Immunology 1984 **52** 733

The acute phase response in parasite infection. *Nippostrongylus brasiliensis* in the mouse

L. R. LAMONTAGNE*, J. GAULDIE[1], A. D. BEFUS[1], K. P. W. J. MCADAM[2], MARILYN L. BALTZ[3] & M. B. PEPYS[3] [1]*Department of Pathology, McMaster University, Hamilton, Ontario, Canada,* [2]*Divisions of Geographic and Experimental Medicine, Tufts-New England Medical Center Hospital, Boston, Massachusetts, U.S.A., and* [3]*Immunological Medicine Unit, Royal Postgraduate Medical School, London*

Accepted for publication 6 March 1984

Summary. Systemic inflammatory reactions are a prominent feature of many parasitic infections and the cellular and humoral components of the acute phase reaction may have an impact on the host-parasite relationship. We examined serum changes of four acute phase reactants: α1-proteinase inhibition (α1Pi); complement C3; serum amyloid A protein (SAA); and serum amyloid P component (SAP), in mice undergoing a primary infection with *Nippostrongylus brasiliensis*. SAA and SAP showed changes within the first 2 days of infection indicating the presence of an acute phase response asociated with inflammation in the lung. α1Pi and C3 serum levels were not altered. However, all four acute phase reactants were synthesized in greater amounts by primary cultures of hepatocytes taken from infected animals at this time. Subsequently, as parasite-mediated inflammatory changes occur in the gut, both serum and hepatocyte cultures demonstrate an acute inflammatory response in all four reactants. It is proposed that the early reaction between parasites and macrophage/monocyte lead to the release of a mediator of inflammation which initiates the hepatocyte response. In this infection, at least one of the APR is shown to localize to the site of inflammation influencing the host–parasite relationship.

*Presently at Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02111, U.S.A.

Correspondence: Dr Jack Gauldie, Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5.

INTRODUCTION

Parasite infections often result in marked tissue destruction as the parasites migrate within the host. Systemic inflammatory reactions are a prominent feature of many parasitic diseases (Befus, Egwang & Gauldie, 1983; Otteson, 1980; Warren, 1982) and it is probable that events associated with the early acute phase of infections may have a significant impact on the host–parasite relationship.

The effect of parasites and parasite-derived products on the activation and inhibition of the humoral pathways of inflammation (e.g. the coagulation, fibrinolytic and complement systems) has recently been reviewed (Leid & Williams, 1979). A prominent feature of the host humoral acute inflammatory response is the rapid increase in the serum concentration of a number of proteins, collectively known as acute phase reactants (APR) (Koj, 1970, 1974; Gordon, 1976; Kindmark, 1976; Pepys & Baltz, 1983), including α1-proteinase inhibitor (α1Pi), serum amyloid A protein (SAA), fibrinogen, C-reactive protein (CRP), ceruloplasmin, α1-acid glycoprotein, haptoglobin and the third component of complement, C3 (Whicher, 1981; Kushner & Feldmann, 1978; McAdam & Sipe, 1976; Koj & Regoeczi, 1978). Other acute

phase proteins include serum amyloid P component (SAP) of mouse (Pepys *et al.*, 1979) and α2-macroglobulin of rat (Hudig & Sell, 1978). While the physiologic role of some APR in the inflammatory reaction is appreciated (e.g. fibrinogen, α1Pi, haptoglobin and C3), the impact of acute phase reactants on the immune response and the non-genetic component of innate resistance/susceptibility to infections is only now becoming apparent.

SAA has been shown to inhibit the specific induction of antibody to T cell-dependent antigens *in vitro* (Benson & Aldo-Benson, 1979) by affecting T cell–macrophage interaction (Aldo-Benson & Benson, 1982), while α1Pi has been shown to suppress the *in vitro* and *in vivo* primary antibody response (Arora, Miller & Aronson, 1979). Reports have claimed that CRP is suppressive *in vitro* to both T and B lymphocyte reactions (Kinsella & Fritzer, 1980; Mortensen, 1979, 1982), however, the findings with T cell modulation have not been reproduced by other groups and have since been modified (James, Hansen & Gewurz, 1981a, b; Vetter *et al.*, 1983; Pepys & Baltz, 1983).

The regulatory function of APR on immunological and inflammatory responses suggests that the study of the induction of APR synthesis in parasitic infections would aid in understanding the host parasite relationship. Using the nematode *Nippostrongylus brasiliensis*, we have characterized the acute phase response in the mouse with respect to the induction of synthesis of the acute phase reactants α1Pi, SAA, SAP and C3. *N. brasiliensis* induces marked inflammatory reactions in the lungs and the small intestine at 2–3 days and 6–12 days post-infection, respectively (Croll & Ma, 1978; Symons, 1965; Wescott & Todd, 1964). Our results show that this parasitic infection leads to the early induction of enhanced synthesis by hepatocytes of all four APR, but paradoxically, this increased synthesis is not necessarily reflected in a marked elevation of serum levels for all APR.

