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# The immune response during a *Strongyloides ratti* infection of rats

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## Abstract

A range of immune parameters were measured during a primary infection of *Strongyloides ratti* in its natural rat host. The immune parameters measured were IL-4 and IFN-γ from both the spleen and MLN cells; parasite specific $IgG_1$, $IgG_{2a}$ and $IgG_{2b}$ in serum and in intestinal tissue; parasite specific IgG and total IgE in serum; parasite specific and total IgA in intestinal tissue and rat mast cell protease II in intestinal tissue. Parasite specific $IgG_1$, $IgG_{2a}$ and total IgE in serum and parasite specific IgA and rat mast cell protease II in intestinal tissue all occurred at significantly greater concentrations in infected animals, compared with non-infected animals. Similarly, the production of IL-4 by MLN cells stimulated with parasitic female antigen or ConA occurred at significantly greater concentrations in infected animals, compared with non-infected animals. In all, this suggests that there is a Th2-type immune response during a primary *S. ratti* infection. These data also show the temporal changes in these components of the host immune response during a primary *S. ratti* infection.

### Keywords

*Strongyloides*; T-helper 2

## Introduction

Host immune responses reduce the fitness of parasitic nematodes. For *Strongyloides ratti* in its rat host, this is manifest as a reduction in the size of the parasitic female stages, a consequent reduction in their *per capita* fecundity, the adoption of a more posterior position in the host gut and, ultimately, the death of these stages1,2,3. That these effects are dependent on the host immune response is shown because these effects do not occur in athymic, nude rats; are reversible if the parasitic stages are transferred surgically to naïve hosts, or if hosts are immunosuppressed1,2,4,5,6. The host immune response is also required for negative density-dependent effects on the survival and *per capita* fecundity of *S. ratti*7. In addition, the host immune response also affects the developmental route of the free-living stages of the *S. ratti* life-cycle8,9. All of these effects mean that, ultimately, the host immune response will have significant effects on the population biology of *S. ratti*.

In the *S. ratti* life-cycle, free-living infective third stage larvae (iL3s) infect hosts by penetrating their skin, after which they migrate *via* the naso-frontal region of the head,10 from where, presumably, they are swallowed; during this migration they moult *via* the L4 stage into parasitic stages. The parasitic stages of *S. ratti* are female only, which reproduce

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by parthenogenesis11; reproduction commences from approximately four days post infection (p.i.). The parasitic females are embedded in the mucosa of the small intestine of the host, through which they migrate, producing eggs which then pass out of the host in faeces.

In *S. ratti* infections in rats there are distinct immune responses that act against the migratory and enteric phases12. The presence of intestinal parasitic females induces protection against parasitic stages that are either implanted directly or develop naturally from subcutaneous iL3s13. However, the presence of the migratory phase of the *S. ratti* life-cycle only induces partial protection against directly implanted parasitic stages14. Protection against migrating larvae can be induced by the transfer of serum from *S. ratti*-infected to recipient *S. ratti*-naïve rats and this protective effect is greater for an $IgG_1$ enriched fraction15. Analogous transfer of mesenteric lymph node (MLN) cells also transfers an anti-*S. ratti* effect that is effective against intestinal parasitic females16.

The immunoglobulin response of rats to *S. ratti* infections is biased towards IgG and IgE isotypes. Temporal analysis of the immune response to repeated *S. ratti* infections of different doses has shown that IgG responses are observed to occur in a dose-dependent manner from approximately two weeks p.i., asymptotically approaching its maximum approximately four weeks p.i.17. The IgE response is also related to dose, but was not observed to develop until three weeks p.i., after which it rapidly increases17. Rats infected with a high dose of *S. ratti* have raised MLN cell and peripheral blood lymphocyte blastogenesis prior to and approximately co-incident, respectively, with the loss of *S. ratti* stages passed in faeces18. In these same animals, anti-*S. ratti* iL3-specific IgG was greatest approximately 30 days p.i., after which it declined18. However, the *S. ratti*-specificity of these effects is not clear because no non-*S. ratti* infected controls were used in these observations. Passive cutaneous anaphylaxis assays have shown that anti-*S. ratti* IgE titres were greatest 30 days p.i., after which they declined though, again, the *S. ratti*-specificity of these effects is not clear because no non-*S. ratti* infected controls were used19. Repeated administration of *S. ratti* iL3s reduced these IgE responses; these responses also appear to be greatest in response to the intestinal parasitic stages, rather than the iL3, or subsequent migrating larval stages19.

