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Proteoglycan components of the intervertebral disc and cartilage endplate: an immunolocalization study of animal and human tissues

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Summary

Monoclonal antibodies have been used to study the presence and distribution of various components of the proteoglycan molecule in the intervertebral disc and cartilage endplate. Link protein, hyaluronic acid binding region, keratan sulphate and chondroitin 4- and 6-sulphate have been investigated in tissues from humans and other mammals. Exposure of the carbohydrate and protein epitopes was enhanced by chondroitinase and trypsin pretreatment respectively. The degree of immunoreactivity varied with location, being greater in the nucleus pulposus than the annulus fibrosus with least reactivity in the cartilage endplate. In addition, there was increased staining in the pericellular domains, particularly in adult tissues. Areas of ectopic calcification exhibited very different immunoreactivity, depending on the type of calcium salt present. Calcium hydroxyapatite deposits showed greater staining for 8A4 (link protein), while calcium pyrophosphate deposits demonstrated greater staining for 3B3(−), 7D4(−) and 3D5 than the surrounding non-calcified matrix. Staining for chondroitin sulphate isomer epitopes 3B3(−) and 7D4(−), indicative of modified chondroitin sulphate chains, was greater in human tissues of degenerate than non-degenerate appearance. This suggests that expression of these epitopes may be an indicator of disease and subsequent reparative procedures in intervertebral disc and cartilage endplate, similar to that seen in articular cartilage degeneration.

Introduction

Proteoglycans are one of the main structural components of the intervertebral disc and other cartilaginous tissues. They directly affect the mechanical and physiological functioning of the tissues via their osmotic properties, due largely to their glycosaminoglycan chains within the molecule (Urban & Maroudas, 1980). Biochemical analyses have demonstrated the presence of the glycosaminoglycans, keratan sulphate (KS) and chondroitin sulphate (CS), in intervertebral disc, but there is little information on their topographical distribution within the tissue. Differential distribution of these glycosaminoglycans may be important to the functioning of the tissue matrix due to physicochemical differences between glycosaminoglycan types. For example, KS has fewer negative charges, rendering a lower fixed charge density, and hence less ability to create osmotic pressure, than an equivalent CS chain. Furthermore, in articular cartilage subtle structural differences occur in the CS glycosaminoglycan chains of proteoglycans isolated from osteoarthritic cartilage that are not present in those extracted from normal cartilage (Caterson *et al.*, 1992). These atypical structures in the CS glycosaminoglycans are recognized by two antibodies, 3B3(−) and 7D4(−), that detect unique terminal and intrachain CS epitopes respectively.

There have been no studies on the detailed distribution of proteoglycan subpopulations in intervertebral disc. In this study we have used a panel of monoclonal antibodies directed against proteoglycan-specific protein or carbohydrate epitopes to determine the occurrence and distribution of a number of proteoglycan components in both the intervertebral disc and the adjacent hyaline cartilage endplate. Tissues from different mammalian species were investigated. Human autopsy and surgical material was studied to establish the effect of age and degeneration, with particular reference to the unusual epitopes.

Materials and methods

Tissues

Intervertebral discs and adjacent cartilage endplates were obtained within 1 h of death from rats, cattle and sheep. Human

Table 1. Details of age, species and pathology of specimens examined

Species	*Age*	*Pathology*
Rat	7 months	
Sheep	7 months	
Cow	Newborn, 18 months	
Human		
autopsy	21, 28, 53, 60, 66, 75, 83 years	
surgical	7 years	Infantile scoliosis
	18 years	Idiopathic scoliosis
	39 years	Posterior annular tear
	40 years	Failed posterior fusion
	44 years	Backache

Table 2. Characteristics of monoclonal antibodies used

Antibody	*Epitope*	*Ig Class*	*Reference*
5D4	KS	G_1	Caterson *et al.* (1983)
2B6	C–4–S, Dermatan SO_4	G_1	Caterson *et al.* (1990a)
3D5	C–4–S, Dermatan SO_4	M	Caterson *et al.* (1990a)
3B3	C–6–S	M	Caterson *et al.* (1985b)
7D4	Native CS	M	Sorrell *et al.* (1990)
8A4	Link protein	G_{2b}	Caterson *et al.* (1985a)
1C6	Binding region	G_1	Caterson *et al.* (1986)

KS = keratan sulphate; DS = dermatan sulphate; CS = chondroitin sulphate; C-4-S and C-6-S = 4- and 6-sulphated CS respectively.

tissue was obtained at autopsy from seven individuals in the age range 21 to 83 years (Table 1), within 36 hours of death. A single lumbar level was studied from all except one of these individuals, where tissue was obtained from the five lumbar levels, to investigate whether any trend could be seen down the lumbar spine. Autopsy specimens were graded according to their macroscopic appearance using a modification of the method described by Galante (1967) whereby a score of 1 denoted minimal and 5 maximal degeneration. Grade 3 and below was considered normal for the age of the individual. Clinically pathological tissue was obtained from patients undergoing anterior fusion for various reasons, such as scoliosis or posterior annular tear (Table 1). Tissue was frozen in hexane cooled in cardice and stored in liquid nitrogen until required for analysis.

Monoclonal antibodies

Histochemistry was performed using monoclonal antibodies directed against keratan sulphate (5D4), chondroitin 4- (2B6 and 3D5) and 6-sulphate (3B3), native CS epitope (7D4), link protein (8A4) and hyaluronic acid binding region (1C6), each diluted at 1:5000, 1:2000, 1:500, 1:1000, 1:1000, 1:5000 and 1:5000 respectively. The epitope characteristics recognized by each of these monoclonal antibodies is summarized in Table 2 and illustrated in Fig. 1.