MATERIALS AND METHODS

Animals

CBA/J female mice from The Jackson Laboratories Inc., Bar Harbor, ME, were fed *ad libitum* and maintained under a 12 hr diurnal light cycle. Animals were used at 14–16 wk of age.

Nippostrongylus brasiliensis *infection*

N. brasiliensis was maintained by serial passage in Sprague-Dawley rats, 150–250 g (Biobreeding, Ottawa, Canada) (Befus, Johnston & Bienenstock, 1979). Infective third stage larvae (L3) were obtained by the hatching of nematode eggs and *in vitro* maturation of L1 to L3 larvae (Jennings, Mulligan & Urquhart, 1963). The larvae were decontaminated with sodium hypochlorite-antibiotic treatment similar to that described by Wescott & Todd (1964). Microbiological studies indicated this procedure considerably reduced any microbial contamination of L3. Mice were infected with 500 L3 larvae by a subcutaneous injection at the back of the neck. This consistently resulted in 150–250 adult worms in the small intestine by days 5–6. Serum samples were obtained at various times by cardiac puncture and tissues were removed, fixed in buffered formalin and embedded in paraffin.

Immunohistochemical localization of α1-protease inhibitor

Mouse α1Pi was identified in tissues using a horseradish peroxidase-conjugated technique that we have previously described (Gauldie *et al.*, 1980). Briefly, liver and lung sections (4 μm) were incubated, in successive steps, with sheep anti-mouse α1Pi, rabbit anti-sheep IgG and horseradish peroxidase-conjugated goat anti-rabbit IgG. The presence of α1Pi was detected by a brown colour reaction subsequent to the addition of the substrate 3,3′-diaminobenzidine tetra-HCl (ICN Pharmaceuticals, Plainview, NY) and H_2O_2. Controls for specificity of immunohistochemical reactions consisted of incubations with buffer only, non-immune sheep serum or sheep anti-mouse α1Pi antiserum absorbed with purified mouse α1Pi.

In vitro *synthesis of acute phase reactants by hepatocyte cultures*

Hepatocytes were isolated from control mice and infected mice by a two-step perfusion procedure similar to that described by Deschenes, Valet & Marceau (1980). The stock perfusate solution consisted of a Ca^{2+}-free HEPES buffer (100 mM HEPES, 67 mM KCl, 1·4 M NaCl, pH 7·3). In the first part of the perfusion procedure, the liver was perfused *in situ* at a rate of 6·6 ml/min, for 6 min, with a 1/10 dilution of the above stock HEPES buffer. The second step consisted of perfusing the liver, at the same rate and volume, with a freshly prepared stock collagenase solution (collagenase, Sigma type I; 54·4 mM $CaCl_2.2H_2O$, 0·12 M NaCl, 10 mM HEPES, pH 7·3) diluted 1/10 with HEPES buffer. The collagenase content ranged from 0·025 to 0·05% and had to be adjusted for optimal

digestion for each batch of collagenase. In both instances, the perfusate was maintained at 37° and a single-pass, non-recirculating perfusing system was used. Upon completion of the digestion, the liver cells were collected in calcium-free HEPES buffer and viable hepatocytes were obtained by sedimenting twice under unit gravity and at room temperature. Viability was assessed by staining with ethidium bromide/fluorescein diacetate (Takasugi, 1971).

Cultures were initiated by adding 5 ml of hepatocyte suspension (3×10^6 viable cells) in William's E medium (Gibco, Grand Island, NY) (supplemented with L-glutamine 2 mM), HEPES (10 mM), agammaglobulinemic horse serum (10%), dexamethasone (1 μM) and penicillin/streptomycin (1%) to 25 cm tissue culture flasks which had previously been coated with rat tail collagen (Sirica *et al.*, 1979). The hepatocytes were allowed to adhere for 1·5 hr at 37°. Non-adherent, dead/damaged hepatocytes and non-parenchymal cells were washed off with 5 ml of medium and 10 ml of fresh medium added. The cultures were maintained at 37° and 6% CO_2 for 24 hr with no significant loss of viability. At various time intervals, a small aliquot (100 μl) was removed from each culture and frozen until further analysis.

Estimation of α1Pi, SAA, SAP and C3

α1-protease inhibitor. Serum α1Pi levels were determined by nephelometry using an automated Fluoronephelometer (Technicon Instruments) with monospecific sheep anti-mouse α1Pi. The reactivity and monospecific nature of the antiserum have been previously described (Lamontagne, Gauldie & Koj, 1981). In hepatocyte culture supernatant, α1Pi was estimated by an enzyme-linked immunosorbent assay (ELISA). The IgG fraction of rabbit anti-mouse α1Pi (5 g/ml) was used to coat microtitre plates (Nunc, Gibco Laboratories, NY). Purified α1Pi standards or supernatant samples were added and the amount of α1Pi bound was measured using sheep anti-mouse α1Pi followed by rabbit anti-sheep IgG conjugated with horseradish peroxidase. The assay had a quantitative range of 10–500 ng α1Pi/ml.

