Changes in chondroitin sulphate structure induced by joint disease

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Chondroitin sulphate is an important component in articular cartilage, where it occurs at high concentration (40–80 mg/ml) primarily on aggrecan, and also in smaller amounts on the leucine-rich proteoglycans, decorin and biglycan (see Hardingham and Fosang 1992). Chondroitin sulphate contains a repeating disaccharide structure of N-acetylgalactosamine and glucuronate and in aggrecan most of the galactosamine residues carry a single sulphate group in the 4 or 6 position, a small proportion (1–5%) are non-sulphated and an even lower proportion (<1%) may be disulphated, with 4-,6-disulphated galactosamine or some 2-sulphated glucuronate residues. As chondroitin sulphate chains are typically Mr 10-30K they contain 20–60 disaccharides and the incidence of disulphated disaccharides is thus usually less than 1 per chain.

Although differences in 4- and 6-sulphation amongst different cartilage sources and changes in sulphation during development and ageing are well documented, there has been little indication of the functional significance of such variations in structure. A big advance in the investigation of chondroitin sulphate distribution was the development of monoclonal antibodies specific for unsaturated 4-, or 6- or non-sulphated disaccharides by Bruce Caterson, Jim Christner and John Baker (University of Alabama at Birmingham) (Caterson et al. 1985). This enabled the detection of chondroitin sulphate in tissues after chondroitinase digestion, which not only created the epitope structures, but also removed most of the chondroitin sulphate chain and made the matrix better penetrated by the antibodies (Couchman et al. 1984). To these monoclonal antibodies have now been added a new generation of antibodies with specificities for parts of chondroitin sulphate structure that are native and are not produced by chondroitinase digestion. This type of antibody was first produced by Bruce Caterson using chick bone marrow chondroitin sulphate proteoglycans as antigen and it is now clear that these proteoglycans contain some epitopes that are expressed at a low level in other tissues such as cartilage (Caterson et al. 1990).

Immunolocalization with these monoclonal antibodies of tissues during embryogenesis showed for the first time quite dramatically that there were distinctive differences in chondroitin sulphate expressed by different cells and the expression showed temporal changes during development associated with cell differentiation and tissue morphogenesis (Sorrell et al. 1988a,b, Caterson et al. 1990). Chondroitin sulphate was thus shown to be synthesised with greater structural control than had been previously suspected and chains contained some selected structural features that formed epitopes for antibodies.

These observations need also to be seen in the context of other research on proteoglycans in which sequences in glycosaminoglycan chains have been identified that have specific biological functions (see Hardingham and Fosang 1992). These include specific sequences in heparin that bind to antithrombin (Thunberg et al. 1982) and more recently sequences in heparan sulphate that bind to basic FGF (see Gallagher 1994). So there is an increasing number of examples which show that glycosaminoglycan chain synthesis, whilst lacking the fidelity of linear templates such as those that relate DNA to RNA to protein, nevertheless contain specific sequences of structure whose expression is under cellular control and is perhaps varied to suit different cellular needs and different biological functions.

The interest in joint disease of changes in the expression of chondroitin sulphate structure can be traced back to observations reporting that the composition of 4- and 6-sulphated disaccharides were changed in degenerative cartilage (e.g. Michelacci et al. 1979). However, it was unclear if this change represented a genuine altered expression in diseased tis-

sue or merely the comparison of the composition of chondroitin sulphate from different depths of cartilage. As cartilage from a degenerative joint may frequently have lost much of its superficial and medium depth zones its composition of chondroitin sulphate may be similar to the deep zone of normal healthy cartilage. In other studies an increase in the ratio of 6-sulphation to 4-sulphation was shown in the joint fluid of patients with OA and traumatic arthritis compared to those with RA (Shinmei et al. 1992). However, more recent results have shown that the ratios from control fluids were also high and similar to those in OA and traumatic arthritis, and that it was the ratios in RA that were unusually low (Shinmei et al. 1995).