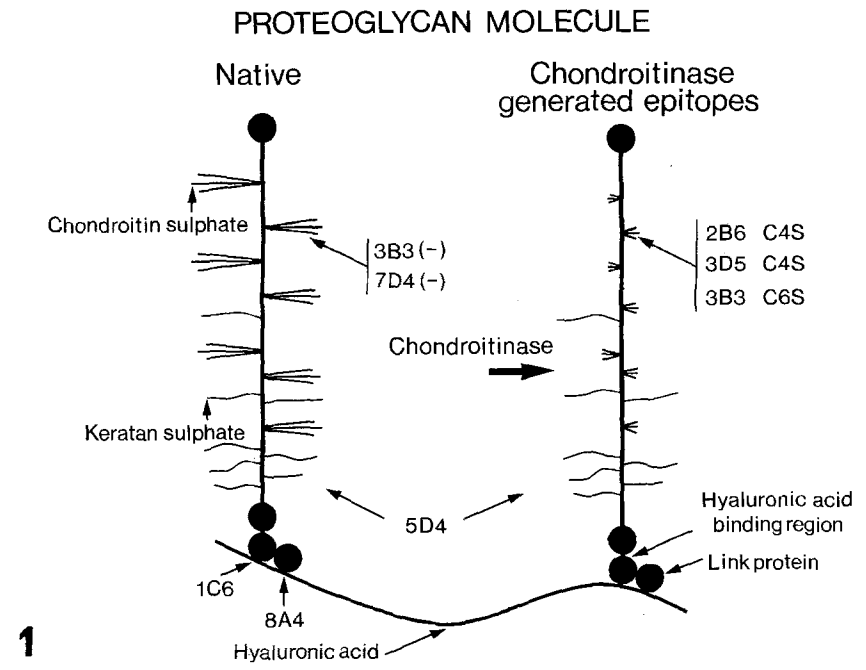
Immunolocalization

Frozen sections (7 μm thick) were fixed with 10% formaldehyde in TRIS-buffered saline (TBS) and either left untreated or digested with chondroitinase ABC or AC II (Seikagaku Kogyo from ICN Biochemicals, UK, and Sigma respectively; 0.25 U cm^{-3} in 0.1 M TRIS–acetate buffer at pH 8.0 for ABC and pH 7.4 for AC II; incubated for 90 min at room temperature (Yamagata *et al.*, 1968)), before incubating with the primary antibody (30 min at room temperature). This procedure was used to differentiate between CS and dermatan sulphate (DS) glycosaminoglycans in tissues (Couchman *et al.*, 1983). Adjacent sections were incubated with TRIS–buffered saline or an inappropriate antibody and used as negative controls. After washing, sections were incubated with biotinylated horse anti-mouse immunoglobulin (diluted 1:200 in TBS with 1.5% normal horse serum). Endogenous peroxidase activity was blocked by incubating with 0.3% hydrogen peroxide in methanol for 30 min at room temperature. Immunoreactivity

Fig. 1. Schematic representation of native and chondroitinase-generated epitopes on aggrecan. The epitopes 3B3(−) and 7D4(−) are detected in native tissue, i.e. that which has not been digested with chondroitinase.

Fig. 2. Pericellular matrix staining in human nucleus pulposus. Sections were stained with monoclonal antibodies 2B6 (a), 1C6 (b) and 3D5 (c) after pretreatment with chondroitinase ABC. Pericellular staining varied with both location and antibody used; this sometimes occurring in the territorial matrix (TM) as in (a), in the pericellular capsule (PC) as in (b). In some cases immunoreactivity resembling rings occurred, see (c). L = cell; ITM = interterritorial matrix.

Fig. 3. Exposure of 8A4 epitope (anti-link protein) after different enzyme pretreatment of human nucleus pulposus sections; (a) chondroitinase and (b) trypsin pretreatment.



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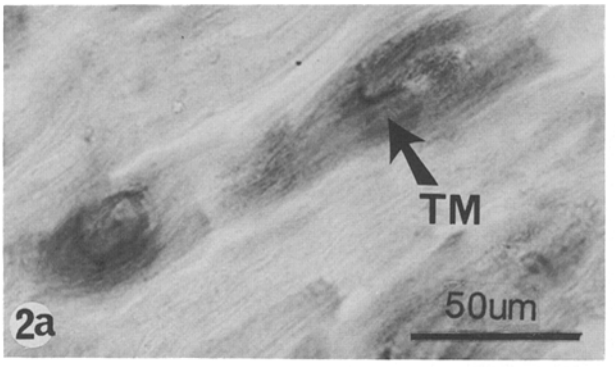
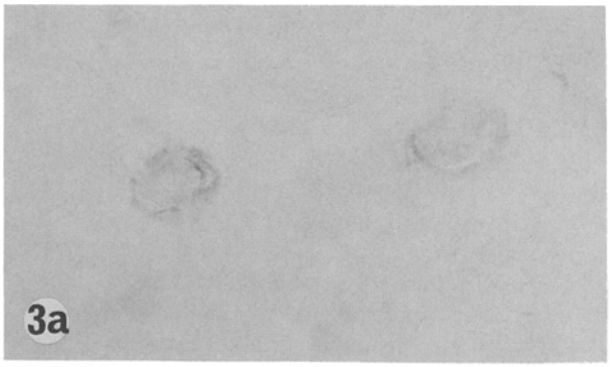


