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# The acute-phase protein response in parasite infection. *Nippostrongylus brasiliensis* and *Trichinella spiralis* in the rat

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## SUMMARY

During acute inflammation, the mammalian liver responds with increased production and secretion of a series of plasma glycoproteins, collectively termed the acute-phase proteins, resulting from the release at the site of inflammation of polypeptide cytokines, including IL-1 and IL-6, which interact with receptors on hepatocytes and alter gene expression. This attribute of the systemic acute-phase response was studied throughout the course of infection with two nematode parasites in rats. Significant increases in serum haptoglobin, $\alpha_1$-acid glycoprotein and $\alpha_1$-cysteine protease inhibitor were detected coincident with episodes of skin, lung and intestinal pathology during *Nippostrongylus brasiliensis*, but were not seen during *Trichinella spiralis*, infection of the rat despite similar intestinal pathology. These changes were seen at both the protein and mRNA levels in the liver. Infection with *T. spiralis* was not anti-inflammatory, as macrophages from various sites could be induced *in vitro* to release inflammatory cytokines, and *in vivo* induction of inflammation by turpentine injection was similar in control and infected animals. However, macrophage populations recovered from animals infected with *T. spiralis* were not activated. Moreover, intestinal infection alone with intestinal stages of *N. brasiliensis* also failed to elicit the systemic acute-phase protein response, requiring an explanation involving skin and lung for the acute-phase response during gut inflammation in a primary infection with *N. brasiliensis*. Taken together, these data suggest that during the intestinal phase of nematode infection, with pathological changes to the gut, the systemic acute-phase response is not elicited through compromise or lack of stimulation of inflammatory cells in the intestine. The systemic parameters of the acute-phase response may not be a component of gastrointestinal pathology.

## INTRODUCTION

The acute-phase response (APR) in inflammation is the mammalian reaction to traumatic injury. The response is characterized by a number of reflexive and homeostatic physiological changes, including fever, leukocytosis, changes in plasma metal levels and increased concentration of a number of hepatocyte-derived plasma proteins, the acute-phase proteins (APP) (Gordon & Koj, 1985; Milanino *et al.*, 1986). These include $\alpha_1$-acid glycoprotein, haptoglobin, fibrinogen, haemopexin, ceruloplasmin, $\alpha_1$-antichymotrypsin, C3 and $\alpha$1 proteinase inhibitor in most species, along with C-reactive protein, Factor B complement component and serum amyloid A and P proteins in man and cysteine proteinase inhibitor and $\alpha_2$-macroglobulin in the rat. The purpose of the APR is confinement of the inflammation, limitation of autolytic damage by phagocytic cells, and ultimately repair of damaged tissues. That some of the APP are clotting factors (Bernuau, Rogier & Feldman, 1983) and others are proteinase inhibitors (Rokita *et al.*, 1985; Lonberg-Holm *et al.*, 1987) is testimony to this homeostatic role for the response.

The occurrence of increased APP concentrations during episodes of inflammation (the acute-phase protein response, APPR) has proven to be a useful indicator of some diseases in humans (Gauldie, Lamontagne & Stadnyk, 1985; Whicher & Dieppe, 1985). Measurements of APP have also been used in determining prognosis and relapses in neoplasia (Rashid *et al.*, 1982), bacterial infection (Whicher, Bell & Southall, 1981) and, with variable results, inflammatory bowel diseases (Shaverymuttu *et al.*, 1986). One inflammatory stimulus for which there is little detail about the subsequent APPR is natural parasitic infection, in either man or animals (Gauldie *et al.*, 1985). This is surprising in view of the considerable inflammation that parasites elicit (Leid & Williams, 1979).

In earlier studies of rodent infection with the nematode *Nippostrongylus brasiliensis*, it has been demonstrated that the message to trigger hepatocyte synthesis of APP arose from the

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site of inflammation, in particular from alveolar macrophages, after the parasite caused trauma in the lung (Egwang, Befus & Gauldie, 1985; Lamontagne *et al.*, 1984). It has been shown that alveolar macrophages from infected rats secrete increased amounts of potent inflammatory mediators, including hepatocyte-stimulating factors (HSF) such as IL-6 (HSF, interferon-beta$_2$; Gauldie *et al.*, 1987) and IL-1 (Lamontagne *et al.*, 1985a). Thus, it is postulated that the macrophage is central to the genesis of the APPR (Gauldie *et al.*, 1987).