It is the advent of monoclonal antibodies to native chondroitin sulphate structures and their application to experimental OA that has provided new evidence that there are changes in the biosynthesis of chondroitin sulphate that accompanies the other metabolic changes that occur in articular cartilage in experimental joint disease (Caterson et al. 1990, Carney et al. 1993, Visco et al. 1993). This is an exciting observation as it suggests that chondroitin sulphate synthesis on aggrecan is modulated as part of a cellular response. It is clear that the changes detected by the antibodies reflect the level of expression of specific structures that are present only rarely in chains, and as such they have little impact on the overall disaccharide composition. The initial results showed that the expression of chondroitin sulphate epitopes recognised by mAbs 3-B-3 and 7-D-4 were increased on proteoglycans from experimental OA cartilage compared to controls from the contralateral joint. The 3-B-3 epitope is interesting as it is a chain terminal structure containing a 6-sulphated galactosamine adjacent to a terminal glucuronate (Caterson et al 1990). This appears to be a very infrequent terminal structure of chondroitin sulphate chains on aggrecan and as the corresponding 4-sulphated terminal structure (epitope to mAb 3-D-5) is similarly rare (Hardingham et al 1994), it appears that most normal chains terminate in N-acetylgalactosamine rather than glucuronate. The increase in 3-B-3 epitope expression on cartilage proteoglycans in experimental OA appears to reflect only a small number of chains as digestion with chondroitinase ABC can greatly increase the level of 3-B-3 expression, suggesting that it creates epitopes on many more chains. In contrast to 3-B-3 the epitope recognised by mAb 7-D-4 is present within the middle of chondroitin sulphate chains (Hardingham et al 1994), and its expression increased 2-4 fold in experimental OA. This showed that the changes in chain structure were not only associated with chain termination.

These initial observations on experimental canine osteoarthritis have now been extended to other experimental models of joint disease and to natural osteoarthritis. The Duncain Hartley guinea pig that develops spontaneous OA of the knee with age also shows an increase in the expression of 3-B-3 epitope in the cartilage of the affected joints, but not in the unaffected hip joints of the same limbs. We have also found an increase in 3-B-3 epitope expression in rabbit articular cartilage following partial meniscectomy (Hazell, Lewthwaite and Hardingham, unpublished results). Experimental injection of IL-1 into rat knee joints was also followed 2 weeks later by the appearance of 3-B-3 epitope in the articular cartilage. The investigation of articular cartilage proteoglycans from human joints of different age showed that there was increased expression of 3-B-3 epitope at 10–20 yr which is during and immediately following the time of rapid growth of long bones, but the expression of 3-B-3 was low in very young (<5 yr) and in old healthy joints (>50 yr)(Bayliss and Davidson, personal communication). The increased staining evident in human OA cartilage was thus against a background of low expression in old non-OA human cartilage.

As the studies in experimental OA showed that proteoglycan fragments were released into synovial fluid that were enriched in 3-B-3 and 7-D-4 epitopes we have extended the studies to investigate their content in synovial fluid from human knee joints following major damage to the cruciate ligaments or menisci (Hazell et al. 1995). Fluid was taken from both knees of each patient so that a comparison of results was possible between a trauma-joint and a contralateral non-trauma joint. This study also differed from many others which measured the content of proteoglycan fragments in joint fluids as it looked for differences in the structure of fragments released rather than differences in their amount. The content of epitope was in each case related to how much proteoglycan was present in the fluid (measured by the total sulphated glycosaminoglycan content). The quality of proteoglycan being released was thus being compared rather than the quantity.

Several chondroitin sulphate monoclonals were used in ELISA to compare the expression of epitopes in the fluids. Epitopes of some, such as 3-B-3 and 7-D-4, showed consistent increases between the control and the trauma joint (Figure 1), but only for 7-D-4 did the trauma joint fluid group have a significantly higher content than the control group. These results contrasted with the assays for keratan sulphate epitope using mAb 5-D-4 which showed that there was a