2a



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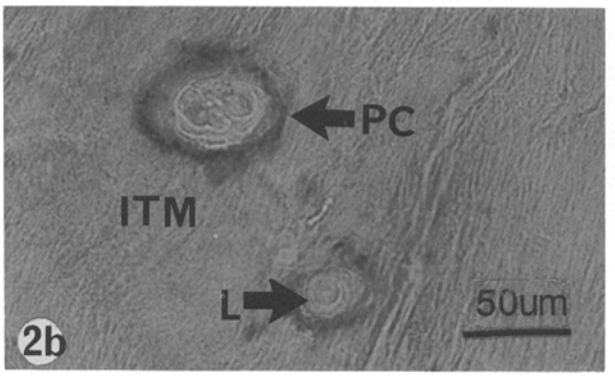

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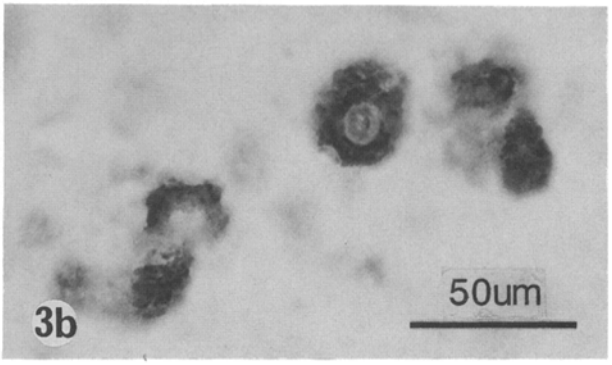

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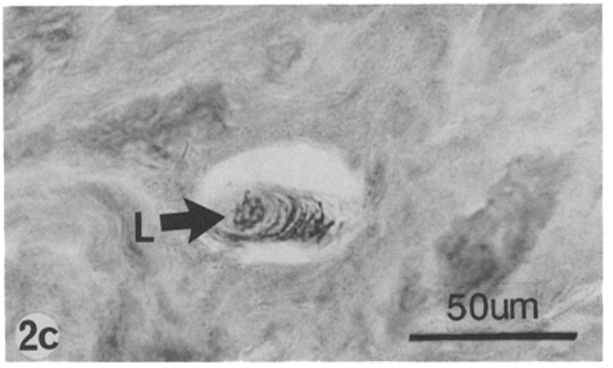


2c

Figs 1–3

was amplified using the biotin–streptavidin complex (Vectastain ABC Elite, Vector Laboratories, UK) and visualized using the peroxidase reaction with 3,3′,4,4′-tetra-aminobiphenyl-hydrochloride.

In addition to being processed as described above, the human specimens were subjected to incubation with 3B3 and 7D4 antibodies without predigestion with chondroitinase, in order to determine whether the native epitopes, 3B3(−) and 7D4(−), were present in the tissue. With the monoclonal antibodies 8A4 and 1C6 additional enzyme treatments were investigated to evaluate potential epitope masking; pretreatments included testicular hyaluronidase (2 mg cm^{-3} in 0.025 M NaCl with 0.05 M sodium acetate buffer, 90 min at 37°C) and trypsin (0.05 mg cm^{-3} in phosphate-buffered saline for 30 min at room temperature).

Results

Intervertebral disc (annulus fibrosus and nucleus pulposus)

In all specimens studied positive immunostaining was seen for each of the monoclonal antibodies investigated following chondroitinase treatment (Fig. 2). For the majority of antibodies strongest immunostaining occurred in the pericellular matrix of adult tissue, particularly in the nucleus pulposus. This staining occurred to varying degrees, the pericellular capsule itself usually being positive (Fig. 2b). In some cases of human tissue concentric rings were seen with several antibodies in the region of the capsule. In addition, positive immunostaining was found in regions outside the pericellular capsules, the territorial matrix, with different monoclonal antibodies. Although this pericellular staining pattern was common, not all the capsules were stained in any one section. Antibody reactivity was also seen throughout the remaining interterritorial matrix (Fig. 2).

Comparative analyses indicated that there was clearly more staining in the nucleus pulposus than in the annulus fibrosus, but little difference was seen with level within the lumbar region. In addition, no consistent trend was seen with age. However, in the younger human surgical specimens, particularly from the 7- and 18-year-olds, there was little pericellular staining in the annulus and nucleus. This was in contrast to disc from the 39- to 44-year-olds, where the usual pericellular staining was present. Similarly, in young bovine tissue the pericellular pattern was absent, whereas it was obvious in the older specimens with most of the monoclonal antibodies tested.

In order to differentiate between the occurrence of CS versus DS in tissue sections, epitopes recognized by 2B6 and 3D5 were compared after differential digestion with chondroitinase ABC or ACII. In all cases there was little difference in staining intensity between the two enzyme treatments, indicating that 4-sulphated CS was more prevalent than DS in these tissues.

Masking of epitopes was investigated after different enzyme pretreatments (Fig. 3). Sections digested with either hyaluronidase or chondroitinase ABC gave a similar staining pattern with monoclonal antibody 8A4 (Fig. 3a). However, with trypsin predigestion (Fig. 3b) there was increased pericellular staining, indicating that the protease pretreatment was unmasking 8A4 epitopes blocked in the link protein associated with proteoglycan aggregates. Similarly, greatly increased staining occurred with monoclonal antibody 1C6 in these conditions, indicating that 1C6 epitope was also masked in the proteoglycan tertiary complex.

Additional studies demonstrated considerable difference between the species investigated. The pattern in sheep tissues most resembled that seen in humans. However, the degree of pericellular staining varied with the different antibodies in all the animals. For example, in adult bovine disc, pericellular staining was strong with 2B6 and 8A4, weaker with 3B3, 7D4, 5D4 and 1C6, whilst absent with 3D5 (Fig. 4). Pericellular staining was weaker in rat tissues.

