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A modified mouse air pouch model for evaluating the effects of compounds on granuloma induced cartilage degradation

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1 Employing rat femoral head cartilage implanted in a 6 day old mouse air pouch, the effects of inflammatory stimuli (i.e. cotton pellets, carrageenan, zymosan) on the loss of proteoglycan and collagen and granuloma formation have been studied.

2 Wrapping of the cartilage in cotton resulted in granuloma formation with accelerated loss of proteoglycan and collagen over the 14 day implantation period. The amount of loss increased with increasing weight of cotton.

3 The effects of different classes of anti-rheumatic drugs on granuloma formation and proteoglycan and collagen loss from cotton wrapped femoral head cartilage in the mouse air pouch have been studied.

4 Non-steroidal anti-inflammatory drugs (NSAIDs) had no influence on granuloma formation, but in general accelerated the rates of proteoglycan and collagen loss.

5 Dexamethasone and prednisolone significantly reduced granuloma formation and had a marked protective effect on cartilage breakdown.

6 Of the slow acting anti-rheumatic drugs examined, only gold sodium thiomalate (GSTM) and dapsone significantly decreased cartilage loss, with an accompanying modest decrease in granuloma formation.

7 The immunosuppressants cyclophosphamide and methotrexate, but not azathioprine, reduced cartilage degradation, but had no effect on granuloma formation.

8 The results for the different classes of anti-inflammatory and anti-rheumatic drugs are discussed in relation to their effects in other animal models and their reported therapeutic activities in man. It is concluded that the mouse air pouch method as described offers advantages as an animal model over existing procedures to predict therapeutic efficacy in man.

Introduction

Many animal models have been established for the evaluation of anti-inflammatory and anti-rheumatic drugs. Various comprehensive reviews on these models (Billingham & Davies, 1979; Billingham, 1983; Lewis *et al.*, 1985) have concluded that no *in vivo* procedure yet exists which is reliably predictive for therapeutic efficacy in man. The development of the air pouch (Edwards *et al.*, 1981; Yoshino *et al.*, 1984; Kimura *et al*, 1985) has represented one of the more recent attempts to overcome this lack of a reliable model. Although there are a number of variations to the method, the procedure adopted by Willoughby and his group (Sin *et al.*, 1984; Sedgwick *et al.*, 1985; Willoughby *et al.*, 1986) would appear to be most applicable to the disease process in man. A similar conclusion was also made by Rainsford (1985). Whereas in initial studies the loss of proteoglycan from mouse xiphoid cartilage implanted in the mouse air pouch was followed (Sedgwick *et al.*, 1984), the more recent studies have employed the rat air pouch (De Brito *et al.*, 1986).

We have made some modifications to the original mouse air pouch procedure and have adapted it for routine screening of novel chemical entities. Results of the studies to optimise this animal model are now presented, amplifying our preliminary communication (Bottomley *et al.*, 1986).

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Employing a similar mouse air pouch model, but using xiphoid cartilage in an inflamed pouch, Sedgwick et al. (1984) found that whereas the non steroidal anti-inflammatory drugs (NSAIDs) inhibited cellular infiltration, the only slow acting anti-rheumatic drug (SAARD) examined, D-penicillamine, had no anti-inflammatory effect, but significantly inhibited the observed reduction in proteoglycan levels. We have now determined the activity of a wide range of known compounds in our model. These compounds were selected from different classes of anti-rheumatic drugs including NSAIDs, steroids, SAARDs and immunomodulators. The results are discussed in relation to their postulated mechanisms of actions in rheumatoid arthritis.

Methods

Animals

Female Charles River CD-1 mice (20–25 g) and male Charles River Sprague-Dawley rats (150–200 g, unless otherwise stated) were used in all experiments.

Preparation of irritants

Carrageenan (0.25–2% w/v) was prepared as a suspension in sterile saline (0.9% w/v). Zymosan A was boiled in 1 M NaOH for 15 min, washed three times in sterile saline and used as a suspension (0.25–2% w/v) in sterile saline. Cotton pellets were prepared from the outer layer of dental rolls which were removed and cut into squares (approx. 1 cm × 1 cm) weighing 5.0 ± 0.2 mg unless otherwise stated. The pellets were sterilised by autoclaving.

