

# Anabolic and catabolic markers of proteoglycan metabolism in osteoarthritis

Bruce Caterson<sup>1</sup>, Clare E Hughes<sup>1</sup>, Peter Roughley<sup>2</sup> and John S Mort<sup>2</sup>

<sup>1</sup>Connective Tissue Biology Laboratories, School of Molecular and Medical Biosciences, University of Wales of Cardiff, Wales, U.K. Tel +44-1222 874593. Fax +44-1222 874594, and <sup>2</sup>Shriners Hospital for Crippled Children, Montreal, Canada. Tel +1-514 849-6208. Fax +1-514 842-7553.

Over the past 16 years our laboratories have focused on the development and use of immunological procedures to study cartilage structure, function and metabolism in health and disease (1). Most recently we have used monoclonal antibody technology to identify subtle changes that occur in the biochemistry of cartilage proteoglycans during the pathogenesis of osteoarthritis (2, 3). Changes in chondrocyte metabolism in OA produce compositional changes in the newly synthesized proteoglycans that occur in an attempt to remodel or repair the tissue in its response to its altered mechanical environment. These subtle changes in cartilage biochemistry can be detected by antibodies that recognize anabolic neopeptides, (*a new epitope that is generated in a biological molecule as a result of either changes in anabolic or catabolic processes in metabolism*), in these newly synthesized proteoglycans. Similarly, increases in proteoglycan degradation (catabolism) also occur in the early pathogenesis of OA; this increased catabolism eventually leading to mechanical destruction of the tissue. Recently we developed monoclonal antibodies that specifically detect newly formed N- or C-terminal sequences (catabolic neopeptides) on proteoglycan degradation products that result from this

increased catabolism (4–6). Identification of these catabolic neopeptides allows one to distinguish between the action(s) of putative proteolytic enzymes involved in normal proteoglycan turnover and specific degradation during the pathogenesis of osteoarthritis. In this review, we will describe work where we have developed and utilized monoclonal antibody technologies to identify both “anabolic” and “catabolic” markers of proteoglycan metabolism that are manifested during the pathogenesis of arthritis.

## Anabolic markers

Several years ago, in collaborative studies with Dr. Tim Hardingham and co-workers at the Kennedy Institute of Rheumatology, our laboratories were the first to describe subtle biochemical differences in the chondroitin sulfate (CS) glycosaminoglycan chains of newly synthesized proteoglycan isolated from osteoarthritic cartilage (7). These differences were detected by two monoclonal antibodies; 3-B-3 and 7-D-4. Antibody 3-B-3 recognizes as its epitope, a non-reducing terminal unsaturated uronic acid residue adjacent to N-acetylgalactosamine-6-sulfate after the CS chains have been digested with chondroitinase (8, 9; Figure 1). This epitope is denoted as 3-B-3(+)

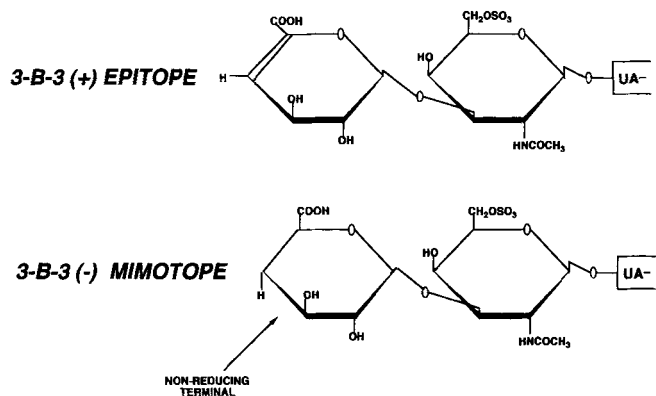


Figure 1. Illustration of the epitope (top) and the mimotope (bottom) that is recognized by monoclonal antibody 3-B-3. The epitope consists of a terminal delta 4, 5 unsaturated glucuronic acid residue adjacent to a 6-sulfated N-acetylgalactosamine residue. The ‘native’ mimotope for antibody 3-B-3 consists of a terminal saturated glucuronic acid adjacent to 6-sulfated N-acetylgalactosamine that occurs at the non-reducing termini of 6-sulfated chondroitin sulfate glycosaminoglycan chains.

