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Proteoglycan biosynthesis as a determinant of patella damage in the murine antigen-induced arthritis model

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Abstract—The incorporation of $^{35}SO_4$ into cartilage proteoglycan has been employed as a measure of patella damage in the murine antigen-induced arthritis model. In a preliminary set of experiments, where both the proteoglycan concentration and $^{35}SO_4$ incorporation were determined in control and arthritic patella over a 2 day to 6 day week period, the arthritic joint contained significantly higher levels of radioactivity compared with the controls. A subsequent study over an extended period of 10 weeks confirmed the earlier results, and indicated that the 6 week samples showed the greatest difference (71%) in $^{35}SO_4$ incorporation between the arthritic and control patella.

One of the most relevant animal models of rheumatoid arthritis, mono-articular arthritis in the rabbit (Dumonde & Glynn 1962), was adapted a number of years ago to the mouse (Brackertz et al 1977). More recently, Hunneyball et al (1986) and Van der Berg et al (1981, 1982) have re-examined and improved the methodology. The latter workers also suggested the use of $^{35}SO_4$ incorporation into cartilage proteoglycan as a measure of chrondrocyte damage (Kruijsen et al 1985). This modification has the potential for replacing the normally employed histological end-point analysis, which although original, is both time consuming and quantitatively subjective. However, to date, only the acute inflammatory phase has been assessed using the radiolabelling procedure (Van der Berg et al 1982).

We have studied the extent of $^{35}SO_4$ incorporation into patella proteoglycan over the duration of the arthritic response. We now report our initial findings on the synthesis and levels of proteoglycan at specific time points (2 days to 6 months) during the inflammatory reaction, and from a larger time course study.

Materials and methods

Induction of arthritis. Male C57BL mice were sensitized following the technique of Hunneyball et al (1986) and Brackertz et al (1977). Briefly this involved intradermal injection into the flank of 100 mL of an emulsion of methylated bovine serum albumin (Met-BSA; 3 mg mL^{-1}; Sigma) in saline with an equal volume of Freund's complete adjuvant (FCA; Difco) containing additional *Mycobacterium tuberculosis* (1 mg mL^{-1}; Weybridge). At the same time an intraperitoneal injection of 2×10^9 organisms of *Bordetella pertussis* (Wellcome Pertussis Vaccine BP) was also administered. One week later these injections were repeated on the same animals and 14 days after the last immunization 6 μL of a sterile solution of Met-BSA (10 mg mL^{-1}) was injected into the right knee joint. The left knee joint served as the control and was injected with 6 μL of sterile saline.

The uptake of $^{35}SO_4$ into patella cartilage. A modified version of the technique described by Kruijsen et al (1985) was used. Following the careful dissection of the arthritic and control patellae free from surrounding connective tissue they were preincubated for 45 min in 5 mL of a medium containing RPMI-HEPES and RPMI-HCO_3 (1:1, v/v; Flow Laboratories) with penicillin (100 u mL^{-1}), streptomycin (100 g mL^{-1}, (Flow Laboratories) and L-glutamine (2 mM, Flow Laboratories). The patellae were transferred to incubation vials containing 0·5 mL of fresh medium plus 5 Ci of ^{35}S-sulphate (Amersham International) and were further incubated for 3 h. All incubations were carried out at 37°C in an atmosphere of 5% CO_2 in air. At the end of the incubation period the patellae were washed three times with 10 mL of saline before digestion at 65°C for 1 h in 1 mL of phosphate buffer (pH 6·5, 0·067 M) containing 2 mM *N*-acetylcysteine, 2 mM EDTA and 20 μL of papain. 0·5 mL of the digestate was added to 10 mL of NE 260 scintillator (Nuclear Enterprises) and the radioactivity was determined by liquid scintillation counting, the results being expressed as d min^{-1}. The remaining 0·5 mL was mixed with 0·5 mL of dimethylmethylene blue (DMB Serva Feinbiochemica, Heidelburg) solution for the measurement of chondroitin sulphate concentration as an estimation of proteoglycan levels (Farndale et al 1982).

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Table 1. $^{35}SO_4$ incorporation into patella proteoglycan at different times. Results are mean values ± s.d. for the number of experiments shown in parentheses with at least 5 mice per group. The proteoglycan concentration and ^{35}S uptake were determined as described in the Methods section. • Significant (paired *t*-test) change from control values $P < 0.05$.

	Proteoglycan concn		^{35}S-Uptake	
Time	Control	Arthritic	Control	Arthritic
2 days	1·13(2)	1·31	2890(2)	2730
2 weeks	1·33 ± 0·09(3)	1·29 ± 0·22	2218(7)	2785*
3 weeks	1·28 ± 0·03(3)	1·42 ± 0·30	1861(13)	2321*
6 weeks	1·24 ± 0·09(3)	1·27 ± 0·03	3120(3)	4100*

Results and discussion

The preliminary studies were designed to follow in-vitro ^{35}S-proteoglycan synthesis, as a measure of chondrocyte death, at specific time points. The results (Table 1) indicate that whereas at the earlier time (2 days) there was no effect on ^{35}S-incorporation, there appeared to be a significantly greater incorporation of radioactivity in the arthritic patella with respect to the control, with time. The lack of a significant effect at

Table 2. Time course experiment for $^{35}SO_4$ incorporation into patella proteoglycan. Results are mean values ± s.d. for 5–7 mice per group at each time point. ^{35}S uptake was determined as described in the Methods section. Significant (unpaired *t*-test) change from control values $^*P < 0.05$, $^{**}P < 0.01$.