Preparation of animals

Subcutaneous air pouches prepared as described by Edwards et al. (1981) were normally employed as the site of cartilage implantation. Experimental procedures were carried out under light ether anaesthesia. On day 0, 5 ml of air was injected subcutaneously into the dorsal surface of mice to form an oval pouch. On day 3 a further 2 ml of air was injected. When appropriate, carrageenan or zymosan (1 ml of a 0.25%, 1% or 2% w/v solution) were injected on day 5. Cartilage was implanted through a small incision in the pouch on day 6 which was sealed with a Michele clip.

Preparation of cartilage

Rats were killed and both femoral head cartilages removed under aseptic conditions, weighed, and placed in 1 ml Medium 199 containing 100 iu ml^{-1} penicillin and 100 $\mu g\,ml^{-1}$ streptomycin. For experiments in which cartilage was wrapped in cotton the sterilised pellets were soaked in Medium 199 as above and the cartilage tightly wrapped in the cotton. The entire procedure was carried out in a laminar flow cabinet and the cartilage was kept in sterile containers until use. The time from removal of cartilage to implantation was usually 90 min. Age-matched rats were used for all experiments and 6–12 control cartilages from the same group were stored at −20°C until analysed at the end of the experiment. (This control group is referred to as day 0 in the time course experiments).

Analysis of cartilage and granuloma

After 7–21 days the mice were killed and the pouch opened. The cartilage was removed, weighed, and digested with papain (20 u ml^{-1}) for 1 h at 65°C. An aliquot was removed and the sulphated proteoglycan content was determined by the method of Farndale et al. (1982). The results were expressed as μg chondroitin sulphate equivalents per mg cartilage since there is a very good correlation between these two parameters in control cartilage ($r = 0.729$, $P < 0.001$, $n = 120$).

The remainder of the sample was freeze dried, digested in 300 μl 6 N HCl at 116° for 16 h and the hydroxy-proline content determined by the method of Berg (1982). The level of hydroxy-proline is a reliable index of collagen content (Berg, 1982) and the results have been expressed as μg hydroxy-proline per cartilage, since there is no correlation between cartilage weight and hydroxy-proline content ($r = 0.112$, $P > 0.1$, $n = 120$).

In experiments in which cartilage was wrapped in cotton, the cotton and surrounding granulation tissue were removed, dried in an oven at 50°C overnight and the dry weight (mg) used as an index of granuloma formation.

Drugs

All drugs were administered p.o., except gold sodium thiomalate (GSTM) which was given i.m., from the day of implantation for 14 days, unless otherwise stated. The drugs used and their sources were: auranofin, BW755C (3-amino-1(m(trifluoro-methyl)-phenyl)-2-pyrazoline) (both synthesized in the Department of Drug Discovery, Hoechst Pharmaceutical Research Laboratories), chloroquine diphosphate, cyclophosphamide monohydrate, ibuprofen, indomethacin, methotrexate, D-penicillamine, pyrithioxin, dexamethasone 21-acetate and prednisolone (all from Sigma, Poole, Dorset). Dapsone and