Serum amyloid A protein. SAA concentrations in serum and in hepatocyte culture supernatants was estimated using a radioimmunoassay as previously described (Sipe *et al.*, 1977). In brief, 10 μl of serum or 200 μl of supernatant were lyophilized and treated with 1 ml of 10% formic acid for 18 hr at 56°. Aliquots of this material were then lyophilized and assayed by solid-phase single antibody radioimmunoassay using affinity purified rabbit antibody to murine AA protein.

Complement C3 and serum amyloid P component. SAP and C3, in serum, were estimated by electroimmunoassay as previously described (Pepys, 1979). SAP and C3 in hepatocyte culture supernatants were estimated by a solid-phase immunoradiometric assay using polystyrene balls coated with affinity purified antibody to SAP or C3 and ^{125}I-labelled antibody to the same antigens as tracer. Sensitivity in both assays was 5 ng/ml of supernatant.

RESULTS

N. brasiliensis-induced inflammation

Infection of the mouse with *N. brasiliensis* was initiated by subcutaneous injection of 500 L3 at day 0. By day 2, L3 migrate via blood and lymph to the lungs where they cause severe local inflammation and destruction of the alveolar septa with hemorrhage and edema. The parasites mature into fourth stage larvae in the lung and migrate to the gastrointestinal tract via the trachea and oesophagus. They are present in the gut from day 3 onwards and an acute inflammatory reaction develops in the small intestine by day 6 causing edema and villous atrophy with crypt-hyperplasia. In the gut, the larvae mature and adult nematodes produce maximum numbers of eggs between days 7–9 and are subsequently expelled by day 14.

Serum levels of α1Pi, SAA, C3 and SAP

α1Pi. During the initial 5 days following infection of mice with *N. brasiliensis*, the serum levels of α1Pi fluctuated within the normal range with a slight increase in serum levels at day 2 (Fig. 1a), a time corresponding to lung inflammation. Subsequently, beginning at day 8, there was a gradual increase in α1Pi serum levels to a maximum of 120% of normal adult by day 10 post-infection, during which time there is considerable gastrointestinal inflammation. The α1Pi levels subsequently subsided to normal as the parasite is expelled from the gut (day 14).

C3. C3 serum levels fluctuated within the normal range up to approximately day 8 post-infection (Fig. 1a). However, during the gut inflammation stage, there was a marked C3 serum acute phase response

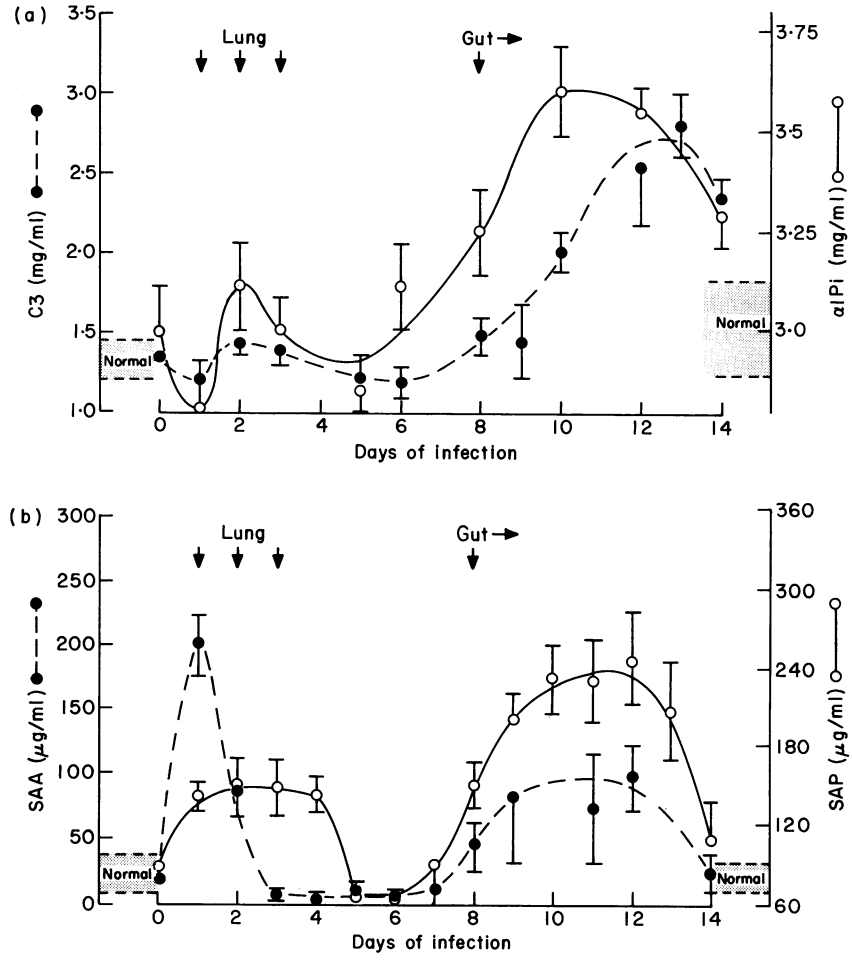

Figure 1. Some levels of acute phase reactants during infection of mouse with *Nippostrongylus brasiliensis*. *Arrows* indicate occurrence of inflammatory pathology in various tissue sites. Normal serum levels are indicated. Values represent mean ± SEM of five mice sera per data point. (a) Complement C3(●); α1-proteinase inhibitor (○). (b) Serum amyloid A (●); serum amyloid P component (○).

with approximately a two-fold increase over normal at day 12 with a subsequent return to normal at day 15.