since one requires that CS-proteoglycans be pre-digested with chondroitinase to generate its specific epitope. However, this antibody also recognizes a natural mimotope, (*a biochemical structure that mimics the epitope recognized by a given antibody* (10)), containing a saturated glucuronic acid residue at the non-reducing terminal (7), that occurs in CS chains of proteoglycans isolated from osteoarthritic cartilage. When antibody 3-B-3 is used without chondroitinase pretreatment immunoreactivity with the native mimotope structure is designated 3-B-3(-). Proteoglycans containing the 3-B-3(-) mimotope at the non-reducing terminal of CS glycosaminoglycans occur at low frequency in proteoglycans isolated from normal cartilage. However, its expression is much more prevalent in proteoglycans extracted from osteoarthritic tissue (7, 11-13). The epitope for antibody 7-D-4 is less well characterized (14-16). However, it appears to recognize subtle combinations of sulfated and non-sulfated disaccharide isomers within the native CS chain (J P Griffin and B Caterson, unpublished observation). Expression of the 7-D-4 epitope in CS glycosaminoglycans is also more prevalent in proteoglycans extracted from osteoarthritic cartilage (7, 12). Studies from another laboratory have also identified yet another monoclonal antibody, that recognizes a native CS epitope (denoted 846), that has increased expression in the CS glycosaminoglycans of proteoglycans extracted from human osteoarthritic cartilage (17). Collectively, these studies have established that subtle changes occur in the biochemistry (i.e. their sulfation and chain termination) of the CS chains of cartilage proteoglycans during the pathogenesis of OA.

Expression of these anabolic neoepitopes in cartilage proteoglycans appears to be a result of the tissues attempt to repair or remodel the damaged cartilage extracellular matrix. Secondly, expression of these epitopes in osteoarthritic cartilage may also reflect a switch in the articular cartilage phenotype to a hypertrophic phenotype. Credence for this hypothesis is provided by (i) expression of the 3-B-3(-) mimotope and the 7-D-4 epitope in the hypertrophic zone (but not other zones) of normal human growth plate (18) and (ii) the observation that type X collagen is synthesized by hypertrophic cell clusters in osteoarthritic cartilage (19).

Monitoring the expression of these anabolic markers of altered proteoglycan metabolism in arthritis has now been widely used in several animal models that mimic the disease in humans (7, 11, 13, 20-22). Similarly, they have also been used in analysis of proteoglycans extracted from human arthritic tissue (7, 12). Their usefulness as diagnostics in detecting pro-

teoglycan metabolites in human body fluids has not yet been definitively established. However, immunochemical detection of 3-B-3(-) mimotope in proteoglycans of cartilage extracts has been used to monitor changes in cartilage metabolism in animal models of OA (21, 22). Recently, expression of this epitope was used to investigate the potential chondroprotective effects of doxycycline treatment in the Hartley strain guinea pig model of natural OA (23).

### Catabolic markers

In past work (4, 5), we have developed and shown the feasibility of using monoclonal antibody technology to study proteoglycan catabolism in health and disease. In earlier work (4), we described methods for producing monoclonal antibodies against catabolic neoepitopes on link protein metabolites generated by the action of different proteinases on cartilage proteoglycan aggregates. More recently, we have used similar technologies to study the mechanisms involved in proteolytic degradation of aggrecan in normal turnover and arthritic diseases. The mechanisms underlying aggrecan catabolism in normal articular cartilage turnover and in diseases such as osteoarthritis and rheumatoid arthritis are thought to involve initial cleavage of aggrecan at two sites within the interglobular region of the molecule between the G1 and G2 globular domains (24, 25). These two catabolic cleavage sites occur between residues 341-342 and 373-374 in the native human aggrecan sequence (26, 27). The former site is hydrolyzed by several of the matrix metalloproteinases (MMP's 1, 2, 3, 7, 8, and 9), while the latter site results from an as yet unidentified proteolytic activity referred to as 'aggrecanase'. In recent publications (5, 6), we have described two new monoclonal antibodies (BC-3 and BC-4) which recognize the new N-terminal in the IGD generated by the unknown enzyme 'aggrecanase' and the new C-terminal in the IGD generated by the MMP's, respectively (Figure 2). In addition, we have recently produced and characterized a new monoclonal antibody (BC-14) that recognizes the N-terminal neoepitope FFG... produced by cleavage of the aggrecan IGD with MMP's 1, 2, 3, 7, 8, and 9 (Figure 2).