	^{35}S-Uptake		
Time	Control	Arthritic	% Change
2 days	2897 ± 513	2617 ± 490	−9·7
1 week	3778 ± 268	3900 ± 430	+3·2
2 weeks	11585 ± 3590	7616 ± 2003	−34·3
3 weeks	4388 ± 1715	6739 ± 2024	+53·6*
4 weeks	1752 ± 105	2909 ± 1138	+66·6*
6 weeks	1985 ± 294	3389 ± 941	+70·7**
10 weeks	3535 ± 1122	5029 ± 1682	+42·7

2 days does not support the findings of Van der Berg et al (1982) who reported impaired ^{35}S incorporation. Possibly reduced proteoglycan synthesis might have been observed with further studies. However, at the 2–6 week times, when far more individual experiments were undertaken, the trend in enhanced proteoglycan synthetic capacity in the arthritic patella was clearly seen. Although the macroscopic appearance of the arthritic patella at the post two week times indicated cartilage damage, there was apparently no differences in the overall proteolgycan concentrations (Table 1).

Based on these early findings, a study was undertaken in which ^{35}S-incorporation was measured in one specific experiment over an extended time course (2 days to 10 weeks). The results (Table 2) confirm the elevated ^{35}S-incorporation in the arthritic patella with time, with the maximum effect being observed at 6 weeks.

Assuming that cartilage damage is associated with chondrocyte death, which in turn can be measured by ^{35}S-proteoglycan synthesis, our results are difficult to interpret. One possibility is that the higher rates of proteoglycan synthesis observed in the arthritic patella represent the laying down of new tissue components to replaced damaged areas. Confirmation of this suggestion will have to await histological analysis.

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The serum amyloid P response in the mouse air pouch

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Abstract—Levels of the acute phase reactant serum amyloid P (SAP) have been measured in the mouse pouch model of rheumatoid arthritis. Implantation of cartilage resulted in a significant and rapid elevation in the SAP concentration, which remained high for the duration of the experiment (14 days). Initial studies with several clinically employed antirheumatic drugs indicated that dexamethasone and cyclosporin A had a marked inhibitory effect.

Subsequent to the original development of the mouse air pouch model by Willoughby and his group (Sedgwick et al 1984; Sin et al 1984), we recently reported a modified procedure, thus allowing the routine evaluation of potential antirheumatic compounds (Bottomley et al 1986). In our model, the inflammatory response (i.e. granuloma formation) and the tissue destructive elements (i.e. proteoglycan and collagen loss from implanted cartilage) could be measured. One of the omissions from the determinants estimated was the role of interleukin-1 (IL-1) in the air pouch during cartilage breakdown.

The levels of the acute phase reactant α_1-glycoprotein in the rat air pouch have been determined as an indirect indication of IL-1 activity (Sedgwick et al 1984; Al-Duaij et al 1986a) since the hepatic synthesis of acute phase proteins is stimulated by IL-1 (Dinarello 1984; Sipe 1985). We have now determined the profile of serum amyloid P (SAP), as an example of an acute phase protein (Pepys et al 1979), over the course of a typical air pouch experiment.

During such an experiment, implanted cartilage is significantly degraded over a 14 day period, with approximately 70 and 30% loss of proteoglycan and collagen, respectively (Bottomley et al 1986).

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In addition, preliminary work has been initiated on the effects of a number of drugs on this profile. The data from these studies are now reported.

Methods

Mouse SAP was measured in serial serum samples obtained from tail vein bleeding using a simple radial immunodiffusion assay. Samples were taken on various days ranging from 6 days before and 14 days after cartilate implantation. For the radial immunodiffusion assay sheep anti-mouse SAP antiserum was mixed with agarose gel (15 μL mL^{-1}) and cast onto gel bond. Wells (2·5 mm diameter) were punched and 5 μL of either normal mouse SAP (5–100 μg mL^{-1}) or serum samples was added. Following incubation for approximately 36 h at 37°C, the plates were washed in 5% NaCl solution for 4 h to remove unprecipitated protein. The pressed and hot air-dried plates were stained with 0·2% (w/v) Coomassie Blue R250 in ethanol–acetic acid–water (9:2:9), washed, destained and finally dried. Serum SAP concentrations were estimated following reference to the generated standard curve.

The mouse air pouch model was that described by Bottomley et al (1986) using Charles River CD1 out-bred mice.

Sheep anti-mouse SAP antiserum (N 474 pool SSP) was kindly supplied by Professor Pepys (Royal Postgraduate Medical School, London).

Serum amyloid P component and normal mouse standard were from Calbiochem. Drugs and suppliers were: ibuprofen, dexamethasone and chloroquine (Sigma); cyclosporin A (Sandoz); clozic acid, BW 775 C (3-amino-1-(*m*-trifluoromethyl)phenyl]-2-pyrazoline) and auranofin (prepared by the Medicinal Chemistry Department, Hoechst UK).

Results and discussion

In a preliminary experiment, the intramuscular administration