More recently, the role of macrophages in the intestine during inflammation has been explored, and nematode infections used as models of inflammatory bowel disorders. Since various hepatocyte-stimulating factors directly induce synthesis and raise the plasma levels of APP, in this study changes in the concentrations of these molecules was examined during episodes of intestinal pathology. The APP concentrations during *Nippostrongylus brasiliensis* and *Trichinella spiralis* infections of rats are described and these changes correlated with alteration in hepatic mRNAs specific for each protein. Infection with *N. brasiliensis* leads to changes in skin, lung and intestines, while that with *T. spiralis* involves intestine and muscle. Both nematode infections elicit marked intestinal pathology, but only infection with *N. brasiliensis* was associated with systemic changes in APP concentrations, and no change in APP synthesis occurred during infection by *T. spiralis*. It is proposed that an infection confined to the intestine alone may fail to provoke the APPR response.

## MATERIALS AND METHODS

*Animals and parasites*

Male Sprague–Dawley rats (Charles River Breeding Laboratories, Ottawa, Canada), weighing between 250 and 300 g, were used in all experiments and were kept in the McMaster animal quarters for at least 1 week before use.

*Nippostrongylus brasiliensis* was maintained by passage through Sprague–Dawley rats. Infective larvae were recovered from parasite eggs and maintained in a charcoal/rat faeces culture as previously described (Egwang *et al.*, 1985). Infective third stage larvae (from 10- to 20-day-old cultures) were collected by Baermann filtration and washed through several changes of sterile phosphate-buffered saline (PBS; 30 mM sodium phosphate, 150 mM NaCl, pH 7·3). Rats were infected with 3000 larave (in PBS) by subcutaneous injection at the nape of the neck, under light ether anaesthesia.

*Trichinella spiralis* (Dr K. Wright, University of Toronto) was maintained by passage through CBA/J strain mice (Jackson Laboratories, Bar Harbor, ME). Infective *T. spiralis* were collected following pepsin/HCl digestion (0·7% each of pepsin and 10 N HCl) of the mouse carcass at 37° for 2 hr. Infective larvae were filtered into plastic 50-ml tubes and washed with fresh PBS. Rats were infected orally with 2000 *T. spiralis* suspended in 0·2% agar, using a feeding tube.

*Adult* N. brasiliensis *transfer experiments*

*N. brasiliensis* larvae collected for transfer experiments were recovered from 3-day infected rat intestines. Baermann collecting funnels were modified to collect the worms in 250 ml volumetric flasks, which were incubated in a shallow water bath maintained at 37°. Hanks' balanced salt solution (HBSS), buffered with 20 mM HEPES, pH 7·3, was used as the collecting media. The rat intestines were left in the water bath for 1·5 hr, then all the worms were pooled and washed repeatedly using sterile HBSS. The concentration of the worms was corrected in order to deliver between 2000 and 3000 in 1-ml volumes in 0·2% agar in HBSS. The worms were then administered by feeding tube to uninfected rats. Recovery of parasite eggs from hosts' faeces was determined to follow the viability of the transferred population.

*Turpentine-induced inflammation*

Undiluted turpentine (Harrisons & Crossfield, Toronto, Canada) was injected subcutaneously in the dorso-lumbar region of rats under light ether anaesthesia. The dose used was 50 $\mu$l per 100 g of body weight.

*Measurement of the acute-phase proteins*

*Serum levels.* Rats were bled every second day via the tail artery using a 23-gauge butterfly infusion needle. A volume of 0·25 ml of whole blood was routinely collected and the serum frozen at $-20°$ until assayed for protein concentrations. Serum concentrations of $\alpha_1$-acid glycoprotein ($\alpha_1$AGP), $\alpha_1$-cysteine proteinase inhibitor ($\alpha_1$CPI) and haptoglobin were determined by rocket immunoelectrophoresis using monospecific rabbit antisera to rat proteins, as previously described (Koj *et al.*, 1985). The results are presented as the mean$\pm$SEM of the relative change from normal to infected levels (infected protein concentration divided by the level for the same animal uninfected). No systemic inflammatory changes were evident in non-parasitized control rats, due to blood collection.

*Measurement of liver acute-phase protein mRNA.* Total cellular RNA was extracted from 1 g of liver from infected rats using guanidine hydrochloride (Glibetic & Baumann, 1986). The purity of the RNA was confirmed by adding RNAase to a sample of the preparation and determining the $OD_{260}$. Ten micrograms of RNA from each liver sample were aliquoted in descending serial dilutions, denatured using formaldehyde, and blotted onto nitrocellulose filters under light vacuum. The filters were then air-dried and baked at 80° for 2 hr. The filters were hybridized to the following $^{32}$P-labelled cDNA plasmids: pIRL-10, rat $\alpha_1$AGP; pIRL-9, rat haptoglobin; and pIRL-3, rat $\alpha_1$ CPI, as described elsewhere (Glibetic & Baumann, 1986).