Cartilage endplate

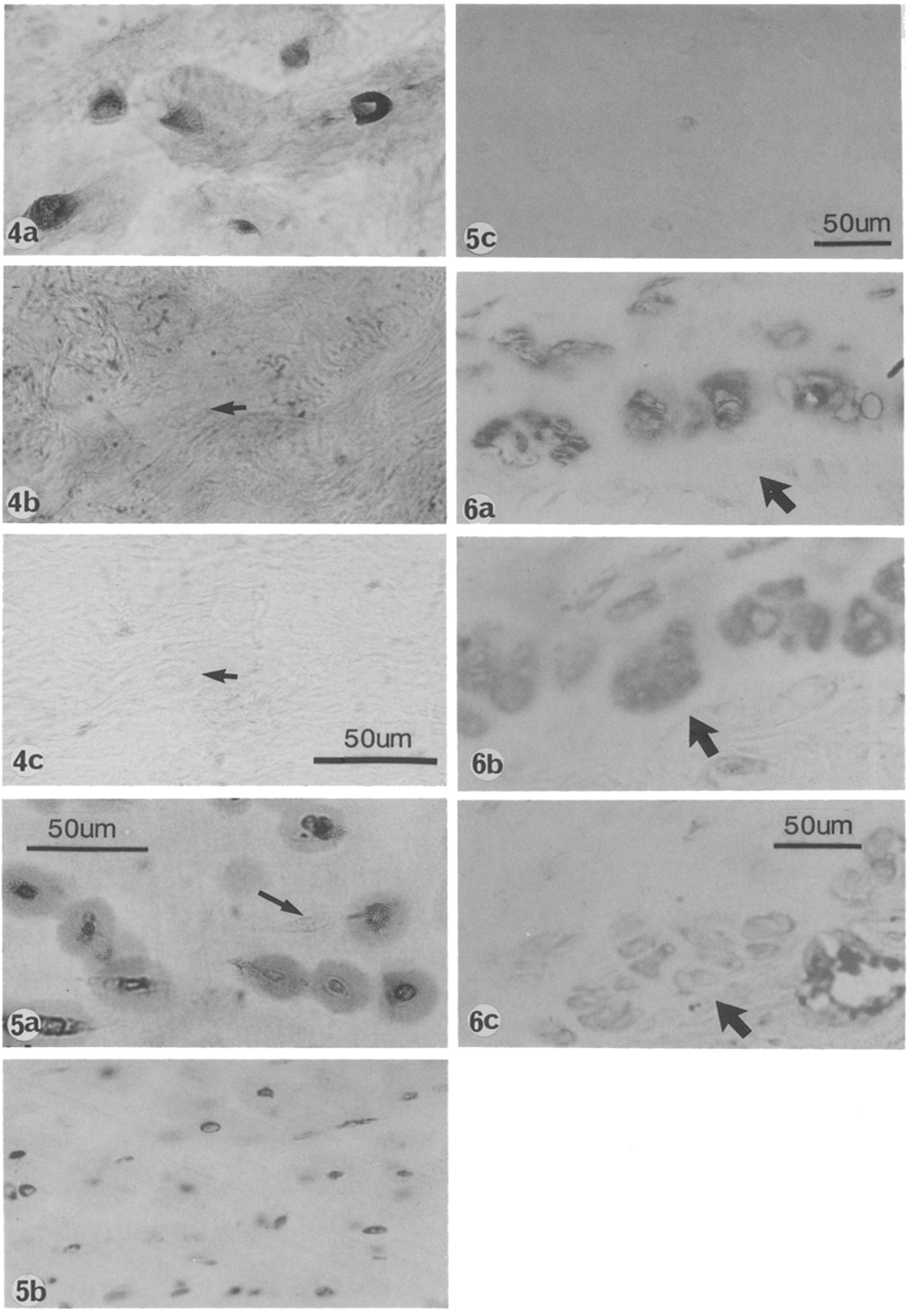
The matrix of the cartilage endplate was generally weakly stained with all the monoclonal antibodies except 2B6. With 2B6 there was often strong staining of the territorial matrix (Fig. 5a). The pericellular capsules, however, were again strongly stained with all the antibodies except that to hyaluronic acid binding region, i.e. 1C6, which was mostly negative (Fig. 5b, c). The junction of the endplate and disc often exhibited stronger staining than elsewhere, as did the amorphous substance occurring in splits of the cartilage endplate.

In two young human surgical specimens, endochondral ossification was observed and some epiphysial growth plate remained within the cartilage endplate. In these samples there was more staining for 2B6, 3D5 and 3B3 in the non-calcified layers, whereas for 8A4 it was stronger in the calcified region. 3B3(−) was also present but only towards the calcified zone. Staining for 1C6 and 8A4 was stronger in the interterritorial matrix. In addition, immunostaining with 2B6 and type IX collagen was similar and closer to the cells in the territorial matrix

Fig. 4. Differences in pericellular staining occur in bovine intervertebral disc tissues. Bovine nucleus pulposus immunostained with monoclonal antibody 8A4 (a), 3D5 (b) and a control without primary antibody (c). Arrows indicate cells.

Fig. 5. Human cartilage endplate immunostained with 2B6 (a), 5D4 (b) and 1C6 (c). The arrow in (a) demonstrates negative immunoreactivity in occasional cells.

Fig. 6. Young human cartilage endplate immunostained with 2B6 (a), anti type IX collagen (b) and 3B3 (c). Arrows indicate the points of cartilage–bone interface in endochondral ossification.



Figs 4–6.