There has been extensive immunological analyses of *S. ratti* infections in mice. Mice are a non-natural host of *S. ratti* and infections of mice are shorter lived compared with infections in rats. The significance of immunological findings in this abnormal host-parasite combination must therefore be in question20.

Experimental work with *S. ratti* infections in mice has implicated intestinal mast cells in limiting the course of such infections, as has been observed for a number of other helminth infections21. Administration of IL-13 both increased small intestine mastocytosis and decreased survival of parasitic stages transferred directly to the gut22. *S. ratti* infections of mast cell deficient mice are more fecund and longer lived compared with infections of normal, control mice23. Administration of IL-13 to *S. ratti*-infected nude mice resulted in an increased loss of *S. ratti* parasitic females concomitant with an increased intestinal mastocytosis, compared with control non-IL-13 treated nude mice24.

Given the relative paucity of immunological analysis of *S. ratti* infections in rats, we have undertaken a comprehensive analysis of the temporal change in a range of immunoglobulin isotypes, in both serum and tissue of the small intestine, as well as cytokine responses of lymphoid cells during a primary *S. ratti* infection. The purpose of doing this was therefore to determine which components of the host immune response changed in response to *S. ratti* infection, at what time these changes occurred and at which sites within a host these changes

occurred. There are no such extant data, of which we are aware, and we therefore wished to determine this baseline information prior to undertaking other immunological analyses of *S. ratti* infections in rats. Immunological analysis of a range of parasitic nematode infections has shown that the immune response generated by these infections is typically of a T-helper 2 (Th2)-type response25,26. In view of this we particularly measured $IgG_1$ and IgA immunoglobulins and the production of IL-4 as indicators of a Th2-type immune response and $IgG_{2a}$ and $IgG_{2b}$ immunoglobulins and the production of IFN-γ as indicators of a Th1-type immune responses. In all, these data therefore provide a firm foundation for other, future immunological analyses of *S. ratti* in its natural rat host.

## Materials and Methods

### Parasites and infections

*Strongyloides ratti* isofemale line ED321 Heterogonic was used throughout and maintained by serial passage in female Wistar rats as previously described27.

To investigate the immune response of rats during a *S. ratti* infection, 28 female Wistar rats (*c.* 100g) (B & K Universal, UK) were randomly allocated equally to one of two groups. The experimental group were infected with 500 iL3s of *S. ratti* by subcutaneous injection27. The control group were given a sham inoculation of phosphate buffered saline (PBS) only. To determine the reproductive output of the infections, faecal material was collected from two animals from each group on days 7, 9, 13, 16, 20, 23 & 27 p.i. and the faeces cultured and maintained for two days at 25°C after which the total number of worms that developed was determined, as previously described7. These analyses therefore encompass the time during which parasitic females are present in the host gut.

To prepare antigen from parasitic females for use in immunological assays (below) parasitic females were recovered from *S. ratti*-infected rats, concentrated by centrifugation and resuspension and cleaned on a Percoll gradient, resuspended in PBS, snap frozen in liquid nitrogen and stored at −20°C until required, as previously described3. Pools of such parasitic females were homogenised, the resulting homogenate centrifuged at 14,000**g** at 4°C for 5 min., the supernatant removed and the protein concentration determined against bovine serum albumin (BSA) standards using Bradford's reagent28. This parasitic female antigen was stored at −20°C until required. Infective third stage larvae antigen was also prepared. To do this, iL3s were obtained from faecal cultures, cleaned by repeated sedimentation and resuspension in water, after which they were resuspended in PBS and then treated as for the parasitic female antigen preparation (above).

To prepare hyperimmune rat serum, rats were infected at least twice with 1,000 *S. ratti* iL3s one month apart, the rats sacrificed one month after the last infection and blood taken by cardiac puncture. This was allowed to coagulate for one hour at room temperature (RT) and was then centrifuged at 14,000**g** at 4°C for 5 min., and the serum harvested and then stored as aliquots in fresh tubes at −20°C.