Quantification of the cDNA hybridization to specific mRNA was performed by cutting out each RNA dot and counting the total radioactivity in a Beckman $\beta$-counter, in liquid scintillant [22 mM 2,5-dephenyloxazole, 0·27 mM di-1,4(phenyl-5-oxazolyl)-2-benzene in toluene]. Radioactivity of infected rat liver mRNA samples (c.p.m.) was related to the counts from uninfected rat liver, and expressed as a percentage of the normal count.

*Measurement of IL-6/HSF and IL-1*

*Macrophage cultures.* Rat alveolar and peritoneal macrophages were isolated from 4 and 7 day *T. spiralis*-infected animals by washing each compartment with PBS (Egwang *et al.*, 1985). The cells from four animals were pooled and their final concentration was corrected to $1 \times 10^6$ viable cells/ml. The plastic-adherent population was isolated following a 2-hr incubation (37°, 5% $CO_2$) in plastic dishes, in RPMI-1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) and 1% of each penicillin/streptomycin, L-glutamine, HEPES and bicarbonate. The adherent monolayer was

cultured overnight in fresh media lacking the FBS. One-half of the cultures had 5 μg/ml lipopolysaccharide W (LPS; *Escherichia coli* 055:B5, Difco Laboratories, Detroit, MI) added as an *in vitro* macrophage-stimulating agent. Following incubation, culture supernatants were collected, filtered, and dialysed (Spectropore tubing, MW cut-off of 15,000) against fresh PBS for 48 hr. The supernatants were then applied to cultures of primary rat liver cells for determination of IL-6/hepatocyte-stimulating activity or mouse thymocytes for measurement of IL-1 activity as lymphocyte-activating factor.

*Primary hepatocyte cultures and IL-6/HSF activity.* The isolation of primary rat hepatocytes has been described elsewhere (Koj *et al.*, 1984). Briefly, the liver of a normal (250 g) rat was perfused *in situ* with $Ca^{2+}$- and $Mg^{2+}$-free HBSS then removed to a collagenase bath. Perfusion with prewarmed collagenase (70 mg/100 ml of media; Sigma Chemical Co., St Louis, MO) was performed for 10 min or until the cells became loose. The entire liver was combed into William's media (Gibco) supplemented with 10% FBS, 1% each of penicillin/streptomycin, HEPES, L-glutamine, bicarbonate and 1 μm insulin and 1 μm dexamethazone. This suspension of cells was plated onto collagen-coated (Vitrogen 100; Collagen Corporation, Palo Alto, CA) plastic dishes and maintained for 3 days at 37°, 5% $CO_2$. Culture medium and exogenous hepatocyte-stimulating agents were diluted into PBS and replaced fresh to the cultures every 24 hr. Hepatocyte culture supernatants were recovered (48–72 hr) and assayed for APP using rocket immunoelectrophoresis. The effect of addition of a standard amount of exogenous IL-6/HSF (a pool of rat lung fibroblast-conditioned media) on primary liver cell synthesis of APP served as an internal control for determining the viability of the cells during the culture period. The results of the hepatocyte-stimulating assay are reported as the ratio of $\alpha_2$-macroglobulin to albumin in the same supernatant, which gives a relative measure of liver synthetic activity (Koj *et al.*, 1985).

*Co-mitogenic assay for IL-1.* IL-1 activity was measured using the C3H/HeJ murine lectin co-mitogenic assay, as described earlier (Lamontagne *et al.*, 1985a). The results are presented as the mean ± SEM of the total radioactivity (c.p.m.) for three determinations for each macrophage supernatant.

## RESULTS

### The serum APPR during infection

Figure 1 shows the changes in serum concentration of several APP during infection of the rat by either *N. brasiliensis* or *T. spiralis*. While the magnitude of changes for each glycoprotein differed in *N. brasiliensis*-infected animals, there was always a small but significant change on Day 2 (1·5–2-fold), followed by a second increase which reached a maximum by Day 8 (3–6-fold). The kinetics of these changes occurred at the same time as the periods of lung inflammation (Day 2) and intestinal pathology (Days 7–10) and coincided with the presence of the parasites in the tissues. In contrast, despite the demonstration of marked pathology in the gut of a similar magnitude to that seen in *N. brasiliensis*, throughout the infection by *T. spiralis* serum APP concentrations remained unchanged. In each case, infection and pathology were confirmed by histological evaluation and the recovery of many viable worms from gut tissues in both infections.