SAA. There was a marked increase in SAA serum levels (13-fold) in the early stages of the infection with the maximum occurring at 1–1·5 days post-infection (Fig. 1b). Subsequently, there was a rapid decline to normal levels by day 3 and at day 8, there was a gradual increase in serum levels with an eight-fold increase at approximately day 12, returning to normal by day 14.

SAP. Serum amyloid P component showed a significant increase in serum levels (two-fold) at approximately 2·5 days post-infection, corresponding to the lung inflammation stage (Fig. 1b). There was a subsequent decline to normal at days 5–7. At day 8, there was a marked increase in SAP serum concentration (three-fold), peaking at day 12 post-infection and returning to normal by day 14.

In vitro hepatocyte synthesis of α1Pi, SAA, C3 and SAP

α1Pi, SAA, C3 and SAP were quantified in culture supernatants of hepatocytes isolated from mice at various times post-infection. For α1Pi, SAA and SAP,

there was a significant increase in synthetic output at day 2 (Table 1), with a subsequent decrease in synthesis by day 5. There was then a progressive increase in synthetic output of all four APR by day 10 post-infection.

Tissue distribution of α1Pi

We have previously used immunohistochemical means to detect α1Pi in mouse liver tissue (Gauldie *et al.*, 1980). We used the same technique to examine other tissues for the presence of α1Pi to determine the tissue distribution of this important acute phase reactant during the infection. Other proteins were not examined and may be distributed in a different manner.

Liver. The localization of α1Pi within the cytoplasm of hepatocytes from normal mice has been described in previous studies from this laboratory (Gauldie *et al.*, 1980) and is shown in Fig. 2a. However, at the onset of an acute inflammatory reaction, such as during the lung stage of *N. brasiliensis* (day 2), there is a remarkable increase in the number of hepatocytes staining intensely for α1Pi (Fig. 2b). In contrast to the normal liver, such intense staining hepatocytes were found surrounding the central vein while the periportal areas contained significantly fewer positive hepatocytes.

Lung. Normal uninfected lung did not show significant interstitial staining for α1Pi and all alveolar macrophages in control animals and in infected animals at times other than day 2 after infection were negative for cytoplasmic α1Pi. In contrast, when we examined the lung at day 2, the time of maximal inflammation and parasite localization, there was obvious staining for interstitial α1Pi. In addition, alveolar macrophages, particularly those adjacent to the nematode, stained intensely for α1Pi (Fig. 3). These same cells were strongly positive for non-specific esterase which was totally inhibited by fluoride ion, confirming their morphologic identity as macrophages.

DISCUSSION

The acute inflammatory response, as characterized by an increase in the plasma concentration of four acute phase proteins, has been investigated during the course of a parasitic infection. Infection of mice with the nematode *N. brasiliensis* is an excellent experimental model of inflammation because of the remarkable and consistent pathology associated with the migration of the parasites within the host. Since these studies were carried out in mice undergoing a primary infection, the inflammatory response during the early stage (lung phase) of the infection may be considered to be initiated by a traumatic non-immune episode while that seen at a later stage (gut phase) likely has both non-specific and immune initiation.

During a primary infection with *N. brasiliensis*, the inflammatory reactions in the lungs (day 2) and the

Table 1. *In vitro* synthesis of acute phase proteins by hepatocytes taken from mice infected by *Nippostrongylus brasiliensis* (ng/ml, mean ± SEM)

Days of infection	α1-Proteinase inhibitor ng/ml	Serum amyloid A (SAA) ng/ml	Serum amyloid P (SAP) ng/ml	C3 ng/ml
Non-infected $n=10$	393 ± 39	24·1 ± 6·8	10·2 ± 3·2	63·5 ± 15·6
Day 2 $n=9$	570 ± 34	49·2 ± 5·7	32·5 ± 8·3	77·0 ± 12
Day 5 $n=5$	325 ± 116	46·4 ± 3·0	11·4 ± 4·4	55·1 ± 14·4
Day 8 $n=5$	1048 ± 102	89·0 ± 16·8	88·8 ± 4·2	331 ± 161
Day 10 $n=5$	780 ± 103	211 ± 13	131 ± 10·3	581 ± 161
Significance day 2 *vs* non-infected	$P<0·005$	$P<0·01$	$P<0·01$	NS