### Serum and tissue sampling

Animals from which faeces were collected were sacrificed on the following day, *i.e.* days 8, 10, 14, 17, 21, 24 & 28 p.i., respectively. Blood samples were taken immediately after sacrifice by cardiac puncture and processed, as described above. The spleen and MLN were recovered by dissection and immediately placed in RPMI media (RPMI 1640, 10% v/v foetal calf serum, 2mM L-glutamine, 100U/ml penicillin, 100ug/ml streptomycin, 0.05 mM 2-mercaptoethanol) (Gibco, UK) on ice. Spleens cells were prepared by gently passing the spleen through a 100μm cell sieve and washing through with ice cold RPMI media; the cell

suspension was centrifuged at 400**g** at 4°C for 5 min. and the sedimented cells resuspended in RPMI media. Red blood cells were lysed with 5ml ACK buffer (155mM $NH_4Cl$, 10mM $KHCO_3$, 0.1mM EDTA, pH 7.2-7.4) for 5 min., diluted with further RPMI media and the cells sedimented by centrifugation as above, after which they were finally resuspended in fresh RPMI media. MLN cells were prepared in the same manner except that there was no treatment with ACK buffer; instead of this step, the cells were sedimented and resuspended in RPMI media. Trypan blue exclusion was used to determine the concentration of viable spleen and MLN cells.

The small intestine was also collected by dissection and stored at −20°C prior to processing. At least four samples from along the small intestine to a total mass of approximately 1g were taken and homogenized in 4ml of ice cold PBS / 1g of tissue; the homogenate centrifuged at 5,500**g** at 4°C for 15 min., the supernatant removed to a fresh tube and then re-centrifuged at 16,100**g** at 4°C for 10 min. and the supernatant stored at −20°C.

## Immunological assays

The components of the rats immune response that were measured are as follows: interleukin-4 (IL-4) and interferon-γ (IFN-γ) from both the spleen and MLN cells; parasite specific $IgG_1$, $IgG_{2a}$ and $IgG_{2b}$ in serum and in intestinal tissue; parasite specific IgG in serum; total IgE in serum; parasite specific and total IgA in intestinal tissue and rat mast cell protease II (RMCPII) in intestinal tissue.

For assays of IL-4 and IFN-γ production from the spleen and MLN cells, $1 \times 10^6$ cells (above) were stimulated, in triplicate, with (i) concanavalin A (ConA) (Sigma, UK), (ii) parasitic female antigen, both at a final concentration of 5μg/ml or (iii) RPMI media only in a total volume of 200μl. Cells were incubated at 37°C in a 5% carbon dioxide atmosphere for 48 hr. after which the resultant supernatants were recovered and stored at −20°C. The concentration of IL-4 and IFN-γ were assayed with commercially available sandwich ELISA kits (GE Healthcare, UK) following the manufacturer's instructions. The mean concentration of IL-4 and IFN-γ of the triplicate assays for the parasitic female antigen and ConA stimulations are reported as above the control background, *i.e.* stimulation with RPMI media only.

The concentration of parasite specific immunoglobulin was assayed using indirect capture ELISAs. Samples were used in duplicate in a doubling serial dilution; 100μl BSA-PBS-Tween (0.5% w/v BSA in 0.1% v/v Tween-20 in PBS) was used for negative controls. ELISA plates were coated with 100μl parasitic female antigen at a final concentration of 5μg/ml in 0.05M carbonate-bicarbonate buffer (pH 9.6) (Sigma, UK) and incubated overnight at 4°C. Plates were then washed in 300μl PBS-Tween three times and tapped dry; they were then blocked with 100μl BSA-PBS-Tween and incubated at RT for 1hr. Plates were washed again (as above) and then 100μl of sample or negative control were added and diluted in BSA-PBS-Tween. The plates were incubated at RT for 2hr. Plates were then washed, as above, and 100μl of detection antibody in BSA-PBS-Tween at the appropriate dilution (1:800, goat anti-rat IgA-Horseradish peroxidase conjugate (HRP); 1:3000, goat anti-rat $IgG_1$, $IgG_{2a}$, $IgG_{2b}$ and IgG-HRP conjugates) (Nordic, Netherlands) were added and the plates incubated at RT for 2hr. Plates were then washed again and 100μl 0.04% w/v o-phenylenediamine dihydrochloride (Sigma, UK), 1.2% v/v hydrogen peroxide in 0.05M phosphate-citrate buffer (pH 5.0) (Sigma, UK) added; plates were kept in the dark at RT for approximately 10 min. Reactions were stopped with 50μl of 3M sulphuric acid per well and optical densities were measured at 490nm with a background reading at 415nm, using a microplate reader (Bio-Rad, UK). For analysis of parasite specific IgA all incubation steps were for 1hr, rather than 2hr (above).