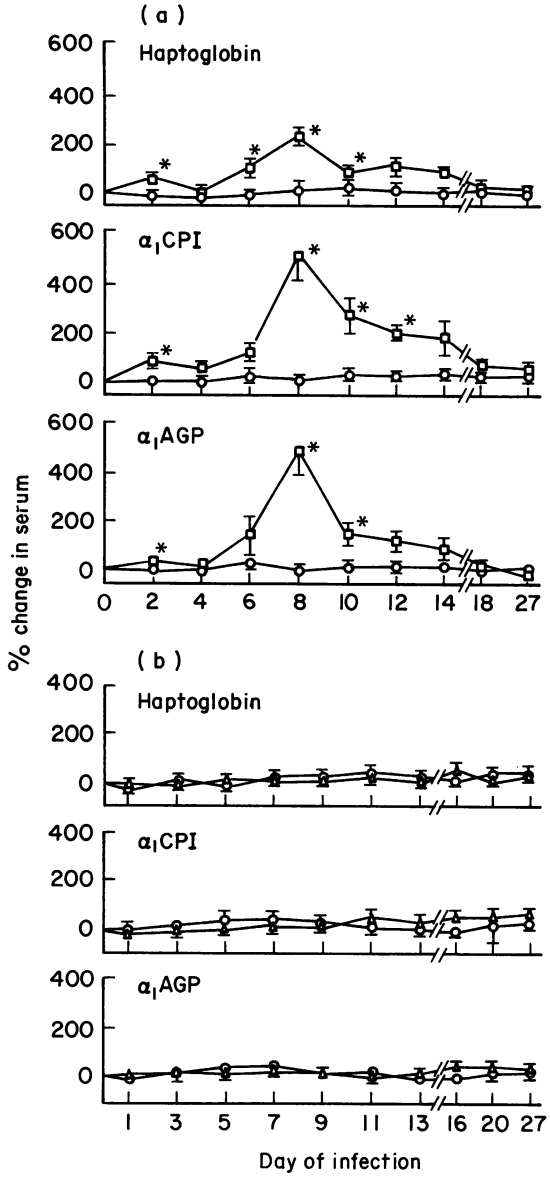


**Figure 1.** Changes in concentrations of serum APP during (a) *N. brasiliensis* (□) and (b) *T. spiralis* (△) primary infection of Sprague–Dawley rats compared to uninfected control rats (○). Each point represents the mean ± SE of five animals bled on the days indicated. Serum protein concentrations were determined using rocket immunoelectrophoresis and monospecific rabbit antisera. * Indicates a significant difference ($P \leq 0{\cdot}05$) between infected and control animals bled on the same day, determined using the Student's *t*-test for two samples.

In addition to the changes shown, increased concentrations of serum $\alpha_2$-macroglobulin, a major acute-phase protein in the rat, were detectable by Day 8 in *N. brasiliensis*-infected rats but not at all in *T. spiralis*-infected rats (data not shown). This protein is undetectable in normal animals. No consistent or significant changes in the APP were detected in similarly bled, uninfected control rats.

During a challenge infection by the homologous parasite, the term of infection is markedly abbreviated by an early host reaction. Figure 2 shows changes in serum APP concentrations during challenge infections with homologous parasites. During

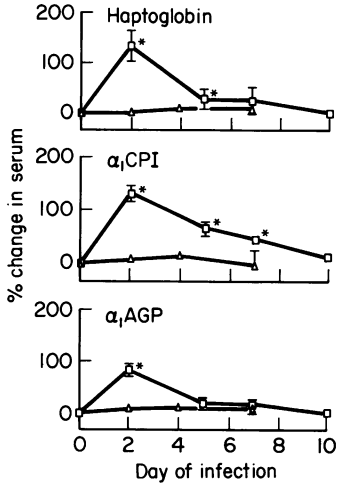


**Figure 2.** Changes in concentrations of serum APP during secondary infections by *N. brasiliensis* (□) or *T. spiralis* (△) of Sprague–Dawley rats. Each point represents the mean ± SE of four or five animals bled on the days indicated. * Indicates a significant difference ($P \leq 0.05$) determined by using the Student's *t*-test on a single sample versus no change on Day 0.