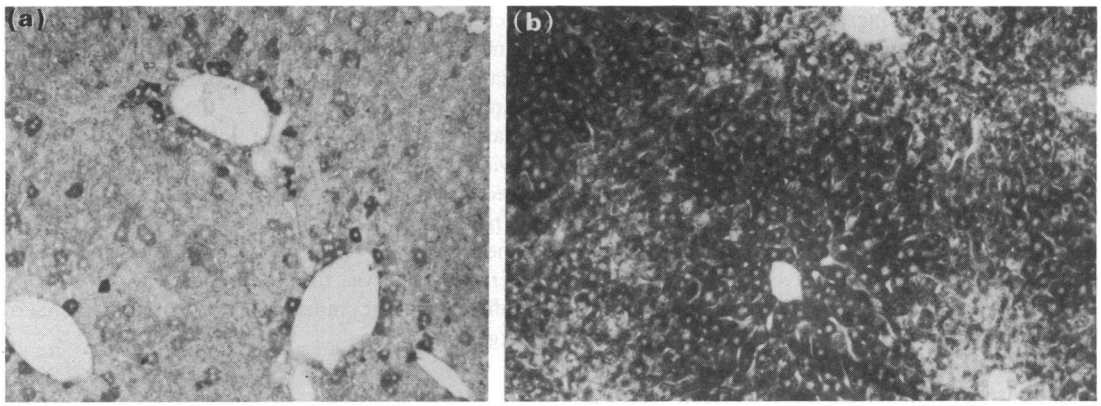

Figure 2. Mouse liver stained with antiserum to α1-proteinase inhibitor and horseradish peroxidase conjugate × 100. (a) Normal uninfected mouse. (b) Mouse infected with *N. brasiliensis* 2 days previously.

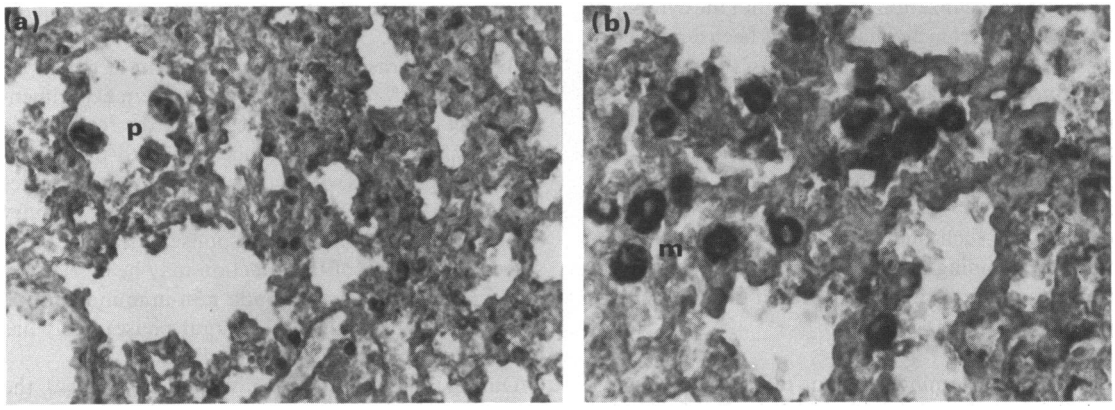

Figure 3. Mouse lung stained with antiserum to α1-proteinase inhibitor and horseradish peoxidase conjugate. Mouse infected 2 days previously with *N. brasiliensis*. (a) Magnification × 100 showing parasite in alveolus (P). (b) Magnification × 400 showing intense alveolar macrophage staining (M).

small intestine (days 6–12) were reflected in a corresponding increase in the serum levels of SAA and SAP (Fig. 1a). Paradoxically, the serum levels of α1Pi and C3 during lung inflammation (Fig. 1b) were not suggestive of increased hepatic synthesis. There was, nevertheless, a significant increase in the serum concentrations of these two acute phase proteins during the intestinal inflammation, similar to that observed with SAA and SAP.

Although there was not a marked increase in the serum levels of α1Pi during lung inflammation, there was an acute phase α1Pi response as indicated by the increase in output by hepatocytes at day 2 (+45%, Table 1) and in addition, as shown in Fig. 2b, most hepatocytes in the liver lobule stain intensely for cytoplasmic α1Pi as compared to normal unstimulated liver (Fig. 2a). The specific localization of α1Pi in the cytoplasm of hepatocytes together with the hepatocyte synthesis data suggests that synthesis as opposed to uptake of denatured or altered α1Pi is occurring in the liver. The validity of this assumption is supported by similar studies with CRP (Kushner & Feldman, 1978), SAA (Benson & Kleiner, 1980) and SAP (Baltz, Dyck & Pepys, 1980), where in the normal animal these APR are not detected in hepatocytes by immunohistochemistry. However, under conditions of inflammation where increased synthesis occurrs, there is a corresponding preponderance of hepatocytes with cytoplasmic staining for these APR.

The apparent lack of significant increased serum

levels of α1Pi may reflect sequestration and utilization at the site of inflammation. The demonstration of α1Pi in infected lung tissue by immunohistochemical means (Fig. 3) is consistent with this hypothesis. Moreover, preliminary studies, using transferred ^{125}I-labelled purified α1Pi, indicate a significant increase of this protease inhibitor in lung tissue and bronchiolar lavage fluid at day 2 post-infection (data not shown).

