Clin. exp. Immunol. (1986) **65**, 311–318

Acute phase protein changes in antigen-induced mono-articular arthritis in rabbits and mice

I. M. HUNNEYBALL, M. SPOWAGE, M. J. CROSSLEY, I. F. ROWE* & MARILYN L. BALTZ* *The Research Department, The Boots Company PLC, Nottingham, UK and *MRC Acute Phase Protein Research Group, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, London, UK*

(Accepted for publication 25 March 1986)

SUMMARY

Acute phase protein levels have been measured during the induction and progression of antigen-induced mono-articular arthritis in rabbits and mice. In rabbits there was a short lived elevation in serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) immediately following intra-articular injection which returned to baseline levels 10–12 days after the injection. In BALB/c mice, serum amyloid P-component (SAP) and the third component of complement (C3) were elevated after intra-articular injection, returning towards baseline levels 6 weeks after the injection. The levels of CRP and SAP correlated with the inflammatory changes in the joints during the acute phase of the arthritic response (7 days after intra-articular injection). During the chronic phase the levels of these acute phase proteins bore no relationship to the degree of connective tissue destruction.

Keywords acute phase proteins experimental arthritis rabbits mice

INTRODUCTION

Serum levels of acute phase proteins are elevated in patients with chronic inflammatory diseases such as rheumatoid arthritis (Hill, 1951; McConkey, Crockson & Crockson, 1972; Mallya *et al.*, 1982). However, whether the level of acute phase proteins reflects the extent of the underlying connective tissue destruction or merely reflects the severity of the acute inflammation is the subject of considerable discussion (Amos *et al.*, 1977; Scott *et al.*, 1984; Sjoblom *et al.*, 1984). Investigation of the changes in serum acute phase protein levels in animals during the induction and progression of experimental arthritis may help to shed light on this question.

It has been shown previously (Hunneyball & Stanworth, 1979) that during the induction of antigen-induced arthritis in rabbits there is a sharp rise in erythrocyte sedimentation rate (ESR) and serum haptoglobin concentration after intra-articular injection, reaching a peak at day 2–4 and returning to baseline levels by day 8. These studies have now been extended to include measurement of serum CRP levels during this period of time. In addition two acute phase proteins, serum amyloid P-component (SAP) (Pepys *et al.*, 1979; Baltz, Dyck & Pepys, 1985) and C3 (Hartveit, Borue & Thunold, 1973; Natsuume-Sakai, Motonishi & Takahashi, 1977) have been measured during the induction of experimental antigen-induced mono-articular arthritis in BALB/c mice. In addition to the changes in the level of these glycoproteins during progression of the arthritis in both of these

Correspondence to: Dr I. M. Hunneyball, The Research Department, The Boots Company PLC, Nottingham NG2 3AA, UK.

models, their relationship to the severity of the joint inflammation as judged by joint swelling measurements and/or histopathology, was also investigated.

MATERIALS AND METHODS

Induction of experimental arthritis in rabbits

Male New Zealand White rabbits weighing 2·0–2·5 kg were obtained from Hacking & Churchill Ltd. Experimental mono-articular arthritis was induced using a modification of the procedure described by Consden *et al.* (1971). Each animal was immunized on four occasions, at fortnightly intervals, by subcutaneous injection of 1 ml (divided) of an emulsion comprising 0·5 ml of a solution of ovalbumin (Sigma) in saline and 0·5 ml Freund's complete adjuvant (FCA, Difco). The first and second immunizations each contained 10 mg ovalbumin; the third and fourth immunizations each contained 5 mg ovalbumin. Ten days after the final immunization the animals were skin tested with 10 and 30 μg of both ovalbumin and tuberculin PPD (Evans Medical Ltd) to confirm the existence of delayed hypersensitivity to both antigens. After a further 12 days each animal received an injection of 0·5 ml of a 20 mg/ml solution of ovalbumin into the right knee joint.

At various times during the induction of arthritis, venous blood samples (both sequestered and clotted) were taken from the marginal ear vein.

Induction of experimental arthritis in mice

Female BALB/c mice, 10 weeks of age, were obtained from Bantin and Kingman Ltd. Arthritis was induced by a procedure similar to that described by Brackertz, Mitchell & Mackay (1977). Mice were sensitized by subcutaneous injection, into the flank, of 100 μl of an emulsion comprising 50 μl of a 2 mg/ml solution of methylated bovine serum albumin (Met-BSA; Sigma) in saline and 50 μl FCA to which had been added *Mycobacterium tuberculosis* (Weybridge) at a concentration of 1 mg/ml. A simultaneous intraperitoneal injection of 2×10^9 *Bordetella pertussis* organisms (Wellcome Pertussis Vaccine BP) was also administered. Seven days later, an identical set of injections was given. Fourteen days after the second immunization, 10 μl of a 10 mg/ml solution of Met-BSA in saline was injected into the left knee joint.