For analysis of total IgE and total IgA, the same procedure was used except that the plates were coated with IgE and IgA capture antibodies, respectively, at 2.5μg/ml (goat anti-rat IgE, IgA) (Nordic, Netherlands); the detection antibody was a 1:1600 dilution of goat anti-rat IgE-HRP conjugate and a 1:800 dilution of goat anti-rat IgA-HRP conjugate (Nordic, Netherlands), respectively, and the incubation steps were 1hr. rather than 2hr., as above.

For analysis of IgG and $IgG_1$ (above), standard curves were constructed using a dilution series of hyperimmune rat serum in place of the experimental samples (above); otherwise, the ELISAs were performed exactly as described above. The concentrations of the experimental samples were expressed as proportions of the hyperimmune serum control. For analysis of $IgG_{2a}$, $IgG_{2b}$, IgA, IgE, standard curves were constructed using a dilution series of purified recombinant antibody. This was done because insufficient of these isotypes were detected in hyperimmune serum. To do this plates were coated with 5μg/ml goat anti-rat $IgG_{2a}$, $IgG_{2b}$ or 2.5μg/ml goat anti-rat IgA or IgE (Nordic, Netherlands), respectively, overnight and in place of the samples (above) known concentrations of recombinant $IgG_{2a}$, $IgG_{2b}$, IgA or IgE (Serotec, UK), respectively, were used; otherwise, the ELISAs were performed exactly as described above. The concentrations of the experimental samples were expressed as equivalents of the standard, recombinant antibody in ng/ml.

The concentration of RMCPII in intestinal tissue was determined using a commercially available indirect sandwich ELISA kit (Moredun Scientific Ltd., UK) which was used following the manufacturer's instructions.

To compare the efficacy of parasitic female antigen and iL3 antigen as the coating antigen in the ELISA, plates were coated with 5μg/ml parasitic female antigen (as above) or with iL3 antigen at a range of concentrations from 1 - 50 μg/ml, inclusive. A dilution series of intestinal tissue homogenates and serum samples from two rats from which hyperimmune serum was prepared (above) were used as the test samples in the ELISA, which, otherwise, was performed exactly as described above; the detection antibody was goat anti-rat IgG-HRP conjugate (Nordic, Netherlands).

### Statistical analysis

ELISA optical density readings below the limit of the respective standard curve were set to zero. This resulted in left censored data for all immune measures, such that a Tobit model29 was used with this skewed distribution. A Tobit model assumes that above the threshold for censored data, the data are independent and normally distributed. Where necessary, data were first normalised using a Box-Cox transformation and then fitted with a Tobit regression model using the Survreg function in *R* (www.r-project.org), according to procedures previously outlined30. For each immunological measure the factors of $_{TREATMENT}$ (*i.e.* infected or non-infected), $_{TIME}$ p.i. and their interaction ($_{TREATMENT}$*$_{TIME\ P.I.}$) were fitted and the minimal model determined by deletion testing to generate a $\chi^2$ statistic. The significance of the effect of these individual factors and their interactions are reported throughout the results section. In addition, for each of the immunological parameters the minimal model, and the significance of the difference between this and the null model, is reported in the figure legends. Day 21 p.i cytokine data were excluded from the analysis due to the interruption of the carbon dioxide supply to these cells during their stimulation.