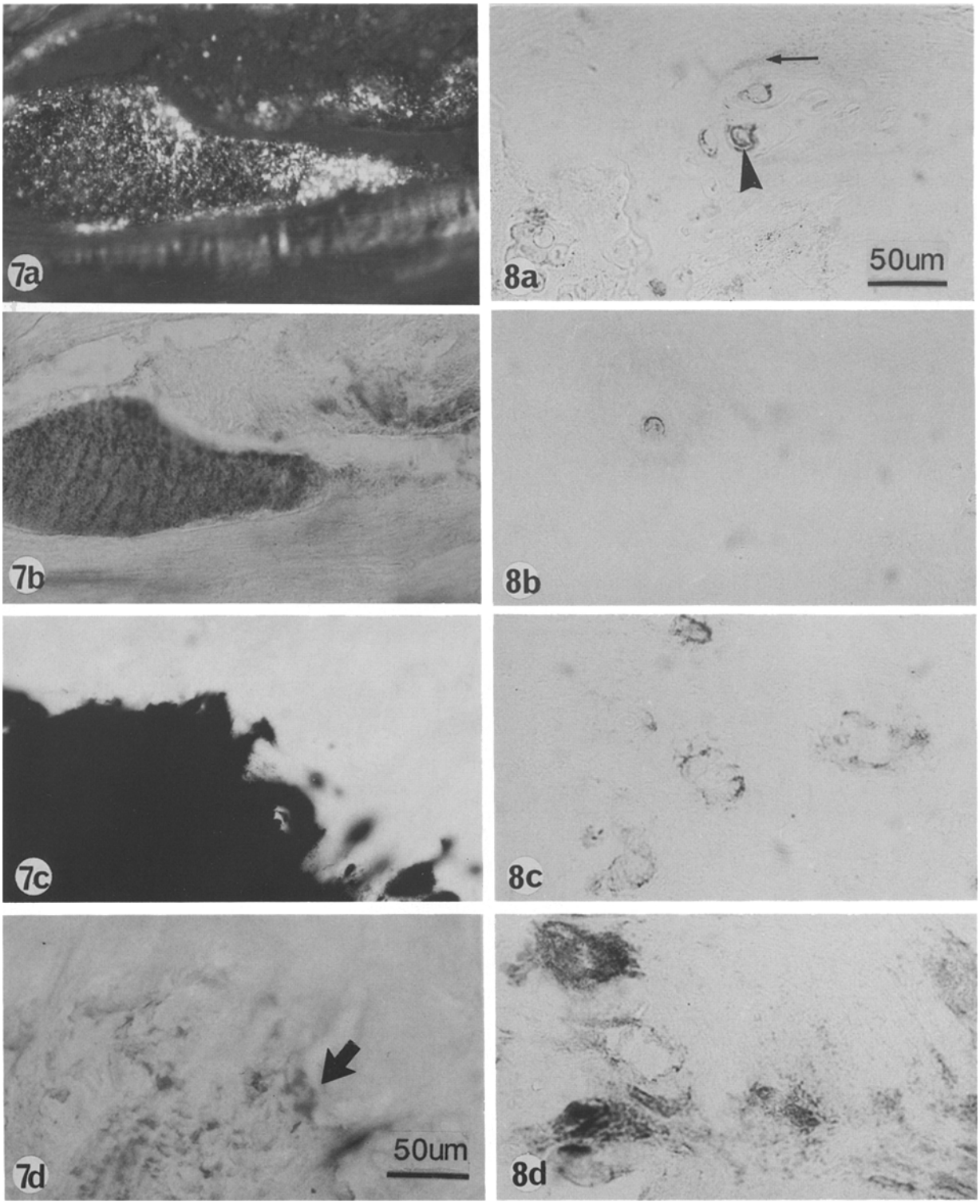


Fig. 7. Calcium crystal deposition and proteoglycan epitope staining. Birefringent analysis showing calcium pyrophosphate crystal deposition in human nucleus pulposus (a); 3D5 immunostaining (b). Von Kossa staining for calcification of human cartilage endplate (c); 8A4 immunostaining (d) with arrow indicating calcification interface.

Fig. 8. Immunostaining of hyperactive chondrocytes with 3B3(−) in scoliotic cartilage endplate (a). Arrowhead and arrow indicate cellular and matrix staining, respectively. Immunostaining of grade 1 (b), grade 4 (c) and grade 5 (d) intervertebral disc cadaveric specimens with antibody 7D4(−).

(Fig. 6a, b). In contrast, 3B3 staining occurred in closer proximity to the cells (Fig. 6c). This data suggests that some of the 2B6 immunoreactivity may be associated with the CS or DS chains of type IX collagen.

Calcification in intervertebral disc and cartilage endplate

Calcium pyrophosphate crystals identified by birefringence (Scott, 1986), were present in the intervertebral disc of four autopsy specimens (Fig. 7a) and immunostaining with 3B3(−), 7D4(−) and 3D5 was markedly increased in this calcified matrix. An example of the staining for 3D5 can be seen in Fig. 7 (a and b). Recent studies (Roberts *et al.*, 1993) indicate that ectopic calcification with the deposition of poorly organized calcium hydroxy apatite crystals occurs in the matrix of cartilage endplate from scoliotic patients. This calcified region demonstrated greater staining for 8A4 than the adjacent non-calcified matrix (Fig. 7c, d). These results suggest that deposition of different calcium salts may be associated with different proteoglycan components.