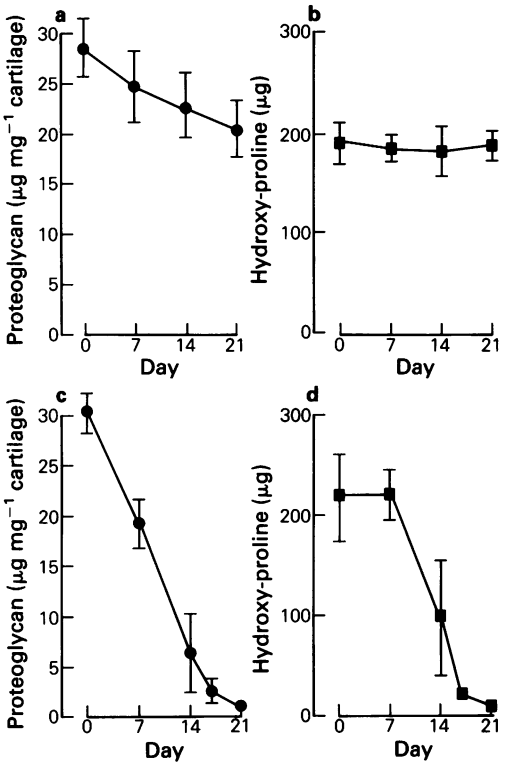

Figure 1 The effect of cotton on the loss of proteoglycan (a and c) and collagen (measured as hydroxyproline) (b and d) from implanted rat femoral head cartilage (a) and (b), unwrapped cartilage; (c) and (d), cotton (5 mg) wrapped cartilage. Each value represents the mean of 6–12 determinations; vertical lines indicate s.d.

GSTM were kind gifts from Wellcome and May & Baker, respectively.

Materials

Materials employed in the present study were obtained from the following suppliers: Medium 199 (containing Earle's salts and 20 mM HEPES buffer) and penicillin/streptomycin (5000 iu ml^{-1}/5000 $\mu g\,ml^{-1}$) (Flow Laboratories, Rickmansworth, Hertfordshire); 1,9-dimethyl-methylene blue (Serva, Heidelburg, F.R.G.); carrageenan-Viscarin GP 109 (Lot no. 170904, Marine Colloids, Springfield, U.S.A.); zymosan A, chondroitin sulphate grade III and papain type III (Sigma, Poole, Dorset). All other reagents were of Analar grade. Dental rolls were obtained from a local dental surgery.

Statistics

Results are expressed as mean ± s.d. Group means were compared by use of Student's unpaired t test with a Bonferroni correction factor for multiple comparisons where appropriate (Wallenstein *et al.*, 1980).

Results

Effect of inflammatory stimuli

Implantation of rat femoral head cartilage into a mouse air pouch led to a progressive loss of proteoglycan (Figure 1a) but not of collagen (Figure 1b). Proteoglycan loss was suppressed in cartilage implanted into air pouches inflamed with carrageenan but in contrast was enhanced if zymosan was used as the phlogistic agent (Table 1). At the higher

Table 1 Effect of inflammation induced by carrageenan or zymosan on proteoglycan and collagen content of rat femoral head cartilage implanted into the mouse air pouch

	Chondroitin SO_4 ($\mu g\,mg^{-1}$)	*OH-proline* (μg)
Stored control	33.8 ± 1.7 (8)	268 ± 38
Non-inflamed	25.6 ± 2.9 (10)	304 ± 42
Carageenan		
0.25%	25.0 ± 3.7 (10)	259 ± 50
1.0%	28.0 ± 3.2 (10)	274 ± 63
2.0%	29.3 ± 1.7 (10)*	260 ± 25
Zymosan		
0.25%	21.6 ± 6.3 (8)	268 ± 86
1.0%	10.4 ± 5.4 (9)***	199 ± 60 ***
2.0%	10.9 ± 7.5 (7)***	127 ± 50 ***

Rat femoral head cartilage was implanted into mice air pouches some of which were injected with 1 ml of carrageenan or zymosan 24 h previously: 14 days later the cartilage was removed and chondroitin sulphate and hydroxyproline content determined. Results show mean ± s.d. of (n) observations.
* $P < 0.05$, *** $P < 0.001$ compared to non-inflamed group. Student's unpaired t test with Bonferroni correction factor for multiple comparisons.

Table 2 Effect of cotton weight on granuloma formation and cartilage degradation in the mouse air pouch

Wt. of cotton (mg)	*Granuloma wt* (mg)	*Chondroitin SO_4* ($\mu g\,mg^{-1}$)	*OH-proline* (μg)
Stored Controls	—	32.6 ± 0.6 (8)	269 ± 19
0	—	25.8 ± 3.4 (6)	293 ± 19
5	29.2 ± 17.3 (5)	9.7 ± 3.7	159 ± 62
10	39.5 ± 5.2 (5)	6.7 ± 2.6	117 ± 32
20	65.2 ± 10.7 (6)	3.5 ± 1.2	85 ± 16

Rat femoral head cartilage was wrapped in cotton pellets of varying weight and implanted into mice air pouches: 14 days later the cartilage was removed and chondroitin sulphate and hydroxy-proline content determined.
The cotton pellet and surrounding granulation tissue was dried in an oven at 50°C overnight and weighed. Results show mean ± s.d. of (n) observations.

zymosan concentrations collagen loss also occurred (Table 1).