In some experiments, mice were killed at various times during the induction of the arthritis and blood samples obtained by cardiac puncture. In other experiments, mice were bled on a regular basis from the tail vein.

Assessment of the severity of the arthritis

The severity of the arthritis in rabbits was monitored by regular measurement of joint diameter using engineering callipers (Vernier). The degree of connective tissue destruction was assessed post-mortem by observations of both gross pathological changes and histological changes in the synovium and cartilage. All assessments were made blind to eliminate observer bias.

The severity of experimental arthritis in mice was assessed solely by histology. The inflammatory changes in the synovium and erosion of cartilage and bone were graded independently on a scale of 0–5.

Measurement of serum CRP levels

The concentration of CRP in rabbit serum samples was measured by single radial immunodiffusion, using purified CRP for calibration, as described previously (Rowe *et al.*, 1984).

Measurement of erythrocyte sedimentation rate

The erythrocyte sedimentation rate of sequestered rabbit blood samples was measured over a period of 1 h in Wintrobe tubes.

Measurement of SAP and C3

The concentrations of SAP and C3 in serum were measured by electroimmunoassay, calibrated with isolated pure protein, as described previously (Pepys, 1979; Baltz *et al.*, 1980). In view of the possible

complication arising from subclinical intercurrent infections in the mouse colony, normal control mice were bled at the same time as the arthritic animals and, in all cases, the values for C3 and SAP were expressed as the difference between the treated animals and normal controls.

Measurement of serum antibodies

Antibodies to ovalbumin and Met-BSA were measured in rabbit and mouse sera respectively by haemagglutination analysis using sheep erythrocytes sensitized with either ovalbumin or BSA. The antigens were coupled to the erythrocyte using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (ECDI; Sigma) as described by Mishell (1980).

RESULTS

Studies in rabbits

As to be expected, a moderate rise in CRP was observed during immunization of rabbits before induction of arthritis (Fig. 1). This was accompanied a by much less marked rise in ESR. Following intra-articular injection of antigen, there was a dramatic, rapid rise in both CRP and ESR, reaching a maximum 2 days after the injection and returning to baseline levels by day 10–12. By contrast, circulating anti-ovalbumin antibody levels fell after intra-articular injection, presumably due to sequestration of antibody within the injected joint, returning to pre-injection levels by day 5.

Joint swelling measurements revealed a marked increase in the diameter of the injected joint during the acute phase of the inflammatory response which, although decreasing to a certain extent, persisted until the animals were killed 12 weeks after intra-articular injection. Post-mortem examination of the injected joints of these animals revealed the presence of active erosive synovitis in all animals according to both macroscopic and histological assessments.