Correlation of expression of 'degenerate' epitopes with macroscopic grading

Staining with monoclonal antibodies 3B3 and 7D4 without chondroitinase pretreatment (3B3(−) and 7D4(−) respectively) has been used to demonstrate early repair processes that occur in the pathogenesis of arthritis (Caterson *et al.*, 1990b; Slater *et al.*, 1992; Visco *et al.*, 1993). These epitopes were also present in several of the operative and autopsy specimens of both intervertebral disc and cartilage endplate, but often being restricted to a small area or a small number of cells in the tissue section. Minor abnormalities, such as disc or bone intrusions, are often seen in the cartilage endplate (Roberts *et al.*, 1989). In these regions enlarged, hyperactive chondrocytes occurred which commonly expressed the 3B3(−) and/or the 7D4(−) epitope (Fig. 8a). This finding suggests that reparative changes similar to those seen in articular cartilage degeneration may also be occurring in disc pathology.

Specimens obtained at autopsy were sampled in a more standardized and controlled manner than those obtained at surgery and could also be graded objectively for macroscopic degeneration. The occurrence and extent of immunostaining for 3B3(−) and 7D4(−) epitopes was assessed in this group (Table 3). These data show that there is little demonstration of 3B3(−) in specimens with a macroscopic grading of 3 or less, compared to those graded 4 or 5. Although there was generally more staining with 7D4(−), the trend was similar, with greater immunoreactivity seen in specimens of a higher degenerative grade (Fig. 8, b–d).

Table 3. Grade of macroscopic degeneration and occurrence of 3B3(−) and 7D4(−) epitopes in autopsy specimens

Age (years)	*Level*	*Grade*	*3B3(−)*	*7D4(−)*
21	L4–5	2	−	+
28	L3–4	2	−	−
53	L2–3	1	(+)	+
60	L4–5	3	−	+ +
66	L5–S1	4	+ +	+
75	L1–2	5	+ +	+ +
	L2–3	4	+	+ +
	L3–4	5	+	+ +
	L4–5	4	+ +	+ +
	L5–S1	4	+	+
83	L5–S1	2	(+)	−

Grading and evaluation of the occurrence of 3B3(−) and 7D4(−) epitopes were performed blind. Degenerative grade according to Galante (1967; see Methods); Immunostaining: + + = strong; + = moderate; (+) = weak; − = no staining.

Discussion

To date there have been no studies describing differences in the topographical distribution of proteoglycans (and their component glycosaminoglycans) in intervertebral disc tissues. The objective of this study was to use immunohistochemistry to describe the differential distribution of proteoglycans within disc tissues with a view to increasing our understanding of the different physiological functions of these tissues. Our investigation has shown that proteoglycan epitope differences occur within pericellular and matrix environment, within species and with the onset of disc pathology.

Chondroitinase treatment is required to both generate epitopes for some of the antibodies and in general to facilitate access to epitopes by removing steric interference from CS glycosaminoglycans. Our studies have also shown that trypsin treatment of formaldehyde-fixed sections facilitates in the exposure of protein epitopes in the link protein and the hyaluronic acid binding region of proteoglycan aggregates. The large increase in binding seen for 8A4 and 1C6 when the tissue was treated with trypsin rather than chondroitinase or hyaluronidase is probably due to the location of the epitopes within the globular regions of the proteoglycan core protein. 1C6 epitopes, for example, are located within the proteoglycan tandem repeat sequence, found in both the G1 and G2 domains of aggrecan (Fosang & Hardingham, 1991). These sites would be more accessible following trypsin digestion of fixed (cross-linked) tissue section.

Pericellular staining was a common finding in this study. A fibrous capsule is known to exist around chondrocytes in articular cartilage (Poole *et al.*, 1988; Modis, 1990). It appears that a similar arrangement occurs around the cells of the intervertebral disc, particularly in the nucleus pulposus (Roberts *et al.*, 1991). The increased pericellular staining we find for many of the monoclonal antibodies used in the present study suggests that certain proteoglycan components are concentrated here. Ratcliffe *et al.* (1984) found a greater amount of labelling for

proteoglycans in this location than in the intercellular region of articular cartilage using a quantitative immunoelectron microscopy method. Although immunolocalization has limitations in that staining can be influenced by other macromolecules masking epitopes, the matrix surrounding the cell of the intervertebral disc and cartilage endplate is obviously very structured. No significant differences occurred in the staining pattern or intensity with antibodies 3D5 or 2B6 when sections were predigested with chondroitinase ABC or chondroitinase AC II. This suggests that there is little or no dermatan sulphate in human intervertebral disc. Indeed, Inerot & Axelsson (1991) detected no dermatan sulphate biochemically in human annulus fibrosus. In contrast, Johnstone *et al.* (1993) have recently reported dermatan sulphate as being the predominant glycosaminoglycan in the small proteoglycans (decorin and biglycan) of the annulus fibrosus. Our results indicate that DS glycosaminoglycans must either be a small proportion of the total glycosaminoglycans present, or that they were inaccessible to chondroitinase or the monoclonal antibodies.

There were considerable differences in staining pattern seen between disc and cartilage endplate. These findings may, in part, result from differences in the packing of the collagen fibres in the two tissues. In the disc, particularly in the nucleus, the collagen fibres are more randomly orientated than in the cartilage endplate, where the collagen fibres are more ordered, lying parallel to the surface of the vertebral endplate (Aspden *et al.*, 1981). In addition, the density of collagen per dry weight is greater in the cartilage endplate than in the adjacent intervertebral disc (Roberts *et al.*, 1989). This packing may physically limit the access of the antibodies to their epitopes. However, biochemical analyses have shown that 2B6 epitope is enriched in cartilage endplate when compared to disc tissues (Caterson, Johnstone & Jahnke, personal communication). In addition, Johnstone *et al.* (1993) have shown that the small proteoglycans of cartilage endplate contain CS rather than DS glycosaminoglycans on both decorin and biglycan.