Induction of a granulomatous response by wrapping the cartilage in cotton greatly enhanced the rate of proteoglycan degradation (Figure 1c) and stimulated collagen loss (Figure 1d). Significant proteoglycan loss occurred prior to that of collagen (7 days versus 14 days). In addition significant cartilage weight loss accompanied the reduction in collagen levels (25% at 14 days, 83% at 21 days) indicating total destruction of cartilage structure. The granuloma dry weight was 19 ± 6 mg (mean ± s.d., $n = 10$) at day 7, and reached a maximum at day 14 (34 ± 8 mg, $n = 12$). Increasing the amount of granulation tissue formed, by increasing the weight of cotton, enhanced proteoglycan and collagen loss after 14 days (Table 2).

We also compared granuloma formation and cartilage degradation in the air pouch with subcutaneous implants. Granuloma formation was significantly greater in the air pouch but despite this proteoglycan and collagen loss were similar in both subcutaneous and air pouch implants (Table 3).

Influence of donor weight on cartilage structure and breakdown

The effect of donor rat weight on cartilage degradation induced by granulation tissue was studied, since changes in cartilage structure are known to occur with age (Hascall & Kimura, 1982). Cartilage from groups of rats weighing 100 to 400 g were wrapped in 5 mg cotton and implanted into the air pouch for 14 days. An apparent progressive decrease in the rate of loss of proteoglycan and collagen was observed with increasing donor rat weight after implantation into recipient mouse air pouches (e.g. ~92% and ~68% loss in 100 g donor rats compared to ~62% and ~24% loss in 400 g rats of proteoglycan and collagen, respectively). In subsequent air pouch studies donor rats of the weight range 150–200 g were employed.

Reproducibility of the model

The absolute chondroitin sulphate and hydroxyproline levels for −20°C stored control cartilage, and cartilage removed from vehicle-treated animals showed only small variations. In 9 separate experiments, each with 6–11 determinations, the ranges of values for chondroitin sulphate ($\mu g\,mg^{-1}$) and hydroxyproline (μg) in stored controls were 29.8–37.5 and 199–267, respectively. In the vehicle-treated mice, with cotton wrapped (5 mg) rat cartilage implanted into air pouches there was a 65–85% reduction in the proteoglycan content of the cartilage compared to the stored control values, and a 31–60% reduction in collagen content over 14 days. Therefore the method is reproducible and the technique can be used to study the effect of drugs on cartilage degradation.

Drug effects on granuloma formation

The steroids dexamethasone and prednisolone reduced the formation of granulation tissue around the cotton pellet (Table 4), and with dexamethasone this occurred even when it was administered 3 or 7 days after implantation. There was no evidence of a fibrous capsule or vascularization of granulomas removed from animals treated with these drugs. Of the other drugs tested only GSTM produced a statistically significant inhibition of granuloma formation (Table 4), although non significant reductions of approximately 20% were observed with ibuprofen, dapsone and azathioprine at the doses used.

Drug effects on cartilage degradation

The drugs which reduced granuloma formation (dexamethasone, prednisolone, GSTM) also decreased proteoglycan and collagen loss from the cartilage. However, protection against collagen loss was always greater than against proteoglycan loss

Table 3 Comparison of granuloma formation and cartilage degradation in subcutaneous or air pouch implants

	Granuloma wt. (mg)	*Chondroitin* SO_4 ($\mu g\,mg^{-1}$)	*OH-proline* (μg)
Stored controls		31.4 ± 2.8	216 ± 34
Air pouch	29.3 ± 10.9 (18)**	6.9 ± 2.4	110 ± 49
Subcutaneous	18.7 ± 6.4 (16)	8.1 ± 2.9	109 ± 50

Rat femoral head cartilage was wrapped in cotton pellets (5 mg) and implanted into mice air pouches or subcutaneously: 14 days later the cartilage was removed and chondroitin sulphate and hydroxy-proline content determined. The cotton pellet and surrounding granulation tissue was dried in an oven at 50°C overnight and weighed. Results show mean ± s.d. of (n) observations. **$P < 0.01$ compared to subcutaneous implants. (Student's unpaired t test).