Although C3 utilization/sequestration was not investigated in these studies, the activation of C3 by stage specific *N. brasiliensis* larvae may account for the apparent lack of increase C3 serum levels during lung inflammation. Previous studies (Mackenzie *et al.*, 1980) have demonstrated C3 on the surface of this nematode and we (Egwang, Gauldie & Befus, 1983) have recently demonstrated the requirement for complement fixation in *in vitro* cell-mediated helminthotoxicity in this model. Scharfstein, Barcinski & Leon (1982), using mice infected with the protozoan parasite *Trypanosoma cruzi* (T strain), have observed a concomitant increase in the serum levels of SAP and C3, 7–11 days post-infection. However, as in this study, there was no rise in serum C3 during the early stages of the infection, at a time when SAP concentrations were elevated.

With all four APR, there was a significant increase in *in vitro* synthetic output by hepatocytes isolated at times corresponding to lung and small intestine inflammation (Table 1). This data is consistent with a previous study (Courtoy *et al.*, 1981) in which simultaneous increases in the serum concentration of various APR, during turpentine-induced inflammation, were observed.

Koj (1974) first suggested that a 'hormone-like' messenger released at the site of inflammation interacts with the liver and induces an increased synthesis of the acute phase reactants. Recent studies have shown that the induction of SAP (Le, Muller & Mortensen, 1982) and SAA (Rosenwasser, Dinarello & Rosenthal, 1979; Selinger *et al.*, 1980; Sipe *et al.*, 1979; Sztein *et al.*, 1981) is caused by a monocyte-derived product believed to be inseparable from interleukin-1 (IL-1) or lymphocyte-activating factor (Oppenheim *et al.*, 1982). On the basis of biological activities and biochemical properties, the molecule(s) shares identity with leukocyte endogenous pyrogen (Bernstein & Walsh, 1978; Murphy, Simon & Willoughby, 1980) and leucocyte enodgenous mediator (Merriman, Pulliam & Kampschmidt, 1977). Our results showing the early induction of SAP and SAA in serum and all four APR in hepatocyte culture indicate the presence of an IL-1-like activity. In concurrent studies (Lamontagne, Stadnyk, Gauldie & Jenkins, manuscript submitted) we have shown that the alveolar macrophage taken from mice infected two days previously are actively secreting significant amounts of IL-1. The suggestion is that this IL-1 or a similar molecule is in part if not wholly responsible for the hepatic acute phase response.

The hepatocyte culture data (Table 1, day 5) shows that the transient return to normal serum levels of SAA and SAP at days 5–7 post-infection (Fig. 1b) is most likely due to decreased synthesis by hepatocytes coupled with normal rapid catabolic disappearance rather than utilization/sequestration at the site of inflammation. The synthetic output of α1Pi and C3 at day 5 is also consistent with this hypothesis.

We have therefore demonstrated that the induction of increased synthesis of acute phase reactants is an integral constituent of the humoral component of the host–parasite relationship. It will be important to determine the impact of the acute phase reactants on this relationship, and more specifically, how these interact with parasites and/or parasite-derived products as well as modulate the host's immune responses. It will also be of significance to investigate if there is any relationship between the presence of cytoplasmic α1Pi in alveolar macrophages at day 2 post-infection (Fig. 3) and the lack of helminthotoxicity of these cells at that time, despite their activated state as measured by other parameters (Egwang, Gauldie & Befus, 1983).

These studies also demonstrate that merely quantifying serum levels of some acute phase reactants may not necessarily be indicative of an ongoing acute phase reaction. Therefore, the determination of an acute phase response should include an examination of the liver for evidence of synthesis and/or secretion of acute phase reactants especially in cases where a dichotomy exists between pathological evidence of inflammatory foci and lack of increased serum levels of acute phase reactants. On examination of the alterations in serum levels and hepatocyte output, SAA and SAP are more sensitive and notable indicators of acute inflammation *in vivo* than is either α1Pi or C3.

This model of parasite-induced inflammation will also allow us to further investigate the role of various acute phase reactants on the initial host–parasite interaction and the subsequent development of immunity. Although the biological functions of α1Pi and C3 are reasonably understood, the roles for SAA and

SAP have not been delineated. This model is amenable to such studies.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council of Canada, National Institutes of Health, U.S.A., and the Medical Research Council, U.K.

The expert technical assistance of Liz Jenkins and editorial assistance of Joan Hickey is gratefully acknowledged.

REFERENCES

ALDO-BENSON M.A. & BENSON M.D. (1982) SAA suppression of immune responses *in vitro*: evidence for an effect on T-cell–macrophage interaction. *J. Immunol.* **128,** 2390.

ARORA P.K., MILLER H.C., & ARONSON L.D. (1979) Alpha-1-antitrypsin is an effector of immunological stasis. *Nature (Lond.)* **274,** 589.

BALTZ M.L., DYCK R.F. & PEPYS M.B. (1980) Amyloid P-component in mice injected with casein: identification in amyloid deposits and in the cytoplasm of hepatocytes. *Immunology*, **41,** 59.

BEFUS A.D., EGWANG T. & GAULDIE J. (1983) Inflammatory and immune responses to parasites. Chapter 10. In: *Immunology of the Lung* (ed. J. Bienenstock), Chapt. 10, (in press). McGraw-Hill, New York.

