs). These collagenases are believed to define the rate-limiting step in normal tissue turnover and in degradative processes, such as in arthritis.

Antibodies have been developed to detect the fragments created by the cleavage of types I and II collagens by these collagenases in humans. The principle behind the technology involved in producing these antibodies is that, as a result of the action of the collagenases on the individual collagen chains, three-quarter and one-quarter length fragments are created with new ends. Antibodies are then prepared to react to the newly created ends or "neopeptides" and not to react to uncleaved collagen chains. An anti-neopeptide antibody, COL2-3/4C<sub>short</sub>, previously generated by Dr. Clark Billingham while

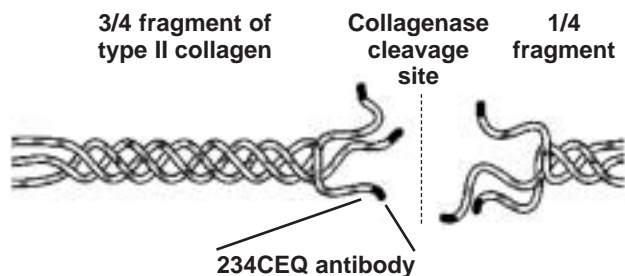
working in Dr. Robin Poole's laboratory in Canada, recognized not only the cleaved three-quarter length fragments of type II collagen of humans, horses and cows, but, as well, the three-quarter length fragments of type I collagen found in skin and bones.

The purpose of this study, done by Dr. Billingham with Dr. Elizabeth Buxton, Dr. Mike Edwards, Megan McGraw and Dr. Wayne McIlwraith, was to develop an antibody, using similar technology to that employed in the production of COL2-3/4C<sub>short</sub>, to identify the neopeptide that is the newly created end of the type II collagen fragment produced by collagenase digestion in horses. Our hypothesis was that by generating this new antibody according to an extended amino acid sequence specific for the type II collagen cleavage site in horses, reactivity only to collagenase-digested type II collagen would result. Using this antibody in an assay, type II collagen cleavage in equine articular cartilage could be identified and quantitated, and the effect of new pharmaceuticals on this degradation could be monitored. Finally, through the use of this antibody in staining tissues removed from diseased joints of horses, increased cleavage of type II collagen in articular cartilage could be identified and localized.

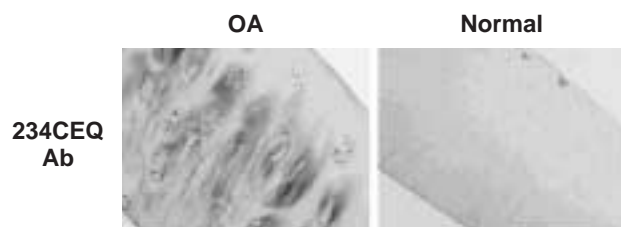
An antibody, called 234CEQ antibody, was produced that recognized the three-quarter length fragments of purified equine type II collagen created by the activity of collagenase (Figure 1), but did not react with uncleaved collagens nor similarly generated fragments of equine type I and III collagens. There was a significant release from articular cartilage in culture of type II collagen fragments bearing the 234CEQ neopeptide in response to the proinflammatory agent interleukin-1 and this could be prevented with an inhibitor of MMPs. Finally, articular cartilage from an arthritic joint of a horse showed increased staining with the 234CEQ antibody compared to cartilage from a normal equine joint (Figure 2).

## Summaries: Focus 3

### Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease



**Figure 1.** The location of collagenase-generated 3/4 fragment neopeptide of equine type II collagen. Shown in the diagram are the 3/4 and 1/4 fragments resulting from the cleavage of a triple helical collagen molecule by MMP collagenases.



**Figure 2.** Sections of articular cartilage removed from an osteoarthritic (OA) and a normal joint of two horses. Both articular cartilage sections were incubated with the 234CEQ collagenase cleavage site antibody. There is evidence of increased staining (dark region) for collagen degradation in the OA cartilage compared to the normal cartilage.

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#### Publications

Billinghurst R, Clark, Buxton E, Edwards M, McGraw M and McIlwraith CW. Use of an antineopeptide antibody for identification of type-II collagen degradation in equine articular cartilage. *Am J Vet Res* 2001; 62: 1031-1039.

Billinghurst RC, Brama P, McGraw M, Van Weeren R and McIlwraith CW. Alterations in serum levels of biomarkers of collagen metabolism in equine osteochondrosis. *Proc Orthop Res Soc*, 2002.

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