(Table 4). Interestingly, when dexamethasone was administered after implantation, although there was still a highly significant reduction in granuloma formation, the protective effect on cartilage degradation was diminished or even abolished.

The NSAIDs ibuprofen and BW755C significantly enhanced cartilage degradation, the effect on collagen content being considerable (Table 4).

The immunosuppressive agents cyclophosphamide and methotrexate and the SAARD dapsone also prevented collagen loss more effectively than proteoglycan loss (Table 4), but in contrast to the steroids did not suppress granuloma formation (Table 4). However the immunosuppressive agent azathioprine had no significant effect on any of the parameters measured, a result confirmed in a second experiment (data not shown).

Discussion

The use of air pouches in rodents as a means of studying one aspect of rheumatoid arthritis, namely cartilage degradation, provides a novel approach to the screening of anti-arthritic drugs. The procedures

Table 4 Effect of drugs on granuloma formation and cartilage degradation

Drug ($mg\,kg^{-1}\,day^{-1}$)	*Granuloma wt* (% inhibition)	*Chondroitin* SO_4 *loss* (% inhibit)	*OH-proline loss* (% inhibit)
Steroids			
Dexamethasone (0.5) Day 0–13	70.5***	18.1*	76.7***
Dexamethasone (0.5) Day 4–13	62.0***	11.1*	30.7
Dexamethasone (0.5) Day 7–13	51.0***	2.2	−4.5
Prednisolone (10)	69.4***	26.2*	98.3***
NSAID			
Indomethacin (1)	−8.9	−5.8	−17.5
Ibuprofen (30)	22.2	7.0	−48.2**
BW755C (50)	13.4	−18.3*	−62.0*
SAARD			
D-Penicillamine (100)	11.1	−1.9	−6.8
Chloroquine (50	−0.9	−2.1	6.0
Pyrithioxin (100)	18.8	1.2	−24.4
GSTM (20)	29.4*	8.6*	37.1**
Auranofin (20)	16.1	−7.4	12.9
Dapsone (30)	21.1	11.7	36.8*
Immunosuppressants			
Cyclophosphamide (10)	7.3	32.6*	94.7***
Methotrexate (0.6)	10.7	16.8	54.0*
Azathioprine (30)	20.8	7.9	7.8

Results show mean percentage inhibition in drug-treated group compared to vehicle-treated group (7–12 animals per group). Statistics were performed on the absolute values for each parameter.
* $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$. Student's unpaired t test with Bonferroni correction factor for multiple comparisons where apropriate.

described in this present study have been adapted from those described by Sin *et al.* (1984), Sedgwick *et al.* (1985) and Willoughby *et al.* (1986).

The inflammatory agents carrageenan and zymosan had contrasting effects on cartilage degradation when pre-injected into the mouse air pouches (Table 1). Carrageenan-induced inflammation resulted in protection against proteoglycan loss, in agreement with Willoughby *et al.* (1985). However, the inflammatory irritant, zymosan, led to increased loss of proteoglycan and collagen at the higher doses used. Carrageenan has been reported to affect a number of responses of the immune system (Thomson *et al.*, 1979) and also to be cytotoxic towards macrophages both *in vitro* (Catanzaro *et al.*, 1971) and *in vivo* (Pugh-Humphreys & Thomson, 1979). The implications of these observations on our present findings are that the higher doses of carrageenan could be inhibiting the immunological factors responsible for cartilage degradation in the air pouch, whereas zymosan is stimulating them.