BEFUS A.D., JOHNSTON N. & BIENENSTOCK J. (1979) *Nippostrongylus brasiliensis*: mast cells and histamine levels in tissues of infected and normal rats. *Exp. Parasitol.* **48,** 1.

BENSON, M.D. & ALDO-BENSON M. (1979) Effect of purified protein SAA on immune response *in vitro*: mechanisms of suppression. *J. Immunol.* **122,** 2077.

BENSON M.D. & KLEINER E. (1980) Synthesis and secretion of serum amyloid protein A (SAA) by hepatocytes in mice treated with casein. *J. Immunol.* **124,** 495.

BERNESTEIN D.L. & WALSH E.C. (1978) Endogenous mediators of the acute-phase reaction. I. Rabbit granulocytic pyrogen and its chromatographic subfractions. *J. Lab. clin. Med.* **91,** 236.

COURTOY P.J., LOMBART C., FELDMANN G., MOGUILEUSKY N. & ROGIER E. (1981) Synchronous increase of four acute phase proteins synthesized by the same hepatocytes during the inflammatory reaction. A combined biochemical and morphologic kinetics study in the rat. *Lab. Invest.* **44,** 105.

CROLL N.A. & MA K. (1978) The location of parasites within their hosts: the passage of *Nippostrongylus brasiliensis* through the lungs of the laboratory rat. *Int. J. Parasitol.* **8,** 289.

DESCHENES J., VALET J.P. & MARCEAU N. (1980) Hepatocytes from newborn and weanling rats in monolayer culture. *In Vitro*, **15,** 722.

EGWANG T.G., GAULDIE J. & BEFUS A.D. (1984) Complement-dependent killing of *Nippostrongylus brasiliensis* infective larvae by rat alveolar macrophages. *Clin. exp. Immunol.* **55,** 149.

GAULDIE J., LAMONTAGNE L., HORSEWOOD P. & JENKINS E. (1980) Immunohistochemical localization of alpha-1-antitrypsin in normal mouse liver and pancreas. *Am. J. Pathol.* **101,** 723.

GORDON A.H. (1976) The acute phase plasma proteins. In: *Plasma Protein Turnover* (eds R. Bianchi, G. Mariani and A. S. MacFarlane), p. 381. Macmillan Press, London.

HUDIG D. & SELL S. (1978) Serum concentrations of alpha-macrofetoprotein (acute-phase alpha-2-macroglobulin) a proteinase inhibitor, in pregnant and neonatal rats with acute inflammation. *Inflammation*, **3,** 137.

JAMES K., HANSEN B. & GEWURZ H. (1981a) Binding of C-reactive protein to human lymphocytes. I. Requirement for a binding specificity. *J. Immunol.* **127,** 2539.

JAMES K., HANSEN B. & GEWURZ H. (1981b) Binding of C-reactive protein to human lymphocytes. II. Interaction with a subset of cells bearing the Fc receptor. *J. Immunol.* **127,** 2545.

JENNINGS F.W., MULLIGAN W. & URQUHART G.M. (1963) Variables in x-ray 'inactivation' of *Nippostrongylus brasiliensis* larvae. *Exp. Parasitol.* **13,** 367.

KINDMARK C.O. (1976) Sequential changes in plasma proteins in various acute diseases, In: *Plasma Protein Turnover* (eds R. Bianchi, G. Mariani and A. S. MacFarlane), p. 395. Macmillan Press, London.

KINSELLA T.D. & FRITZLER M.J. (1980) CRP: An immunoregulatory protein. *J. Rheumatol.* **7,** 272.

KOJ A. (1970) Synthesis and turnover of acute-phase reactants. In: *Energy Metabolism in Trauma* (eds R. Porter and J. Knight), p. 79. J. & A. Churchill, London.

KOJ A. (1974) Acute phase reactants. In: *Structure and Function of Plasma Proteins* (ed. A. C. Allison), p. 73. Plenum Press, London.

KOJ A. & REGOECZI E. (1978) Effect of experimental inflammation on the synthesis and distribution of antithrombin III and alpha-1-antitrypsin in rabbits. *Br. J. exp. Pathol.* **59,** 473.

KUSHNER I. & FELDMANN G. (1978) Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. *J. exp. Med.* **148,** 466.

LAMONTAGNE L., GAULDIE J. & KOJ A. (1981) Ontogeny and tissue distribution of alpha-1-antitrypsin of the mouse. *Biochim. biophys. Acta*, **662,** 15.

LE P.L., MULLER M.T. & MORTENSEN R.F. (1982) Acute phase reactants of mice. I. Isolation of serum amyloid P-component (SAP) and its induction by a monokine. *J. Immunol.* **129,** 665.

LEID R.W. & WILLIAMS J.F. (1979) Helminth parasites and the host inflammatory system. Chem. Zoology, 229.

MCADAM K.P.W.J. & SIPE J.D. (1976) Murine model for human secondary amyloidosis-genetic variability of the acute phase serum protein SAA response to endotoxins and casein. *J. exp. Med.* **144,** 1121.