Greater staining was found in the calcifying than the non-calcifying epiphysial cartilage for antisera against link and core protein (Poole *et al.*, 1982), resembling that seen in the present study for 8A4. Although only two specimens here had epiphysial regions present, staining was similar for the glycosaminoglycans keratan sulphate and chondroitin sulphate, to that reported by Byers *et al.* (1992). The presence of the 3B3(−) epitope in the calcified, hypertrophic zone of these young individuals supports other evidence that it is expressed, not only in degeneration, but also in development (Caterson *et al.*, 1990b; Byers *et al.*, 1992). The pattern of staining for 3B3 and 2B6 differed in the calcified region, with that for 2B6 located in the territorial matrix, similar to that seen with antibody to type IX collagen. This may be due to type IX collagen having a glycosaminoglycan chain attached, which is commonly chondroitin-4-sulphate in chick cartilage (Yada *et al.*, 1990).

Areas of calcification within these tissues also exhibited different staining patterns for some other epitopes. There was greater staining for 8A4 (anti-link protein) in both the calcified zone of the epiphysial plate and region of ectopic calcification within the cartilage endplates of scoliotic patients. In contrast, where calcification occurred in the intervertebral disc, the main difference was a greater staining for 3B3(−), 7D4(−) and 3D5 here, compared to the surrounding non-calcified matrix. This may reflect different processes of calcification being involved in the two locations. Certainly different types of calcium salts are involved, there being calcium pyrophosphate in the disc but hydroxyapatite in the cartilage endplate (Roberts *et al.*, 1993).

Following chondroitinase treatment, 3D5 and 2B6 recognize the same epitope, that is, the non-reducing terminal hexuronate residue adjacent to the penultimate 4-sulphated *N*-acetylgalactosamine (Caterson *et al.*, 1990a). Hence it is to be expected that the distribution of staining would be similar with these two antibodies in chondroitinase-treated sections. However, this is not the case in cartilage endplate, where there is more 2B6 than 3D5 staining, and vice versa in calcified matrices. This may be due to these antibodies being different classes of immunoglobulins, 3D5 being IgM while 2B6 is an IgG_1 (Table 2). IgM is a much larger molecule than IgG and hence may be more prone to physical exclusion in the cartilage endplate where the collagen fibres are tightly packed. Alternatively the antibodies, 2B6 and 3D5, may have different affinities.

There is increasing evidence that 3B3(−) and 7D4(−) epitopes occur more in osteoarthritic articular cartilage than in normal tissues, both in an experimental animal model (Caterson *et al.*, 1990b; Carney *et al.*, 1992; Ratcliffe *et al.*, 1993; Visco *et al.*, 1993) and in human clinical material (Caterson *et al.*, 1992; Slater *et al.*, 1992). Results from the present study indicate that the presence of these same epitopes is related to some extent to the degree of degeneration in mature intervertebral disc and cartilage endplate. Caterson (1991) proposed that the expression of these epitopes may prove useful in the future as markers and monitors of joint disease. If they are to be used as serological markers then the tissue source must be able to be identified (Brandt, 1989), for example to distinguish between disc degeneration and osteoarthritis of the hip. Perhaps by characterizing other epitopes further this will be possible and may provide a non-invasive, biochemical means of assessing disc degeneration.

Acknowledgements

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References

ASPDEN, R. M., HICKEY, D. S. & HUKINS, D. W. L. (1981) Determination of collagen fibril orientation in the cartilage of vertebral endplate. *Conn. Tissue Res.* **9,** 83–7.

BRANDT, K. D. (1989) A pessimistic view of serologic markers for diagnosis and management of osteoarthritis. Biochemical immunologic and clinicopathologic barriers. *J. Rheum.* **16,** 39–42.

BYERS, S., CATERSON, B., HOPWOOD, J. J. & FOSTER, B. K. (1992) Immunolocation analysis of glycosaminoglycans in the human growth plate. *J. Histochem. Cytochem.* **40,** 275–82.

CARNEY, S. L., BILLINGHAM, M. E. J., CATERSON, B., RATCLIFFE, A., BAYLISS, M. T., HARDINGHAM, T. E. & MUIR, H. (1992) Changes in proteoglycan turnover in experimental canine osteoarthritic cartilage. *Matrix* **12,** 137–47.

CATERSON, B. (1991) Immunological aspects of markers of joint disease. *J. Rheum.* **18,** 19–23.

CATERSON, B., CHRISTNER, J. E. & BAKER J. R. (1983) Identification of a monoclonal antibody that specifically recognizes corneal and skeletal keratan sulphate. *J. Biol. Chem.* **258,** 8848–54.

CATERSON, B., BAKER, J. R., CHRISTNER, J. E., LEE, Y. & LENTZ, M. (1985a) Monoclonal antibodies as probes for determining the microheterogeneity of the link proteins of cartilage proteoglycan. *J. Biol. Chem.* **260,** 11 348–56.

CATERSON, B., CHRISTNER, J. E., BAKER, J. R. & COUCHMAN, J. R. (1985b) Production and characterization of monoclonal antibodies directed against connective tissue proteoglycans. *FASEB* **44,** 386–93.

CATERSON, B., CALABRO, T., DONOHUE, P. J. & JAHNKE, M. R. (1986) In *Articular Cartilage Biochemistry* (edited by KUETTNER, K.), pp. 59–73. New York: Raven Press.

CATERSON, B., GRIFFIN, J., MAHMOODIAN, F. & SORRELL, J. M. (1990a) Monoclonal antibodies against chondroitin sulphate isomers: their use as probes for investigating proteoglycan metabolism. *Biochem. Soc. Trans.* **18,** 820–3.