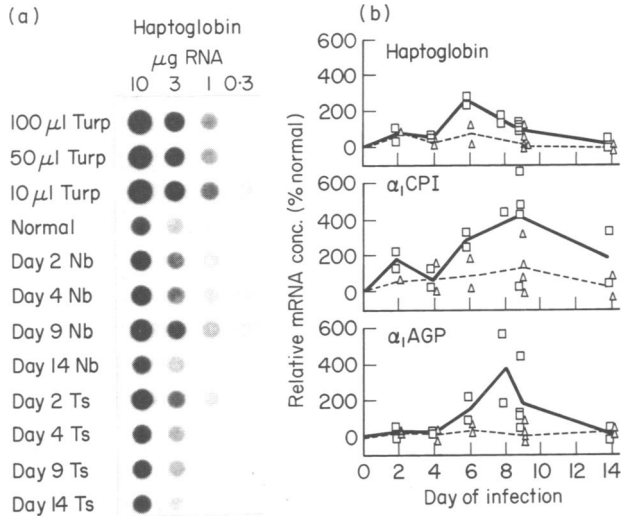


**Figure 3.** Dot-blot hybridization of rat liver RNA from *N. brasiliensis*- or *T. spiralis*-infected Sprague–Dawley rats. (a) Autoradiograph of hybridization of $^{32}$P-labelled haptoglobin-specific cDNA to liver RNA on nitrocellulose. For comparison, the mRNA changes due to turpentine-induced inflammation (turp) are shown. (b) Summary of radioactive counts for all molecular hybridizations for three APP during either infection. The figure is a summary of five separate experiments of various days post-infection and each experiment included an uninfected animal. The results are expressed as the percentage change of the total radioactivity of *N. brasiliensis*- (□) or *T. spiralis*- (△) infected animal samples compared to uninfected samples.

*N. brasiliensis* secondary infection there was a single peak of increased APP, reaching maximal concentrations by Day 2 post-infection and declining thereafter. No changes were detected in serum APP during secondary infection with *T. spiralis*, even though there was evidence of previous exposure.

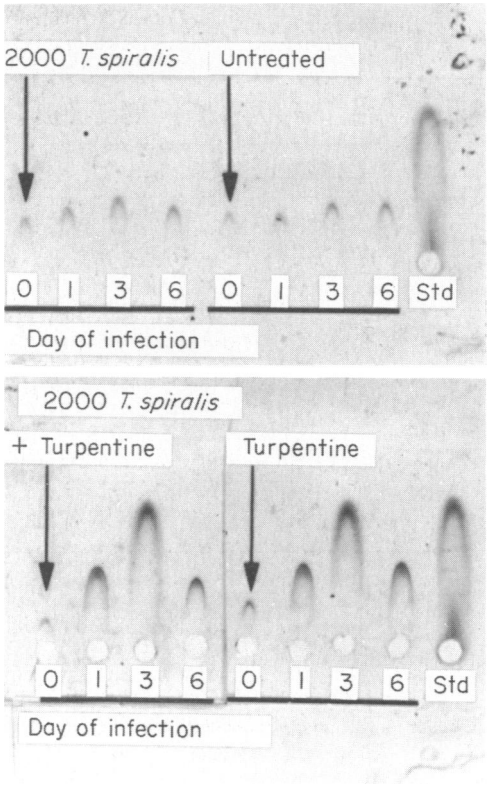


**Figure 4.** Rocket immunoelectrophoresis showing changes in serum APP concentrations of Sprague–Dawley rats during concurrent *T. spiralis* infection and turpentine inflammation. The turpentine (50 μl per 100 g body weight) was administered on the same day as the parasite (2000 infective larvae). Animals were bled on the days indicated and rocket immunoelectrophoresis performed on the serum. The gel shown is that for $\alpha_1$AGP; similar results were observed for the other APP. Std is a protein mixture containing a standard amount of each APP.

### Liver mRNA for APP

The liver of nematode-infected rats was examined for changes in specific mRNAs for each APP to determine whether there was increased hepatic APP production. Figure 3 shows the relative changes in specific mRNA, determined as increased hybridized radioactivity in RNA samples from infected RNA samples compared with normal liver RNA. Each point represents one rat and the figure is a composite of five experiments, each with an uninfected control. The mRNA increased at times of infection corresponding to increased serum APP in the *N. brasiliensis*-infected animals. With the exception of haptoglobin on Day 2 of infection, there were no consistent changes seen during the period of greatest intestinal inflammation due to *T. spiralis* (Fig. 3a). This result mirrors the change or lack of change in serum APP during each infection and rules out the possibility that the failure to see plasma increases was due to increased turnover of the respective protein.

### *T. spiralis* does not inhibit the APPR

It has been reported that an infection with *T. spiralis* in the rat is anti-inflammatory for a second, unrelated stimulus, delivered to a site other than the intestine (Castro, Malone & Smith, 1980). It was tested whether this anti-inflammatory activity included suppression of the APPR by treating *T. spiralis*-infected rats