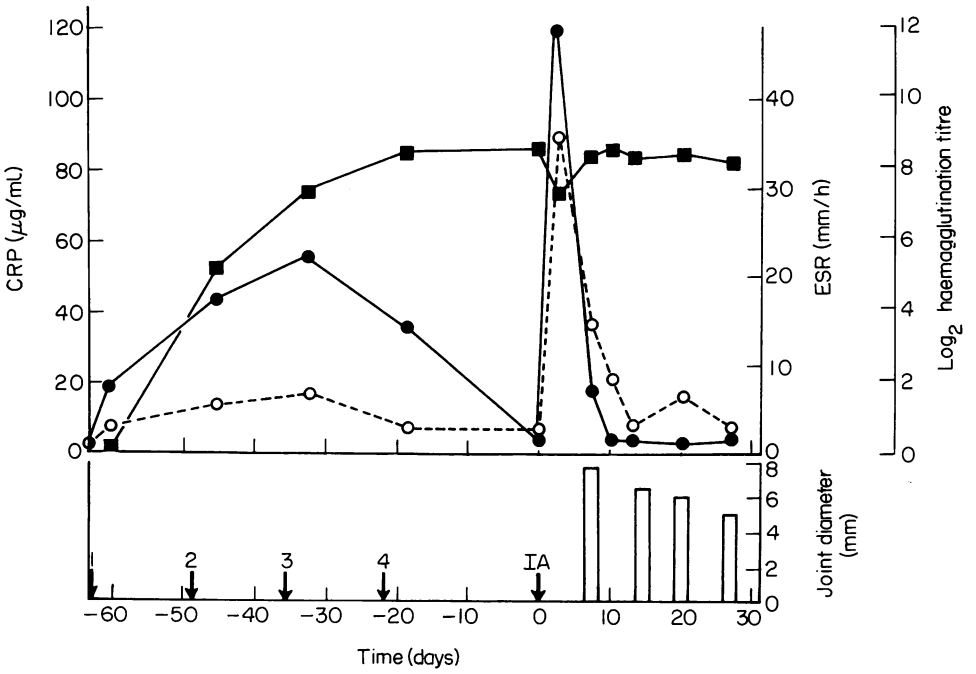

Fig. 1. Erythrocyte sedimentation rate (ESR: ○), serum C-reactive protein (CRP: ●) and serum anti-ovalbumin (log_2 haemagglutination titre ■) levels during the induction of experimental mono-articular arthritis in rabbits. Each point represents the mean of five animals. Arrows 1–4 indicate immunizations; IA, intra-articular injection.

Table 1. Correlation between peak serum CRP level (Day 2) and ESR, joint swelling, macroscopic score and histological score in rabbits with antigen-induced mono-articular arthritis

		Correlation coefficient
Day 2 CRP vs:	Day 2 ESR	0·813*
	Day 7 joint swelling	0·836*
	Day 10 joint swelling	0·797*
	Day 14 joint swelling	0·198
	Day 29 joint swelling	0·339
	Day 43 joint swelling	0·212
	Day 80 joint swelling	0·017
	Macroscopic score (Day 84)	−0·038
	Histological score (Day 84)	0·267

$n = 5$.
* $P < 0{\cdot}005$.

The peak serum CRP level at day 2 correlated well with the ESR at the same time (Table 1). Furthermore, peak serum CRP correlated well with swelling of the joint at day 7 and to a lesser extent at day 10. However, there was no correlation with joint swelling at times beyond day 10, nor was there any correlation between peak CRP level and the level of connective tissue destruction as judged by either macroscopic or histological indices.

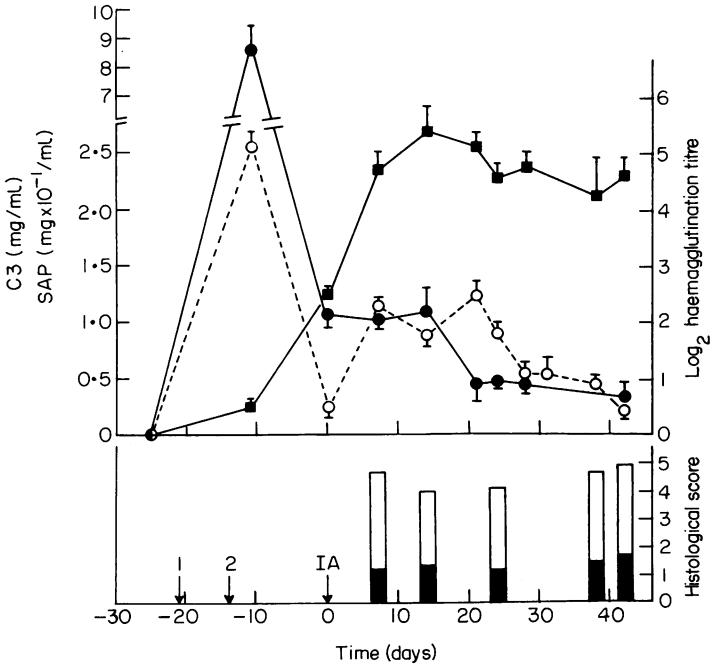

Fig. 2. Circulating SAP (●), C3 (○) and anti-BSA antibody (■) levels during the induction of chronic experimental mono-articular arthritis in BALB/c mice. The results are expressed as the difference between treated and normal mice (SAP and C3 values in normal mice were 85–100 μg/ml and 1·35–1·85 mg/ml respectively). The histogram represents the total joint histology score, the open sections representing synovitis and the closed sections representing erosions. Each point represents the mean ± s.e.m. of 9–10 mice. Arrows 1 and 2 indicate immunizations. IA, intra-articular injection.