In recent studies (5, 28-31), we have used these antibodies to identify specific catabolites of 'aggrecanase' and MMP's 1, 2, and 3 as well as putative candidate enzymes that exhibit 'aggrecanase' activity. In bovine explant cultures treated with a single dose of IL-1 the appearance of 'aggrecanase'-generated metabolites (BC-3 positive) was first evident after 8 hours of exposure suggesting that 'aggrecanase' was a newly synthesized proteolytic

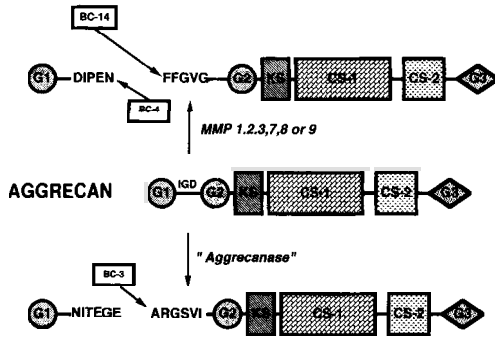


Figure 2. Schematic representation of aggrecan cleavage sites generated by either matrix metalloproteinases (MMP's) 1, 2, 3, 7, 8 and 9, or 'aggrecanase'. The specificities of new N-terminal and C-terminal catabolic neopeptide peptides recognized by monoclonal antibodies BC-3, BC-4 and BC-14 are indicated as ARGSVI..., ...DIPEN and FFGVG..., respectively. G1, G2 and G3 depict the globular domains of aggrecan; IGD, the interglobular domain; and KS, CS-1 and CS-2 the keratan sulfate and chondroitin sulfate glycosaminoglycan attachment domains of aggrecan.

activity or that it took time to activate the enzyme. In the untreated cultures the appearance of BC-3 positive catabolites occurred at very low levels, and only after 24 hours of culture (31). Analysis, with monoclonal antibody BC-14 (anti FFG...; Figure 2) showed no evidence of MMP-generated degradation products. Other studies (29), have shown that with porcine explant cultures exposed to IL-1 or retinoic acid for 72 hours, that 'aggrecanase'-generated (BC-3 positive) catabolites were the predominant metabolites. However, both control and IL-1 or retinoic acid treated cultures showed immunopositive staining for matrix metalloproteinase generated catabolites (as shown using antibody AF28, (29, 32)). Exposure of the porcine cartilage explants to IL-1 or retinoic acid, in fact, reduced the immunopositive signal for MMP-generated aggrecan catabolites. Collectively, these data suggest that there are at least two different mechanisms of aggrecan metabolism occurring in control versus IL-1 or retinoic acid stimulated cultures i.e. normal turnover using MMP's and cytokine-induced degradation elicited by 'aggrecanase'. These differences may also distinguish mechanisms of normal turnover versus pathological degradation in joint disease.

We have also used these antibodies in studies aimed at identifying potential enzymes with 'aggrecanase' activity. In collaborative studies (28), we used monoclonal antibody BC-3 to demonstrate that neutrophil collagenase (MMP-8) could catalyze 'aggrecanase' activity; this unexpected result sug-

gesting that other MMP's may also exhibit this activity. This result also left open the question as to whether or not chondrocytes could synthesize MMP-8. Recently, Cole and Kuettner (see their Chapter) have used immunohistochemical and in situ hybridization techniques to demonstrate the presence and expression of MMP-8 in IL-1 stimulated and human osteoarthritic cartilage.

In addition, we have recently used antibody BC-3 to detect 'aggrecanase'-generated proteoglycans in synovial fluids of human patients with both osteoarthritis and rheumatoid arthritis (unpublished work). Collectively, our development and use of neopeptide monoclonal antibody technology to study mechanisms of matrix degradation shows considerable promise for use in technologies either diagnosing or monitoring the progression of arthritic diseases.

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