**Table 1.** Hepatocyte-stimulating activities of macrophages recovered from *T. spiralis*-infected Sprague–Dawley rats

| | IL-6† | | IL-1‡ | |
|---|---|---|---|---|
| Macrophages* | −LPS | +LPS | −LPS | +LPS |
| Normal alveolar | 0·23 | 0·64 | 16,349 ± 1961 | 35,447 ± 11,166 |
| 4-day infected alveolar | 0·24 | 0·80 | 10,738 ± 953 | 26,052 ± 13,084 |
| 7-day infected alveolar | 0·23 | 1·14 | 20,878 ± 3104 | 26,662 ± 12,458 |
| Positive control§ | — | 1·60 | — | 140,607 ± 2841 |
| Normal peritoneal | 0·12 | 0·17 | 13,995 ± 4882 | 39,059 ± 1755 |
| 4-day infected peritoneal | 0·14 | 0·14 | 16,649 ± 4253 | 23,967 ± 2260 |
| 7-day infected peritoneal | 0·13 | 0·16 | 17,176 ± 3639 | 27,021 ± 4399 |
| Positive control§ | — | 1·60 | — | 123,913 ± 5406 |

* Alveolar cells were pooled from four animals and peritoneal cells from three animals. Plastic adherent cells were cultured for 24 hr.

† Results shown for IL-6 are the ratio of $\alpha_2$-macroglobulin synthesis/albumin in the hepatocyte stimulation assay.

‡ Results shown are the radioactive c.p.m. determined from a PHA co-mitogenic assay using C3H/HeJ thymocytes and are mean ± SE of three replicates.

§ Human peripheral blood mononuclear cells cultured in the presence of LPS.

with low levels of (subcutaneous) turpentine at the nape of the neck. The turpentine dose chosen stimulated a significant APPR. Figure 4 shows that rats concurrently infected with *T. spiralis* and inflamed with turpentine underwent an APPR of equal magnitude to the response induced by turpentine alone. Thus, the liver of infected rats is capable of responding to inflammatory signals with increased APP synthesis.

Although the hepatocytes of *T. spiralis*-infected rats may be responsive to inflammatory signals, the cells sending the mediators may be different from those involved in turpentine inflammation and may be inhibited by the infection. Table 1 shows that secretion of hepatocyte-stimulating factors (IL-6/HSF, IL-1) by alveolar and peritoneal macrophages from infected animals is normal and shows no activation by the infection and, furthermore, that the cells can be stimulated into *in vitro* production of the cytokines by LPS.

A concurrent infection of rats, by both parasites, resulted in a response which resembled that due to an infection by *N. brasiliensis* alone (data not shown). These data further imply that *T. spiralis* fails to elicit or suppress the APPR.

### Direct intestinal infection with *N. brasiliensis*

These results suggest that the intestinal stages of *T. spiralis* do not elicit the APPR. To determine whether this was a common phenomena among intestinal infections, an intestine-only infection with *N. brasiliensis* using transferred worm populations recovered from donors was investigated. Viable parasites were collected from the lungs of 3-day *N. brasiliensis*-infected animals, washed, and transferred to the intestine of a second set of rats using a feeding tube. The viability of the transferred worms was determined by following parasite egg production in

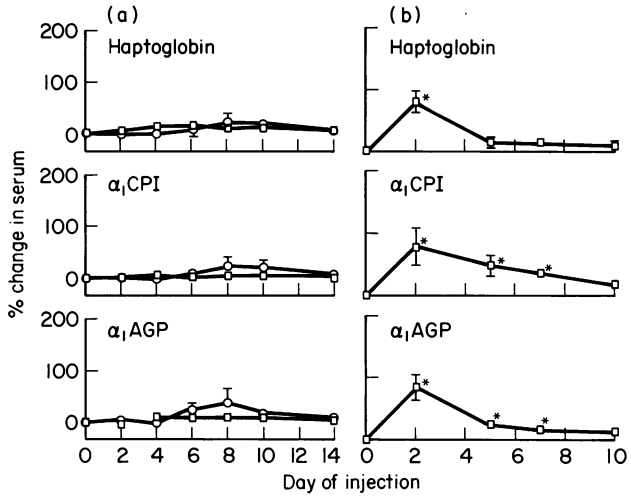


**Figure 5.** (a) Changes in concentrations of serum APP during infection of Sprague–Dawley rats with transferred, 3-day-old *N. brasiliensis* worms (□). Donor rats were infected with 3000 $L_3$ *N. brasiliensis* and killed 3 days later. Their intestines were removed, the worms ($L_5$ larvae) collected and washed, and their concentration corrected to 2000 worms/ml of 0·2% agar. Egg production by the transferred $L_5$ population began on Day 4 post-infection during a primary infection. Control animals (○) received heat-killed worms. (b) Serum changes during challenge infection with *N. brasiliensis* $L_3$ of rats immunized by transferred $L_5$. The rats used in (a) were used for this experiment. The challenge infection delivered on Day 30 post-immunizing infection indicates a significant difference ($P \leq 0{\cdot}05$) between secondary infected animals and control (primed) animals, determined using the Student's *t*-test for two samples. One experiment was performed in which the rats were not bled during the primary infection, in order to determine whether the repeated bleeding during two periods of infection had an effect on serum APP levels. This experiment showed that there was no effect due to the bleeding in either infection.

the faeces of the host. Eggs were detected on Day 4 of infection by transferred worms, 2 days earlier than during a normal infection. Worm recoveries were approximately 50% of that obtained with primary administration of 3000 larvae, but detectable APP changes were seen in infections with as few as 500 larvae. Despite this evidence of a successful intestinal infection, Fig. 5 shows that the serum concentrations for the APP did not change significantly following oral infection of rats with the intestinal stage of *N. brasiliensis*.