Table 2. Correlations between acute phase proteins (APP) and histological assessment of disease in BALB/c mice with experimental mono-articular arthritis

		SAP	C3
Experiment 1	Day 7 APP vs Day 7 histology	0·778*	0·108
	Day 14 APP vs Day 14 histology	−0·185	−0·127
	Day 28 APP vs Day 28 histology	−0·008	−0·586
	Day 42 APP vs Day 42 histology	−0·572	0·065
Experiment 2	Day 7 APP vs Day 42 histology	0·626†	0·217
	Day 42 APP vs Day 42 histology	0·226	0·248

$n = 8$–10.
*$P < 0{\cdot}001$.
†$P < 0{\cdot}005$.

Studies in mice

The first experiment with BALB/c mice was designed to investigate the changes in acute phase protein levels during the progression of chronic arthritis. Mice were killed at various stages during the study; blood was removed by cardiac puncture for serological estimations and the knee joints removed for histology. Normal mice were bled at each time point to provide the baseline value for SAP and C3 for the colony. This ranged from 85–100 μg/ml for SAP and from 1·35–1·85 mg/ml for C3 during the course of the experiment.

Both SAP and C3 showed a dramatic rise after immunization and rapidly returned towards baseline values (Fig. 2). C3 levels reached preimmunization levels before intra-articular injection whereas SAP remained somewhat elevated. Following intra-articular injection, C3 levels rose slowly, reaching a maximum at day 21 and returning to baseline levels by day 42. SAP levels remained elevated until day 14 after which they declined, although they had not returned to baseline levels by day 42. By contrast, circulating levels of anti-BSA antibody showed a much slower rise reaching a maximum at day 14.

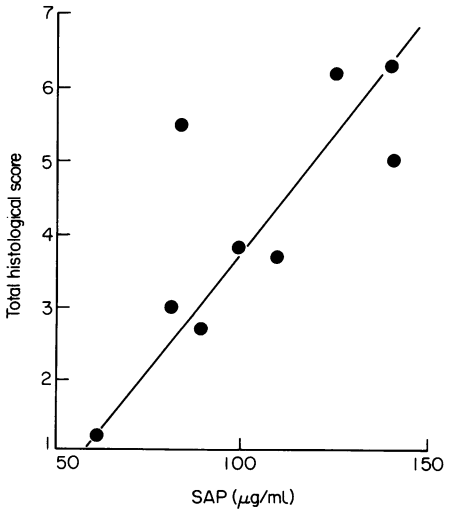

Fig. 3. Correlation between SAP at day 7 and total histological score at day 7. SAP levels are expressed as the difference between arthritic animals and normal controls (normal SAP levels were 85–100 μg/ml). Correlation coefficient = 0·778

Histological examination of the knee joints of these animals revealed the presence of active synovitis in all animals, with erosive changes detectable as early as day 7. As reported previously (Hunneyball, Crossley & Spowage, 1986), synovitis followed a biphasic course whereas erosions showed a rapid onset but slow progression. As shown in Table 2, serum C3 levels showed no positive correlation with joint histology at any stage during the study. In contrast, SAP concentrations determined on day 7 showed some correlation with the joint histology score at that time (Fig. 3), although as the arthritis progressed this correlation was not maintained (Table 2).

A parallel experiment was performed in which the animals were bled sequentially from the tail vein throughout the experiment and killed on day 42. The changes in SAP, C3 and anti-BSA antibody observed in this experiment were essentially the same as those observed in the first experiment. The histological scores of sections taken on day 42 showed some correlation with SAP level at day 7 but not at day 42 (Table 2).

In view of the rapid rise and fall in ESR and CRP observed in the rabbit study, we decided to look in detail at the changes in C3 and SAP occurring during the first 8 days after induction of arthritis. Both SAP and C3 rose following intra-articular injection of antigen reaching levels similar to those seen in Fig. 2. In neither case was there a dramatic rise and fall as observed with CRP following intra-articular injection in rabbits.

DISCUSSION

The antigen-induced model of mono-articular arthritis in rabbits, originally described by Dumonde & Glynn (1962), bears a strong resemblance to rheumatoid arthritis in terms of its synovial histology, gross pathology and drug responsiveness (Glynn, 1968; Hunneyball, 1984). The comparable mouse model shares many of the features of the rabbit model. Histologically, both models have relatively distinct acute and chronic phases (Steinberg *et al.*, 1973; Hunneyball *et al.*, 1986); the acute phase is characterized by polymorphonuclear (PMN) cell infiltration into the synovium whereas the chronic phase is characterized by a predominantly mononuclear cell infiltrate. In both species the erosion of cartilage and bone results primarily from the action of synovial pannus and erosive disease persists virtually indefinitely following a single intra-articular injection.