MACKENZIE C.D., JUNGERY M., TAYLOR D.M. & OGILVIE B.M. (1980) Activation of complement, the induction of antibodies to the surface of nematodes and the effect of these factors and cells on worm survival *in vitro*. *Eur. J. Immunol.* **10,** 594.

MERRIMAN C.R., PULLIAM L.A. & KAMPSCHMIDT R.F. (1977) Comparison of leukocyte pyrogen and leukocytic endogenous mediator. *Proc. Soc. exp. Biol. Med.* **154,** 224.

MORTENSEN R.F. (1979) C-reactive protein (CRP)-mediated inhibition of the induction of *in vitro* antibody formation. I. T-cell dependence of the inhibition. *Cell. Immunol.* **44,** 270.

MORTENSEN R.F. (1982) Inhibition of the polyclonal antibody plaque-forming cell response of human B lymphocytes by C-reactive protein (CRP) and CRP complexes. *Cell. Immunol.* **66,** 99.

MURPHY P.A., SIMON P.L. & WILLOUGHBY W.F. (1980) Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte-activating factors made by rabbit alveolar macrophages. *J. Immunol.* **124,** 2498.

OPPENHEIM J.J., STADLER B.M., SIRAGANIAN R.P., MAGE M. & MATHIESON B. (1982) Lymphokines: their role in lymphocyte responses. Properties of interleukin 1. *Fed. Proc.* **41,** 257.

OTTESON E.A. (1980) Immunopathology of lymphatic filariosis in man. *Springer Semin. Immunopathol.* **2,** 373.

PEPYS M.B. (1979) Isolation of serum amyloid P-component (protein SAP) in the mouse. *Immunology*, **37,** 637.

PEPYS M.B., & BALTZ M.L. (1983) Acute phase proteins with special reference to C-reactive protein and related proteins (Pentaxins) and serum amyloid A protein. *Adv. Immunol.* **34,** 141.

PEPYS M.B., BALTZ M., GOMER A., DAVIES A.J.S. & DOENHOFF M. (1979) Serum amyloid P-component is an acute-phase reactant in the mouse. *Nature (Lond.)* **278,** 259.

ROSENWASSER L.J., DINARELLO C.A. & ROSENTHAL A.S. (1979) Adherent cell function in murine T-lymphocyte antigen recognition. IV. Enhancement by human leukocytic pyrogen. *J. exp. Med.* **150,** 709.

SCHARFSTEIN J., BARCINSKI M.A. & LEON L.L. (1982) Induction of the acute-phase protein serum amyloid P in experimental Chagas' disease. *Infect. Immun.* **35,** 46.

SELINGER M.J., MCADAM K.P.W.J., KAPLAN M.M., SIPE J.D., VOGEL S.N. & ROSENSTREICH D.L. (1980) Monokine-induced synthesis of serum amyloid A protein by hepatocytes. *Nature (Lond.)*, **285,** 498.

SIPE J.D., MCADAM K.P.W.J., TORAIN B.F. & POLLACK P.S. (1977) Isolation and structural properties of murine SAA: the acute phase serum precursor of amyloid A. *Immunol. Commun.* **6,** 1.

SIPE J.D., VOGEL S.N., RYAN J.L., MCADAM K.P.W.J. & ROSENSTREICH D.L. (1979) Detection of a mediator derived from endotoxin-stimulated macrophages that induces the acute phase serum amyloid A response in mice. *J. exp. Med.* **150,** 597.

SIRICA A.E., RICHARDS W., TSUKADA Y., SUTTLER C.A. & PITOT H.C. (1979) Fetal phenotypic expression by adult rat hepatocytes on collagen gel/nylon meshes. *Proc. natn. Acad. Sci. U.S.A.* **76,** 283.

SYMONS L.E.A. (1965) Kinetics of the epithelial cells, and morphology of villi and crypts in the jejunum of the rat infected by the nematode *Nippostrongylus brasiliensis*. *Gastroenterology*, **49,** 158.

SZTEIN M.B., VOGEL S.N., SIPE J.D., MURPHY P.A., MIZEL S.B., OPPENHEIM J.J. & ROSENSTREICH D.L. (1981) The role of macrophage in the acute-phase response: SAA inducer is closely related to lymphocyte activating factor and endogenous pyrogen. *Cell. Immunol.* **63,** 164.

TAKASUGI M. (1971) An improved fluorochromatic cytotoxic test. *Transplantation*, **12,** 148.

VETTER M.L., GEWURZ H., HANSEN B., JAMES K. & BAUM L.L. (1983) Effects of C-reactive protein on human lymphocyte responsiveness. *J. Immunol.* **130,** 2121.

WARREN K.S. (1982) Mechanisms of immunpathology in parasitic infection. In: *Immunology of Parasitic Infections* (eds S. Cohen and K. S. Warren), p. 116. Blackwell Scientific, Oxford.

WESCOTT R.B. & TODD A.C. (1964) A comparison of the development of *Nippostrongylus brasiliensis* in germ-free and conventional mice. *J. Parasitol.* **50,** 138.

WHICHER J.T. (1981) The role of the acute phase proteins. *Diagnostic Med.* **4,** 52.