CATERSON, B., MAHMOODIAN, F., SORRELL, J. M., HARDINGHAM, T. E., BAYLISS, M. T., CARNEY, S. L., RATCLIFFE, A. & MUIR, H. (1990b) Modulation of native chondroitin sulphate structure in tissue development and in disease. *J. Cell Science* **97,** 411–7.

CATERSON, B., HUGHES, C. E., JOHNSTONE, B. & MORT, J. S. (1992) Immunological markers of cartilage proteoglycan metabolism in animal and human osteoarthritis. In *Articular Cartilage and Osteoarthritis* (edited by KUETTNER, K. E., *et al.*), pp. 415–27. New York: Raven Press.

COUCHMAN, J. R., CATERSON, B., CHRISTNER, J. E. & BAKER, J. R. (1984) Mapping by monoclonal antibody detection of glycosaminoglycans in connective tissue. *Nature* **307,** 650–2.

FOSANG, A. J. & HARDINGHAM, T. E. (1991) 1-C-6 epitope in cartilage proteoglycan G2 domain is masked by keratan sulphate. *Biochem. J.* **273,** 369–73.

GALANTE, J. O. (1967) Tensile properties of the human lumbar annulus fibrosus. *Acta Orthop. Scand.* (suppl) **100,** 30–1.

INEROT, S. & AXELSSON, I. (1991) Structure and composition of proteoglycans from human annulus fibrosus. *Conn. Tissue Res.* **26,** 47–63.

JOHNSTONE, B., MARKOPOULOS, M., NEAME, P. & CATERSON, B. (1993) Identification and characterization of glycanated and non-glycanated forms of biglycan and decorin in the human intervertebral disc. *Biochem. J.* **292,** 661–6.

MODIS, L. (1990) Structure of the hyaline cartilage matrix. In *Organisation of the Extracellular Matrix: a Polarization Microscopic Approach* (edited by MODIS, L.), pp. 75–97. Boca Raton: CRC Press.

POOLE, A. R., PIDOUX, I. & ROSENBERG, L. (1982) Role of proteoglycans in endochondral ossification: immunofluorescent localization of link protein and proteoglycan monomer in bovine fetal epiphyseal growth plate. *J. Cell. Biol.* **92,** 249–60.

POOLE, C. A., WOTTON, S. F. & DUANCE, V. C. (1988) Localisation of type IX collagen in chondrons isolated from porcine articular cartilage and rat chondrosarcoma. *Histochem. J.* **20,** 567–74.

RATCLIFFE, A., FRYER, P. R. & HARDINGHAM, T. E. (1984) The distribution of aggregating proteoglycans in articular cartilage: comparison of quantitative immunoelectron microscopy with radioimmunoassay and biochemical analysis. *J. Histochem. Cytochem.* **32,** 193–201.

RATCLIFFE, A., SHURETY, W. & CATERSON, B. (1993) The quantitation of a native chondroitin sulphate epitope in synovial fluid lavages and articular cartilage from canine experimental osteoarthritis and disuse atrophy. *Arthritis Rheum.* **36,** 543–51.

ROBERTS, S., MENAGE, J. & URBAN, J. P. (1989) Biochemical and structural properties of the cartilage endplate and its relation to the intervertebral disc. *Spine* **14,** 166–74.

ROBERTS, S., MENAGE, J., DUANCE, V., WOTTON, S. & AYAD, S. (1991) Collagen types around the cells of the intervertebral disc and cartilage endplate: an immunolocalisation study. *Spine* **16,** 1030–8.

ROBERTS, S., MENAGE, J. & EISENSTEIN, S. M. (1993) The cartilage endplate in scoliosis: Calcification and other sequelae. *J. Orthop. Res.* **11,** 747–57.

SCOTT, J. T. (1986) Gout. In *Copeman's Textbook of the Rheumatic Diseases* (edited by SCOTT, J. T.), p. 895. Edinburgh: Churchill Livingstone.

SLATER, R. R., CATERSON, B., LACHIEWICZ, P. & BAYLISS, M. T. (1992) The occurrence of 3-B-3(−) and 7-D-4 epitopes as markers of arthritis in humans. *Proc. Orthop. Res. Soc.* **38,** 277.

SORRELL, J. M., MAHMOODIAN, F., SCHAFER, I. A., DAVIS, B. & CATERSON, B. (1990) Identification of monoclonal antibodies that recognise novel epitopes in native chondroitin/dermatan sulphate glycosaminoglycan chains: their use in mapping functionally distinct domains of human skin. *J. Histochem. Cytochem.* **38,** 393–402.

URBAN, J. & MAROUDAS, A. (1980) The chemistry of the intervertebral disc in relation to its physiological function and requirements. *Clin. Rheum. Dis.* **6,** 51–76.

VISCO, D. M., JOHNSTONE, B., HILL, M. A., JOLLY, G. A. & CATERSON, B. (1993) Immunohistochemical analysis of 3-B-3(−) and 7-D-4 epitope expression in canine osteoarthritis. *Arthritis Rheum.* **36,** 1718–25.

YADA, T., SUZUKI, S., KOBAYASHI, K., KOBAYASHI, M., HOSHINO, T., HORIE, K. & KIMATA, K. (1990) Occurrence in chick embryo

vitreous humor of a type IX collagen proteoglycan with an extraordinarily large chondroitin sulphate chain and short α1 polypeptide. *J. Biol. Chem.* **265**, 6992–9.

YAMAGATA, T., SAITO, H., HABUCHI, O. & SUZUKI, S. (1968) Purification and properties of bacterial chondroitinases and chondrosulfatases. *J. Biol. Chem.* **243**, 1523–35.