## Results

### Infections

The mean *S. ratti* reproductive output was greatest at 7 days p.i., the first day of sample, after which it declined (Figure 1). At days 23 and 27 p.i., no *S ratti* stages were detected in faeces. Similarly, none were detected in the control, non-infected group at any time.

## Immunological assays

**Serum—**Parasite specific IgG, $IgG_1$, $IgG_{2a}$ and $IgG_{2b}$ and total IgE were all detected in serum. Infected animals had a significantly greater concentration of parasite specific $IgG_1$, compared with non-infected animals, which increased during the course of the infection to a maximum from day 24 p.i. ($_{TREATMENT}\ \chi_1^2 = 34.55$, $p < 0.0001$; $_{TIME}$ p.i. $\chi_1^2 = 30.17$, $p < 0.0001$) (Figure 2a). There was no statistically significant difference in the concentration of IgG between infected and non-infected animals (Figure 2a). However, visual inspection of the data suggests that there is some small increase in IgG during an infection in infected animals reaching its maximum at days 24 and 28 p.i. (Figure 2a). The temporal profile of $IgG_1$ and IgG (Figure 2a) is qualitatively similar, which may suggest that $IgG_1$ is the dominant feature of the measured IgG response.

Infected animals also had a significantly greater concentration of parasite specific $IgG_{2a}$, compared with non-infected animals, which increased during the course of the infection ($_{TREATMENT}\ \chi_1^2 = 15.43$, $p < 0.0001$; $_{TIME}$ p.i. $\chi_1^2 = 4.32$, $p = 0.037$) (Figure 2b). There were no significant effects of $_{TREATMENT}$ or $_{TIME}$ p.i. on parasite specific $IgG_{2b}$ (Figure 2b). The absolute concentration of parasite specific $IgG_{2b}$ was very low compared with $IgG_{2a}$ (Figures 2b).

There was no difference in the concentration of total IgE produced by infected and non-infected animals, but the concentration did change during the experiment ($_{TIME\ P.I.}\ \chi_1^2 = 4.24$, $p < 0.039$) (Figure 3). Visual inspection shows that there is an apparent maximum production of IgE at the start of the experiment in both groups, perhaps due to the subcutaneous administration of material (parasites or PBS) (Figure 3).

**Intestinal tissue—**Infected animals had a significantly greater concentration of parasite specific IgA, compared with non-infected animals ($_{TREATMENT}\ \chi_1^2 = 6.83$, $p < 0.0089$) (Figure 4). Visual inspection of the data suggest that this also increased during the infection (Figure 4). There was no difference between infected and non-infected animals in the concentration of total IgA, though there is a suggestion that this increased during the course of the experiment in both groups of animals (Figure 4).

Infected animals had a significantly greater concentration of RMCPII, compared with non-infected animals ($_{TREATMENT}\ \chi_1^2 = 49.53$, $p < 0.0001$) and this increased during the course of the infection ($_{TIME\ P.I.}\ \chi_1^2 = 33.42$, $p < 0.0001$; $_{TREATMENT*TIME\ P.I.}\ \chi_2^2 = 23.90$, $p < 0.0001$) (Figure 5). Essentially no parasite specific $IgG_1$, $IgG_{2a}$, or $IgG_{2b}$ was detected in intestinal tissue (data not shown).

**Comparison of parasitic female and iL3 antigen sources—**The use of parasitic female antigen resulted in greater detection of IgG, compared with the use of iL3 antigen (Figure 6). This shows either that there is IgG immunological cross reaction between these two antigen sources and, or that the rat anti-*S. ratti* IgG immune response is made in response to both the iL3 and parasitic female stages, but with the latter predominating.

**Cytokines—**MLN cells of infected animals produced a significantly greater concentration of IL-4, compared with non-infected animals, when stimulated with *S. ratti* parasitic female antigen ($_{TREATMENT}\ \chi_1^2 = 16.02$, $p < 0.0001$) (Figure 7a). This production of IL-4 increased rapidly from approximately day 10 p.i., to an apparent maximum on day 17 p.i. The MLN cells of infected animals also produced a significantly greater concentration of IL-4, compared with non-infected animals, when stimulated with ConA; this also increased during the infection ($_{TREATMENT}\ \chi_1^2 = 12.49$, $p = 0.0004$; $_{TIME\ P.I.}\ \chi_1^2 = 9.73$, $p = 0.0018$) (Figure 7a). The absolute concentration of IL-4 produced in response to ConA stimulation was much lower compared to that produced in response to stimulation with parasitic female antigen.