A secondary infection with third stage ($L_3$) *N. brasiliensis* of the intestine-primed animals (transfer of lung worms) resulted in serum changes similar to those seen in $L_3$-immunized and challenged rats (compare Fig. 5 to Fig. 2). These results, in combination with the failure of the *T. spiralis* infection to evoke the APPR, indicate that intestinal helminth infections generally do not involve the systemic hepatic response.

## DISCUSSION

A number of systemic inflammatory changes occur in nematode-infected rats. Changes occur in cell numbers and types, with significant increases in neutrophils, eosinophils and basophils (mast cells) in the blood (Roth & Levy, 1980; Lee & Best, 1983a, b) and lungs (Egwang, Gauldie & Befus, 1984). Certain plasma macromolecular concentrations increase, including cor-

tico-steroids and gastro-intestinal hormones (Ovington, Bacarese-Hamilton & Bloom, 1985), immunoglobulins and, in the rat, mast cell protease (Woodbury *et al.*, 1984).

APP changes due to nematode infection of the rat have been established in this study. Two nematode species were examined, both of which are resident in the intestine as adults. In the case of *N. brasiliensis*, having penetrated the skin and migrated to the lung, the migrating larvae breech the mucosal barrier in the lungs before reaching the intestine. The infectious larvae also moult in the host's lung. Infectious *T. spiralis* arrive in the intestine as larvae, having existed in the stomach. It is interesting to note that the adult of each species occupy a different habitat in the host's intestine; *N. brasiliensis* remains in the lumen while *T. spiralis* matures and mates intra-epithelially (Gardiner, 1976). Despite these differences in life cycle, the infections cause similar gross pathological changes in the intestine (Russel & Castro, 1987).

Both infections elicit eosinophil, mast cell and goblet cell hyperplasia in the lamina propria and/or epithelium of the host intestine (Leid & Williams, 1979). The precise timing of the hyperplasia of each cell type is rat-strain dependent. Increases in leucocyte numbers are concurrent with shortened turnover time of the intestinal epithelium and crypt hyperplasia, the outcome of which is a characteristic atrophy and flattening of the intestinal villi (Symons, 1965), often referred to as intestinal inflammation. Hence, the parasite infections make ideal models for studying intestinal pathophysiology.

During the examination of the systemic inflammatory changes due to these intestinal infections, it was observed that APP changes occurred during periods of inflammation due to *N. brasiliensis*, but that there were no or minimal changes due to infection with *T. spiralis*. It was important to extend the data assessing serum APP changes with a measure of specific liver RNA for each protein. Although increased APP-specific mRNA is characteristic of turpentine-induced inflammation (Schreiber *et al.*, 1986; Northemann *et al.*, 1983), it is unknown whether any of these molecules are sequestered at the site of inflammation, as has been shown previously for murine $\alpha_1$-protease inhibitor ($\alpha_1$Pi) during the lung stage of *N. brasiliensis* infection, resulting in a normal serum level of $\alpha_1$Pi despite increased hepatic synthesis (Lamontagne, Stadnyk & Gauldie, 1985b). Using cDNA probes specific for each rat APP, it could be shown that there were no significant increases in APP mRNA during infection by *T. spiralis* but that obvious changes occurred in the liver of *N. brasiliensis*-infected animals during the second peak of increased serum APP. These changes in liver mRNA's, although less easy to detect and less quantitative, correlate with the quantitative changes seen in the protein serum levels.

Macrophages are a major source of hepatocyte-stimulating factors, including IL-6 and IL-1 (Gauldie *et al.*, 1987), and it was important to assess the role of these cells during an infection when the APP did not change. There is evidence from other work showing that macrophages are activated during infection by *T. spiralis* (Roth & Levy, 1980; Lee & Best, 1983a). Macrophages are also thought to be important in expulsion of the worms (Wing & Remington, 1978). Animals whose macrophages were stimulated using a heterologous agent prior to infection with *T. spiralis* had lower peak worm burdens than animals whose macrophages were not stimulated, although the time of expulsion of the infection was unchanged (Wing & Remington, 1978). The finding that neither alveolar nor peritoneal macrophages from *T. spiralis*-infected rats were secreting greater than normal amounts of IL-1 or IL-6 suggests that these cells were not stimulated *in vivo* by this infection. Their helminthocidal properties *in vitro* were not examined, and perhaps the failure to detect cell activation is a function of the assay used here, although each cell type could be activated *in vitro* by endotoxin (Table 1).