The acute phase response in other animal models of arthritis has been investigated previously. Induction of adjuvant arthritis in rats produces a biphasic elevation of acute phase protein levels corresponding to the primary and secondary phases of the arthritis (Billingham, 1983). A similar rise in acute phase protein levels has been demonstrated during the development of type II collagen-induced arthritis in rats (Billingham *et al.*, 1981). In MRL/l mice which have a spontaneous lymphoproliferative disorder with an arthritic component, a very marked increase (up to 400 μg/ml) in SAP level was observed coincident with the appearance of clinical disease (Rordorf *et al.*, 1982). In contrast, NZB/W mice showed no change in SAP during the appearance of the spontaneous lupus erythematosus-like syndrome (Rordorf *et al.*, 1982), a situation closely resembling that with regard to CRP in SLE (Pepys & Baltz, 1983).

Interpretation of the results from studies with MRL/l mice is complicated by the influence of the lymphoproliferation and the other autoimmune manifestations such as polyarteritis and lupus nephritis which develop at the same time as the arthritic lesions (Murphy & Roths, 1978; Andrews *et al.*, 1978; Hang, Theofilopoulos & Dixon, 1982). Similar complicating factors exist in the adjuvant arthritis model. In contrast, the antigen-induced models of arthritis do not contain a significant extra-articular component and hence have advantages over these other models for investigating the relationships between acute phase proteins and erosive progression.

In the present study, the observed elevation in CRP in rabbits within the first 5 days after intra-articular injection was identical to that reported previously for haptoglobin (Hunneyball & Stanworth, 1979). In agreement with these observations, the ESR was also elevated during the same period of time but took longer to return to baseline levels. These results, together with those of Hunneyball & Stanworth (1979), indicate that in this model of arthritis in rabbits, changes in acute phase proteins reflect the severity of the acute phase of the arthritis but bear no relationship to the

progression of the disease in terms of either the joint swelling or the histological index of erosive disease activity.

Although both SAP and C3 were raised in mice during the acute phase of the arthritis they showed different temporal profiles and whereas SAP showed some relationship to the severity of the arthritis during the acute phase, C3 bore no such relationship at any time during the study. The difference between these two proteins may be explained by differences in their site of synthesis, i.e. SAP appears to be synthesized exclusively in the liver (Baltz *et al.*, 1985), whereas C3 may also be synthesized by macrophages at the inflammatory foci (Colten, 1976; Whaley 1980; Lachmann & Peters, 1982). However, this may not be the only explanation and differences in the catabolic rates for the two proteins may also play a role.

Although the factors responsible for regulating the synthesis of acute phase proteins have yet to be fully elucidated, interleukin-1 (IL-1) has been postulated to play an important role (Pepys & Baltz, 1983). Detection of IL-1 in joint fluids of patients with erosive arthropathies (Bendtzen *et al.*, 1985) has implicated this mediator as a marker of the chronic phase of arthritis. Therefore, the lack of correlation between serum acute phase protein levels and the chronic phase of arthritis in our animal models merits discussion with respect to the role of IL-1.

One possible explanation is that the elevated levels of serum acute phase proteins in both rabbit and mouse arthritis models are a consequence of IL-1 production by Kupffer cells in the liver rather than IL-1 production within the arthritic joint. The observed increases in acute phase protein levels after intra-articular challenge could reflect the release of antigen or immune complexes from the injected joint into the circulation. These complexes may then stimulate IL-1 production by liver Kupffer cells and consequently hepatocyte acute phase protein synthesis. The higher levels of acute phase proteins in the acute phase as opposed to the chronic phase in both rabbit and mouse may therefore reflect antigen levels in the arthritic joints. In fact, our data correlate well with the antigen levels in the joints of arthritic rabbits and mice observed by Van den Berg *et al.* (1982) and van Beusekom *et al.* (1982).

Alternatively, acute phase protein synthesis may be stimulated directly by IL-1 production within the arthritic joint. However, if this is the case some explanation is required to account for a diminution in IL-1 synthesis in the chronic phase of the arthritis. Clearly, further studies are required before any firm conclusions can be drawn.

In conclusion, we have shown that acute phase protein levels are elevated following the induction of antigen-induced experimental mono-articular arthritis in rabbits and mice. In rabbits this response is very short lived, whereas mice produce a more protracted response. Although there is evidence for correlation between certain acute phase proteins and the severity of the arthritis during the acute phase, in the chronic phase these proteins appear to bear no relationship to the degree of connective tissue destruction in these models.

We are grateful to Dr M. C. Lancaster and the staff of the Pathology Department of The Boots Company PLC, Research Department, for the preparation of the histological sections.

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