An enhanced granulomatous response induced a greater loss of proteoglycan and collagen from the cartilage; this was observed with the higher cotton pellet weights (Table 2). This agrees with the work of De Brito *et al.* (1986). The greater density of the cotton pellet, as well as eliciting a greater irritant effect, provides a thicker mesh for retaining the cells responsible for secreting the cartilage degrading enzymes. The demonstration that significant weight loss accompanied significant collagen losses is in agreement with studies on cartilage degradation in tissue culture (Saklatvala & Dingle, 1980).

The comparison of results for cartilage implanted either subcutaneously or into the air pouch showed a significant difference in dry granuloma weights but no difference in proteoglycan or collagen content (Table 3). These results were somewhat surprising since the degree of cartilage breakdown appears to be related to the extent of granuloma formation (Table 2). However, data were only obtained for a single time point and also dry weight measurements might not be a reliable index of the cartilage degrading capacity of the granuloma.

The earlier work using mouse xiphoid cartilage in the inflamed mouse air pouch (Sedgwick *et al.*, 1984) suggested the potential of this animal model for studying the effect of drugs on cartilage degradation. This conclusion was derived from the observation that D-penicillamine, which can be considered a disease modifying agent (Huskisson, 1983), had a protective effect on proteoglycan loss, whereas the non-steroidal anti-inflammatory drugs only reduced the inflammatory response. Using our modified air pouch model (i.e. the implantation of cotton wrapped rat femoral heads in non inflamed pouches), we have examined a range of drugs, often prescribed for the treatment of rheumatoid arthritis, for effects on granuloma formation and weight, as well as on the loss of proteoglycan and collagen.

The selected compounds can be empirically divided into a number of categories, namely NSAIDs, steroids, SAARDS and immunomodulators. Within these categories the present results will be considered in relation to the postulated mechanisms of action, activities in other animal models, and in relation to their therapeutic efficacy in rheumatoid arthritis.

The two NSAIDs tested, indomethacin and ibuprofen, as well as the dual cyclo-oxygenase and lipoxygenase inhibitor, BW755C, had no significant effect on granuloma formation. However, there were reduced levels of collagen compared to control values, and this difference reached significance with ibuprofen and BW 755C. The latter compound also increased the rate of loss of proteoglycan from the cartilage. In the carrageenan rat paw oedema, a classical animal model of anti-inflammatory activity, the two NSAIDs show good activity (Lewis *et al.*, 1985). BW755C also exhibits an effect in the rat paw oedema assay, even when administered 2 h after the carrageenan (Holsapple & Yim, 1984). Employing the *in vivo* rat models associated with chronic inflammation, adjuvant polyarthritis (Goldberg & Godfrey, 1980; Zinnes *et al.*, 1982) and type II collagen-induced arthritis (Billingham *et al.*, 1980; Probert *et al.*, 1984) the NSAIDs including indomethacin were found to be protective. In addition granuloma formation induced by cotton pellets is inhibited, although the data are far from unequivocal, with the results depending on the actual NSAID employed (Di Rosa, 1979). Finally, in the procedure most resembling our model, namely the rat air pouch, Willoughby *et al.* (1985) found that indomethacin at the higher dose of $3\,mg\,kg^{-1}\,day^{-1}$ showed protection against the loss of proteoglycan from rat femoral heads.

The results obtained with NSAIDs in the present mouse air pouch method are therefore substantially different from those determined in other animal models for anti-arthritic activity. Since it is now generally accepted that this class of compound does not modify the disease process in rheumatoid arthritis (Wright & Amos, 1980; Pinals, 1983), the lack of effect represents a positive feature of the model.

The steroids, dexamethasone and prednisolone, at doses which reflected their clinical efficacy, were the most active compounds in inhibiting proteoglycan and collagen loss as well as reducing granuloma formation. Similar results were recently obtained in a related model (De Brito *et al.*, 1986). However, decreased or no protection against cartilage degra-

dation was observed when dexamethasone administration was commenced on either day 4 or 7, although the reduction in granuloma formation remained very high. This dosing regime is more applicable to the patient situation where the disease is on-going. Although the observed reduction of cartilage destruction in the mouse air pouch could be solely due to an anti-inflammatory effect, an argument against this as the fundamental mechanism has recently been presented (Willoughby *et al.*, 1985). These latter authors suggested that the protection of the cartilage by dexamethasone could be due to anti-proliferative activity and the ability to inhibit cellular secretion of cytokines (e.g. interleukin-1).