The finding of two peaks of increased serum APP during infection by *N. brasiliensis* indicates that there are two periods of liver activation: one occurring after skin penetration and coincident with the presence of lung pathology; and the second coincident with the presence of intestinal infection. The first peak is likely to be due to a combination of inflammation at both the skin and lung. It was not possible to detect APP changes when an intestine-only model of *N. brasiliensis* was examined, even though such an infection is sufficient stimulation to immunize the recipient for a challenge infection (Ogilvie, 1965; Jenkins, 1972). These data are similar to findings of no changes during intestinal *T. spiralis* infection (compare Figs 1 and 5a), and it is speculated that both the first and second peak of APP changes seen in *N. brasiliensis* infection originate because of inflammation in the skin and the lungs, perhaps due to immune mechanisms against residual worm antigens in the tissues. The earlier observation that alveolar macrophages recovered from 8-day infected animals secrete greater amounts of IL-1 and IL-6 than lung cells recovered from 2-day infected rats, long after the parasite has cleared the lung, supports this contention (Egwang *et al.*, 1985). The single peak of APP changes, seen in the challenge infection with *N. brasiliensis*, probably represents the outcome of early immune challenge and parasite destruction that occurs in the tissues, resulting in decreased worm fecundity and elimination of the few worms that do reach the intestine by Day 6 post-infection (Lee & Best, 1983a; Bell & McGregor, 1979).

There is evidence from other work that mediators, in addition to IL-1 and IL-6, may be effecting the two peaks of APP. Ovington (1985) reported two periods of host inappetence during infection of rats with *N. brasiliensis*; an initial decline in food intake at Day 2 and a second at Day 8. It is interesting to speculate a role for tumour necrosis factor (TNF) in this inappetence, as this inflammatory mediator has recently been implicated in sepsis-induced anorexia (Le & Vilcek, 1987; Tracey *et al.*, 1988). TNF$\alpha$, similar to IL-1, has hepatocyte-stimulating properties, most notably detected *in vitro* in the depression of albumin synthesis (Koj *et al.*, 1987). A single period of albumin depression was detected, maximal by Day 8, similar to the observations of others (Ash, Crompton & Lunn, 1985; Ovington, 1987).

The significance of the findings presented here in relation to the detection of similar human diseases is not immediately clear. There are few studies of the systemic APPR and intestinal infections in humans. One report of a cross-sectional sample of Egyptian boys reported a decline in albumin concentrations and body weight due to a number of parasites including *Ascaris lumbricoides* and *Hymenolepis nana* (Cole *et al.*, 1982). A second study involved following the success of anthelminthic treatments and APP concentrations among a sample population of immigrants (Blom, Prag, Norredam, 1979). A significant decline in circulating $\alpha_1$AGP, $\alpha_1$-anti-trypsin and cerulo-plasmin (to normal levels) was reported for those patients treated for most helminths. Both of these studies suggest the APPR had occurred

in parasitized humans, although the studies were not controlled for multiple parasite species, including hookworms which have a life cycle similar to *N. brasiliensis*.

Using the assays outlined here it was not possible to demonstrate an anti-inflammatory effect in *T. spiralis* infections, as has been identified by others (Castro *et al.*, 1980). The significance of these models of intestinal inflammation to human inflammatory bowel disease (IBD) is speculative; however, the lack of APP response in these intestinal models may reflect the lack of evidence of inflammation in IBD (fever, ESR, C-reactive protein) prior to exacerbation of the disease, despite histological evidence of intestinal pathology.

The hepatic APPR in the rat requires exogenous delivery of cytokine mediators such as IL-6 and IL-1 to the liver (J. Gauldie, G. Fey and W. Northemann, manuscript submitted for publication). The fact that this response is not activated during these two episodes of intestinal inflammation without evidence of systemic suppression must reflect the absence of mediator production by the gut.

The macrophage/monocyte is the main cellular source of cytokines such as IL-1, and a preliminary study indicates that macrophages do not comprise a major portion of the cell population of the rat intestine (Stadnyk, Befus & Gauldie, 1989). Whether these cells or others in the gut are capable of releasing cytokines, or are locally suppressed by the presence of helminth parasites, is unknown. It will be important to examine these cells directly in normal and diseased conditions to determine whether the intestine represents a 'priviledged' site for inflammation in general, or only during helminth infections. The cellular events in the infected intestine are currently being examined in order to understand this situation better.

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