The MLN cells also produced IFN-γ in response to stimulation with ConA, but there was no difference between infected and non-infected animals, and this production did change during the experiment (Time $\chi_1^2 = 14.9$, p = 0.0001). The MLN cells of infected animals produced a significantly greater concentration of IFN-γ, compared with non-infected animals, when stimulated with parasitic female antigen ($_{\text{TREATMENT}}\ \chi_1^2 = 7.1$, p = 0.0077) (Figure 7b). However, the absolute concentration of IFN-γ produced by MLN cells in response to parasitic female antigen was very low compared to that produced in response to ConA (Figure 7b).

Spleen cells stimulated with ConA produced IFN-γ, though there was no difference between infected and non-infected animals, and this did not change during the experiment (Figure 7c). There was essentially no such response to stimulation with parasitic female antigen (Figure 7c). Spleen cells stimulated with ConA or with parasitic female antigen produced a very low concentration of IL-4 (data not shown).

## Discussion

These results describe, for the first time, the immune response elicited by a primary *S. ratti* infection in rats, the host species in which the parasite has evolved. Overall, the observed immune response is consistent with the generation of a Th2-style response that becomes manifest some two to three weeks p.i. This is consistent with the now widely reported Th-2 response induced by helminth infections26.

There is a circulating specific anti-*S. ratti* $IgG_1$ response that increases during the infection, but which peaks after the worms have been expelled (Figures 1 and 2)3. These data are consistent with the previous demonstration of the passive transfer of anti-*S. ratti* immunity with serum, principally $IgG_1$15. There is also a circulating specific anti-*S. ratti* $IgG_{2a}$ response that increases during the infection (Figure 2a). This $IgG_{2a}$ response is not fully consistent with a Th2-type immune response. Comparison of iL3 and parasitic female antigen sources, show that there is a greater measurable IgG response against the parasitic female antigen source (Figure 6), which is consistent with this being the main source and target of the host's IgG response. In intestinal tissue, there is a specific anti-*S. ratti* IgA response which increases during the infection, and which like $IgG_1$, is at its maximum when the *S. ratti* infection is terminated (Figure 4). In the same tissue, there is an increase in the concentration of RMCPII, which approaches its maximum from approximately 17 days p.i., after which it remains elevated (Figure 5). This finding is consistent with the previous implication of mast cells in the clearance of *S. ratti* infections23,24. We observed an apparent total IgE response to both *S. ratti* infection and control sham infection (Figure 2). Previous studies also observed an increase in IgE titre following *S. ratti* infection, though, in view of our findings and the absence of control, non-infected animals in this previous work, this is unlikely to be *S. ratti*-specific19.

The principal cytokine response to stimulation with *S. ratti* parasitic female antigen of *S. ratti*-infected animals was the production of IL-4 by MLN cells, with this maximum at day 17 p.i., before the *S. ratti* infection is cleared (Figure 7a). MLN cells from *S. ratti* infected rats also produced a low level IL-4 in response to stimulation with ConA. In contrast, the dominant production of IFN-γ by these MLN cells was only in response to ConA, and this did not differ between infected and non-infected animals.

These findings have implications for understanding how hosts limit and terminate *S. ratti* infections. The peak anti-*S. ratti* circulating $IgG_1$ and intestinal IgA responses are temporally co-incident with the elimination of *S. ratti* infections, suggesting a causal link. Antibody-containing plugs in the oesophagus of *S. ratti* have been observed1 which have

been hypothesised to prevent feeding, and thereby, presumably, to reduce survival and reproduction; $IgG_1$ and IgA may therefore be directly involved in this. The intestinal RMCPII response may also be an anti-*S. ratti* effector response21.

