

tributed over a much narrower range in all synovial fluids, the values were slightly higher for the NGOA and RA groups.

When the $\Delta\text{di-6S}:\Delta\text{di-4S}$ ratios were compared, significantly lower values were obtained for all patient groups compared to those measured in normal synovial fluid. However, when the $\Delta\text{di-6S}:\Delta\text{di-4S}$ ratios for the three OA patient groups were analysed the lowest values obtained were seen in patients with NGOA and these were comparable to the ratios measured in RA synovial fluids.

Discussion

The finding that chondroitin sulphate levels of OA and RA joint fluids were significantly lower than those in normal fluids, suggests that the total mass of articular cartilage was decreased. However, the contribution of extra-articular tissues such as the meniscus, cruciate ligament and synovium to the synovial pool should not be underestimated. These tissues also have chondroitin sulphate-containing proteoglycans in their extracellular matrix and the metabolism of these molecules would affect the composition of synovial fluid. Similar mechanisms may also account in part for the lower $\Delta\text{di-6S}:\Delta\text{di-4S}$ ratio in OA and RA

fluids compared to those of healthy volunteers.

The only additional clinical feature that was used to differentiate the NGOA group from those patients with knee OA, was the presence of nodal OA of the hands. Even so, this was enough to significantly decrease the $\Delta\text{di-6S}:\Delta\text{di-4S}$ ratio seen in these patients. Although the mean synovial fluid values for NGOA patients were very similar to those obtained for RA patients, suggesting that similar biological mechanisms may possibly be involved, it is worth noting that the presence of clinical inflammation in the OA groups did not affect the disaccharide values. This is the first biochemical difference that has been detected in subsets of OA at the same site, and is further justification for considering NGOA as a subset with a possibly different aetiopathogenesis.

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Reference

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Characterization of proteoglycan populations separated by agarose electrophoresis

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The aim of this study is to develop methods for characterization of small amounts of proteoglycans available from human samples.

Methods

Proteoglycans (PGs) from 4M GuHCl extracts of normal or arthrotic human knee joint cartilage and from synovial fluids from patients with reactive arthritis or arthrosis were quantitated and purified by precipitation with Alcian Blue (1). The proteoglycans were then separated according to size by agarose electrophoresis (2) and recovered from the gel by capillary blotting.

Results

Binding and detection on different membranes. Conditions of transfer and type of membrane affected both binding and detection of PGs. The PGs were detected on PVDF-P by a reversible Toluidine Blue

staining. Isolated glycosaminoglycans did not bind to PVDF-P. PGs with low protein content did bind to PVDF-P only when blotted at high ionic strength.

Antigenic epitope identification. Comparison between Toluidine Blue and immunostaining of the same electrophoretic lane simplifies identification of antigenic epitopes. Recognition of GAG or protein core epitopes with mab 2B6, 5D4, 7D1 and 1C6 often requires deglycosylation.

Protein core characterization. The efficacy of deglycosylation with Chondroitinase ABC and Keratanase II of the different stained bands of normal human knee cartilage PGs was evaluated by SDS-PAGE. PGs were either deglycosylated when immobilized on PVDF-P or first released by treatment with guanidine-HCl/propanol and then deglycosylated in solution. Yields of 50–95% depending on type of membrane and staining method were obtained. Largest size proteoglycan population was monodis-

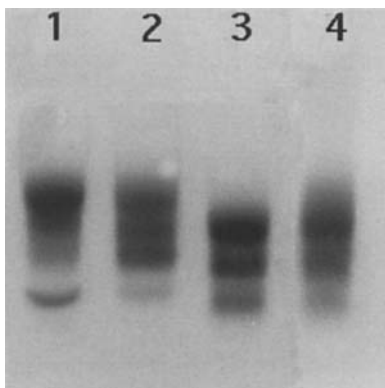


Figure 1. Proteoglycans from arthrotic cartilage (1), normal cartilage (2), reactive arthritis synovial fluid (3) and arthrosis synovial fluid (4), blotted to PVDF-P at high ionic strength and stained with Toluidine Blue.

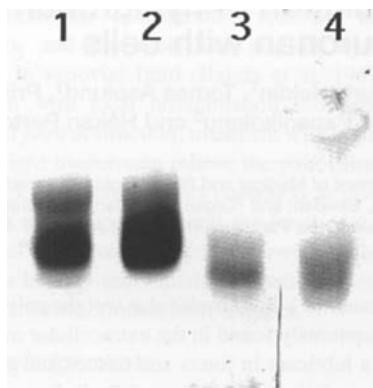


Figure 2. Mab 1C6, same samples as Figure 1. N-terminal globular domains G1 and G2 were detected after reduction and alkylation and digestion of the immobilized proteoglycans with chondroitinase ABC and Keratanase II. Equal amounts (10 µg) of glycosaminoglycan was applied in each lane. Cartilage PGs contain both G1 and G2, while the synovial fluid PGs possibly contains only G2.

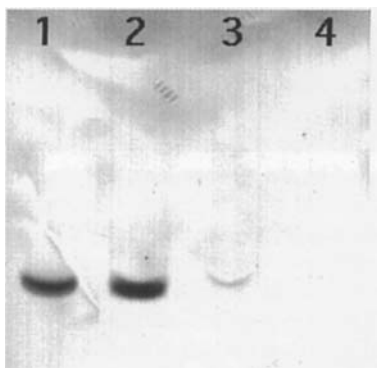


Figure 3. Mab 2B6, same samples as in Figure 1. Detection of unsaturated disaccharides of chondroitin-4-sulfate in native proteoglycans. Only the small proteoglycans, and not aggregate, of the cartilage extracts contain the exposed epitope. Neither synovial fluid contains the epitope.

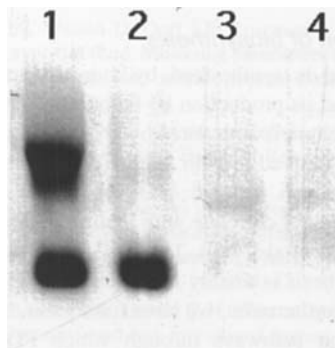


Figure 4. Mab 2B6, same samples as in Figure 1. Detection of unsaturated disaccharides of chondroitin-4-sulfate after digestion with Chondroitinase ABC of the immobilized proteoglycans. The aggrecan from arthrotic cartilage, but not from normal cartilage exhibits this epitope. Neither synovial fluid exhibits the epitope after chondroitinase.

perse with an extrapolated M_w of 360 kD. An intermediate polydisperse population had a M_w of 270 kD. The third, most abundant population, had a M_w of 170 kD. Deglycosylation was more efficient with released PGs than with immobilized PGs (not shown).

Conclusion

A 10-µg sample is sufficient for separating PGs from knee joint cartilage or synovial fluid into three different populations with subsequent characterization of their antigenic epitopes or core protein sizes. Both synovial fluid populations contained 1C6-epitope

and are thus C-terminal fragments of aggrecan cleaved in the interglobular domain. The most abundant population in normal human knee cartilage had a core protein M_w of 180 kDa, that was considerably lower than the most abundant proteoglycan population in synovial fluid which had a core protein M_w of 260 kDa. The large size fragments in synovial fluid may be derived from a separate, newly synthesized pool of large, untruncated molecules.

References

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2. Björnsson S. *Anal Biochem* 1993; 210: 292–8.