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.

References

Brackertz, D., Mitchell, G. F., Mackay, I. R. (1977) Arthritis Rheum. 20: 841–850

Dumonde, D. C., Glynn, L. E. (1962) Br. J. Exp. Pathol. 43: 373–383

Farndale, R. W., Sayers, C. A., Barrett, A. J. (1982) Conn. Tiss. Res. 9: 247–248

Hunneyball, I. M., Crossley, M. J., Spowage, M. (1986) 18: 384–393

Kruijsen, N. B., M. W. M, Van der Berg, W. B., Van der Putte, L. B. A. (1985) Arthritis Rheum. 28: 813–819

Van der Berg, W. B., Kruijsen, M. W. M., Van de Putte, L. B. A., Van Beusekom, H. J., Van der Sluis-Van der Pol, M., Zwarts, W. A. (1981) Br. J. Exp. Pathol. 62: 308–316

Van der Berg, W. B., Bensekom, H. J., Van de Putte, L. B. A., Zwarts, W. A., Van der Sluis-Van der Pol, M. (1982) Am. J. Pathol. 108: 9–16

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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

of turpentine (50 μL) to non-pouched mice produced a rapid increase in SAP levels (data not shown), which was maximal at 24 h and had decreased to background values by day 8. A similar result for α_1-acid glycoprotein in rats had previously been reported (Jamieson et al 1972). These results demonstrated that an SAP response could be adequately monitored in the strain of mice employed for the subsequent studies.

The levels of SAP in air pouched mice are shown in Fig. 1.

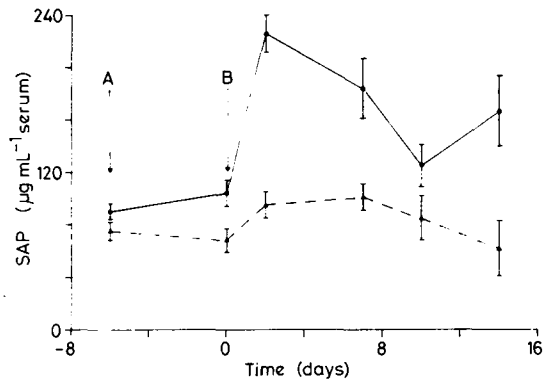

FIG. 1. The time course of SAP levels in the mouse air pouch following implantation of cotton-wrapped rat femoral heads (●) or following sham implantation (▲). Each point is the mean of 12 determinations ± s.e.m. Air pouch formed at A, cartilage implanted at B.

Formation of the air pouch produced a small, but non significant increase in the SAP concentration before cartilage implantation at day 0. However, following the introduction of the cotton wrapped femoral head, there was a marked elevation in the acute phase protein levels. At the first time point determined (2 days) the SAP concentration appeared to be maximal, and it remained above the control value even at day 14. Thus it would appear that the implanted cartilage and the associated inflammatory reaction causes a long lasting elevation in acute phase protein response suggesting raised IL-1 levels accompanying the ongoing cartilage destruction.

A number of drugs showing anti-inflammatory or anti-rheumatic activity were examined (Table 1).

Table 1. The effect of drugs on serum amyloid P levels and collagen loss in the mouse air pouch. Mice (12 per group) were dosed once daily with drug for 14 days unless indicated. SAP levels were compared with vehicle dosed controls.

Drug	Dose (mg kg^{-1})	Route	% Inhibition serum amyloid P	Collagen loss (% protection)
Dexamethasone	0·5	i.p.	115*▲	100†
Clozic acid	30	i.p.	50▲	0
Auranofin	20	p.o.	0	13
Cyclosporin A	25	p.o.	123*	100†
BW755C	50	p.o.	−28	−62†
Ibuprofen	50	p.o.	8	−48*

† Statistically significant ($P < 0.02$, unpaired Wilcoxen test) from controls, *statistically significant ($P < 0.05$, unpaired t-test) from control values. ▲ Sampled at day 9.

Dexamethasone and cyclosporin A produced significant inhibition of SAP levels and also gave total protection against collagen loss. Clozic acid, which has been reported to reduce acute phase reactants in the rat adjuvant arthritis model (Billingham 1983), lowered the SAP concentration in the mouse air pouch model, although this result was not statistically significant. However there was no protection against collagen loss. The anti-inflammatory agents BW 755C and ibuprofen accelerated cartilage breakdown, and with the former compound there was also an elevated serum SAP concentration at 14 days. This failure of cyclooxygenase inhibitors to protect cartilage confirms the previous findings of Willoughby et al (1985) and Al-Duaij et al (1986b), who also showed that exudative inflammation may protect cartilage from breakdown and that indomethacin enhanced breakdown in this type of model. Auranofin had no effect on either the cartilage-induced elevation in SAP levels or on collagen loss.

In conclusion, the implantation of a cotton wrapped rat femoral head in the mouse pouch raises SAP levels over 14 days. From this initial study, inhibition of collagen loss from the cartilage would appear to be associated with suppressed SAP levels.

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References

Al-Duaij, A. Y., de Brito, F., Moore, A. R., Sedgwick, A. D., Willoughby, D. A. (1986a) Acute phase response is a model of chronic inflammation, 2nd World Conference on Inflammation, Monte Carlo, Abstract A414

Al-Duaij, A. Y. De Brito, F. B., Holmes, M. J. G., Law, K. A., Willoughby D. A. (1986b) Indomethacin, D-penicillamine and dexamethasone in a model of cartilage breakdown by a granulomatous reaction. Br. J. Pharmacol. 89: 567P

Billingham, M. A. (1983). Models of arthritis and the search for anti-arthritic drugs. Pharmacology 21: 389–428

Bottomley, K. M. K., Griffiths, R. J., Norris, D. B., Rising T. J., Steward, A. (1986) Cartilage degradation in a mouse air pouch model, Br. J. Pharmacol. 88: Proceedings supplement. 401P

Dinarello, C. A. (1984). Interleukin I and the pathogenesis of the acute phase response. New. Eng. J. Med. 311(22): 1413–1419

Jamieson, C., Ashton, F. E., Friesen, A. D., Chou, B. (1972) Studies on acute phase protein of rat serum II. Determination of the contents of α_1-acid glycoprotein, α_2-macroglobulin, and albumin in serum from rats suffering from induced inflammation. Can. J. Biochem. 50: 871–880

Pepys, M. B., Baltz, M., Gomer, K., Davies, A. J. S., Doenhoff, M. (1979) Serum amyloid P-component is an acute-phase reactant in the mouse. Nature 278: 259–261

Sedgwick, A. D., Moore, A. R., Al-Duaij, A., Sin, Y. M., Landon, B., Willoughby, D. (1984) Effects of therapeutic agents on cartilage degradation in-vivo. J. Pharm. Pharmacol. 36: 706–710

Sin, Y. M., Sedgwick, A. D., Willoughby, D. A. (1984). Studies on the mechanism of cartilage degradation. J. Pathol. 142, 23–30

Sipe, J. D. (1985) Interleukin 1 target cells and induced metabolic changes. In: Gordon, A. H., and Koj, A. (eds) Research monographs in cell and tissue physiology 10, Elsevier, Amsterdam, pp 51–67

Willoughby, D. A., Al-Duaij, A. Y., De Brito, F. B., Sedgwick, A. D., Moore, A. R. (1985) A hypothesis for the mode of action of anti-rheumatic drugs in a model of cartilage destruction. J. Pharm. Pharmacol. 37: 931