There is no evidence that glucocorticosteroids alter the course of the disease process in rheumatoid arthritis (Huskisson, 1983). Therefore prophylactic dosing of steroids in the present air pouch model, as in the normal battery of animal models, can be accused of giving false positives as far as anti-rheumatic activity is concerned. However, administration at a time when the cartilage degradation process has been initiated, although producing marked anti-inflammatory activity, reduces or abolishes the protective effect.

For the purpose of this discussion, gold preparations, D-penicillamine, pyrithioxin, chloroquine and dapsone can be categorised as SAARDs. Chloroquine had no effect on the parameters measured, a similar result to that normally found in other anti-arthritic animal models (Pearson & Chang, 1978; Billingham & Davies, 1979). Surprisingly in view of the results of Sedgwick *et al.* (1984), D-penicillamine was without effect in our system. Although this SAARD has been shown to be active in the antigen-induced arthritis model in the rabbit (Hunneyball *et al.*, 1977), in all other animal systems it affords no protection (Arrigoni-Martelli & Bramm, 1975; Pearson & Chang, 1978; Lewis *et al.*, 1984). The result with the gold compounds was interesting in that inhibition of collagen loss was observed with GSTM but not auranofin. Despite a large number of studies with these two compounds in a variety of acute and chronic models of inflammation, there is no universal agreement on their relative activities. In general however, auranofin appears to inhibit most parameters, whereas GSTM is either inactive or far less potent (Billingham & Davies, 1979; Pearson & Chang, 1978; Walz *et al.*, 1982; Blodgett *et al.*, 1984). One notable exception is in rat collagen-induced arthritis where GSTM and not auranofin is active (Lewis *et al.*, 1984). Dapsone, which has not been widely examined in other models of inflammation, appeared to inhibit proteoglycan and the subsequent collagen loss.

Of the SAARDs tested, there is evidence that GSTM (O'Duffy & Luthra, 1984) and possibly D-penicillamine (Camp, 1981; Lyle, 1984) retard the development of radiological erosions in rheumatoid arthritis. Similar findings have not been demonstrated with the other compounds. The positive result found in the mouse air pouch with GSTM is encouraging, and it will be of interest to obtain data from radiological progression studies with auranofin when this compound has been employed clinically more widely.

Within the remaining class of anti-rheumatic drugs, the immunomodulators, both cyclophosphamide and methotrexate significantly protected against proteoglycan and collagen loss in the mouse air pouch, although there was no effect on granuloma weight. However, azathioprine produced no changes in the measured parameters. In other animal models, the three immunomodulators not only inhibit adjuvant-induced arthritis but also display anti-inflammatory activity (Hurd, 1973; Pearson & Chang, 1978). Whether these observed effects are due to a common mechanism is not clear, since azathioprine, cyclophosphamide and methotrexate all modulate T-lymphocyte function but apparently not in an identical way (Otterness & Chang, 1976; Koutounakis & Kapusta, 1976; Dimitriu & Fauci, 1979).

Whereas azathioprine and cyclophosphamide appear clinically to be superior to placebo (Bunch & O'Duffy, 1980), only the latter drug shows evidence of retardation of radiological erosions (Iannuzzi *et al.*, 1983). Clinical studies with methotrexate appear to give positive results, but no definite assessment of the remission-inducing potential has yet been undertaken (Willkens, 1985). Although two members of this class of compound gave positive results in the mouse air pouch, this is not surprising in view of their broad immunomodulating properties.

There are two general conclusions that can be made from the present study. Firstly, the actual experimental procedure is relatively simple and produces an acceptable degree of replication of data for a whole animal model. Secondly, the generated results appear to be predictive for anti-rheumatic activity (e.g. systemic gold, cyclophosphamide), whilst giving negative results for compounds which are only anti-inflammatory (e.g. indomethacin). Thus, the mouse air pouch model described here would appear in many ways to offer advantages over more classical methods, including the type II collagen-induced arthritis model as recently reviewed by Phadke *et al.* (1985).

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