The statistical analyses of these data have identified significant differences between infected and non-infected animals, despite there being only two animals within each group at each time point. With the exception of the RMCPII data, we did not detect significant interactions between $T_{REATMENT}$ and $T_{IME\ P.I.}$. However, visual inspection of many of these data suggest that these interaction effects may exist for some of the parameters measured (*e.g.* $IgG_1$, $IgG_{2a}$), but which may require greater sample size to be detected statistically.

The data presented here suggest that the relevant immunological measures of an *S. ratti* infection of this nature and duration are serum concentrations of parasite specific $IgG_1$ and $IgG_{2a}$; RMCPII and parasite specific IgA in intestinal tissue and IL-4 production by MLN cells stimulated with parasitic female antigen. Previously we have shown that there are immune-dependent negative density-dependent effects that act on *S. ratti*7 and it will therefore be important to investigate how the anti-*S. ratti* immune response described here is affected by parasite dose.

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## Abbreviations

| | |
|---|---|
| **BSA** | bovine serum albumin |
| **ConA** | concanavalin A |
| **HRP** | Horseradish peroxidase |
| **IFN-γ** | interferon gamma |
| **iL3s** | infective third stage larvae |
| **IL-4** | interleukin 4 |
| **MLN** | mesenteric lymph node(s) |
| **PBS** | phosphate buffered saline |
| **p.i.** | post infection |
| **RMCPII** | rat mast cell protease II |
| **RT** | room temperature |
| **Th2** | T-helper 2 |

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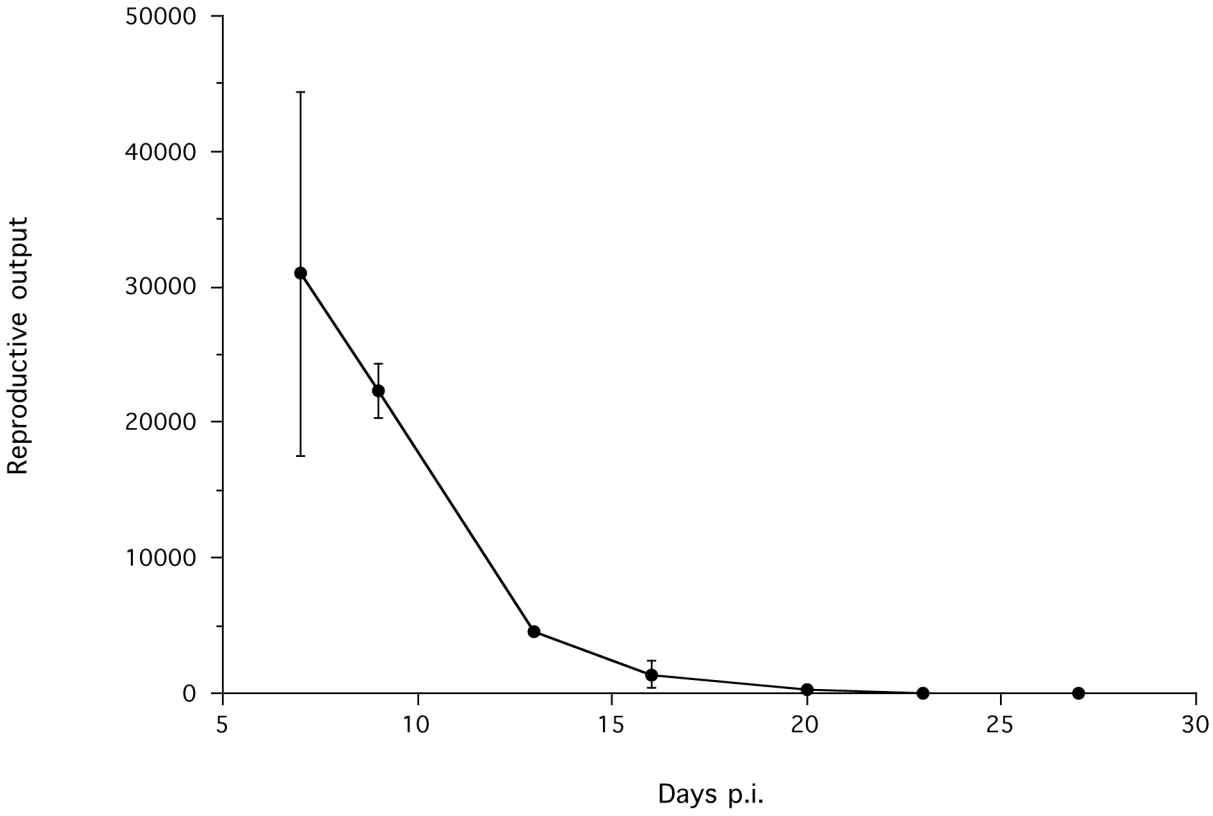