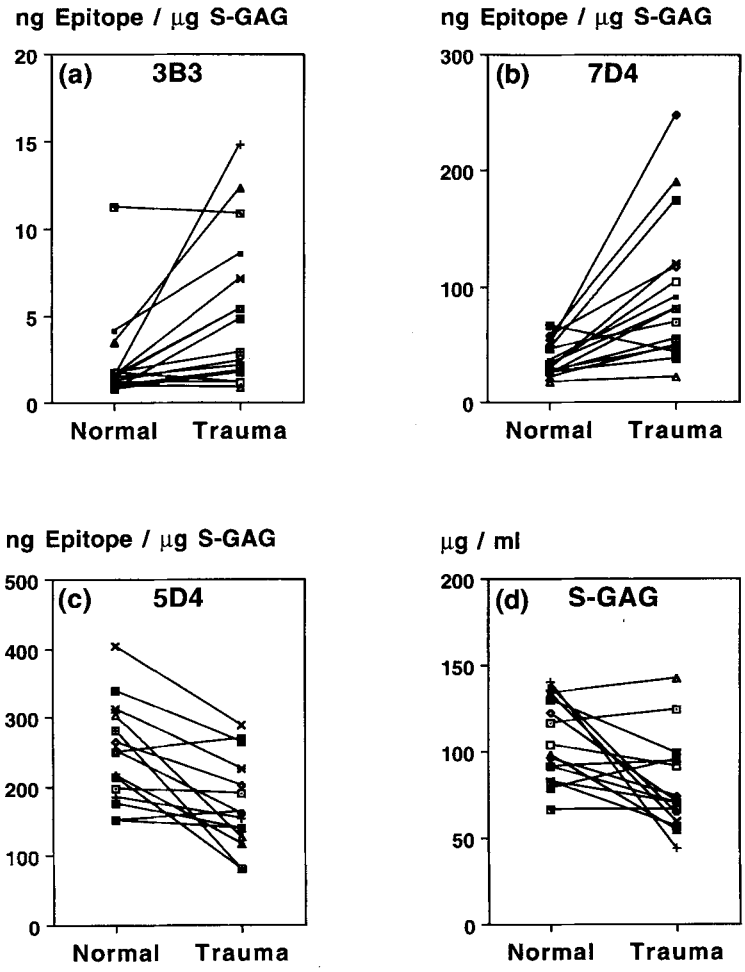

Figure 1. Results from paired normal and injured (trauma) knees. Each patient is represented by 2 symbols joined by a line. For each ELISA the results are shown as equivalent weight of antigen standard per weight of sulphated glycosaminoglycan (S-GAG: in ng/ml) for (a) 3B3, (b) 7D4, and (c) 5D4. The total S-GAG concentration (in mg/ml) is shown in (d).

small fall in KS-epitope in trauma joint fluids (Figure 1).

Analysis of the results with time after injury showed that many of the largest increased in CS-epitopes were in samples taken early after injury (<3 months), although one patient had a large increase at 5 years after injury. The difference in epitope levels between normal and injured knee joint fluids for the 5-D-4 (KS) epitope was relatively small in most cases and did not vary with time after injury. These limited results on patients following joint trauma are broadly comparable with the results from experimental OA and suggest that similar processes are occurring in human articular cartilage in response to joint damage. From the biochemical and immunohistochemical studies on experimental OA, the results suggest that the expression of 3-B-3 and 7-D-4 epitopes reflect part of the hypermetabolic response by chondrocytes, which may increase matrix synthesis and help to repair matrix damage. It is interesting that these increases in chondroitin sulphate epitopes are not accompanied by similar increases in keratan sulphate epitope. Biochemical studies also suggest that newly synthesised proteoglycans in experimental OA are of lower keratan sulphate content.

On the basis of the evidence so far, the increased expression of CS-epitopes depends on a sustained response of the chondrocytes in articular cartilage. It may be hypothesised that if the joint recovers well following injury and successfully adapts to resume good function, then the chondrocytes response would cease and the expression of epitopes would return to

normal. However in those joints with a continuing problem, and the beginnings of mechanical damage, the expression would continue, although if there were damage to articular cartilage, resulting in chondrocyte death and eventual significant loss of tissue, as is typically found in late stage clinical OA, the expression of CS-epitopes may not then be detected. The response in articular cartilage that leads to an increase in CS-epitope expression may thus be most commonly found in early (preclinical) OA, but more studies are necessary to establish if the differences in expression are consistent and if they are sufficiently large to be of clinical use in diagnosis and monitoring of osteoarthritis.

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