**Figure 1.**
The mean reproductive output of *S. ratti* infections. There were no *S. ratti* stages detected in non-infected control animals (not shown). Error bars are ±1 S.E.

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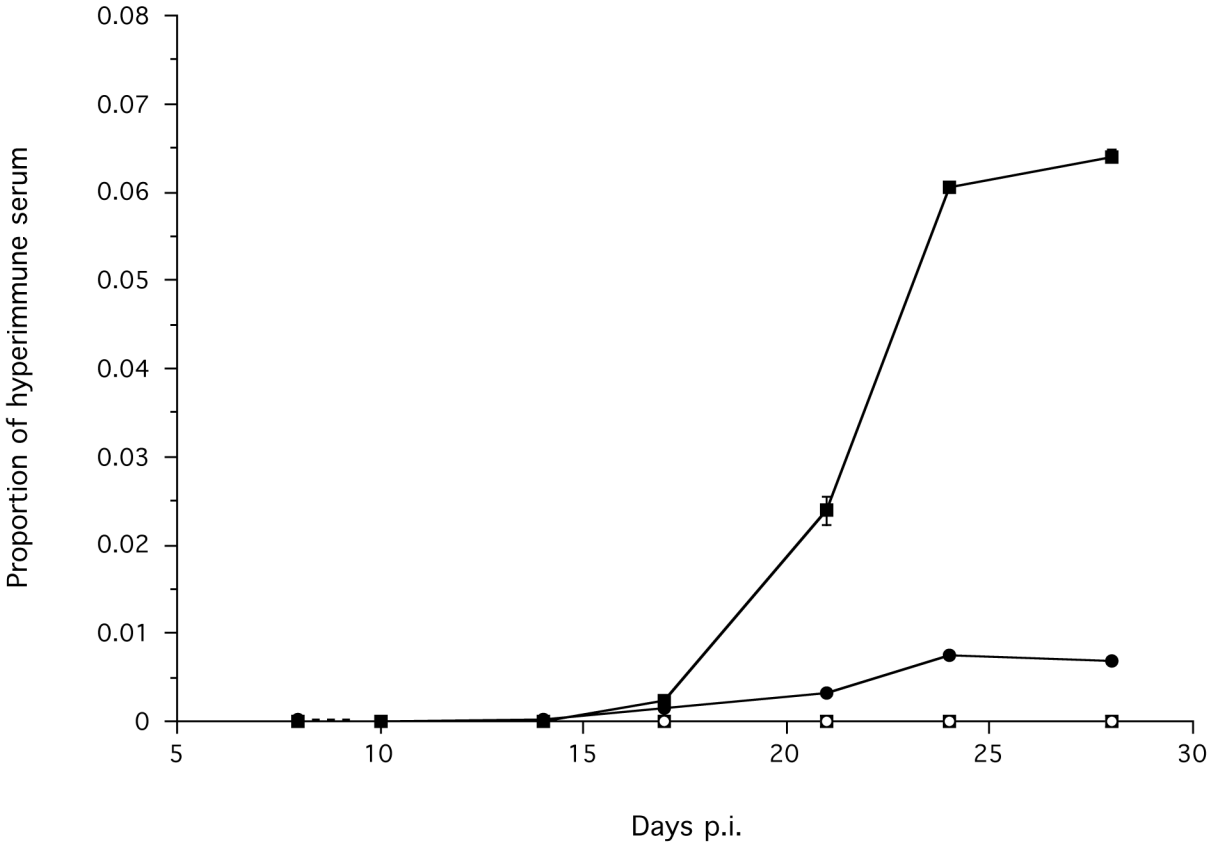
**2a**



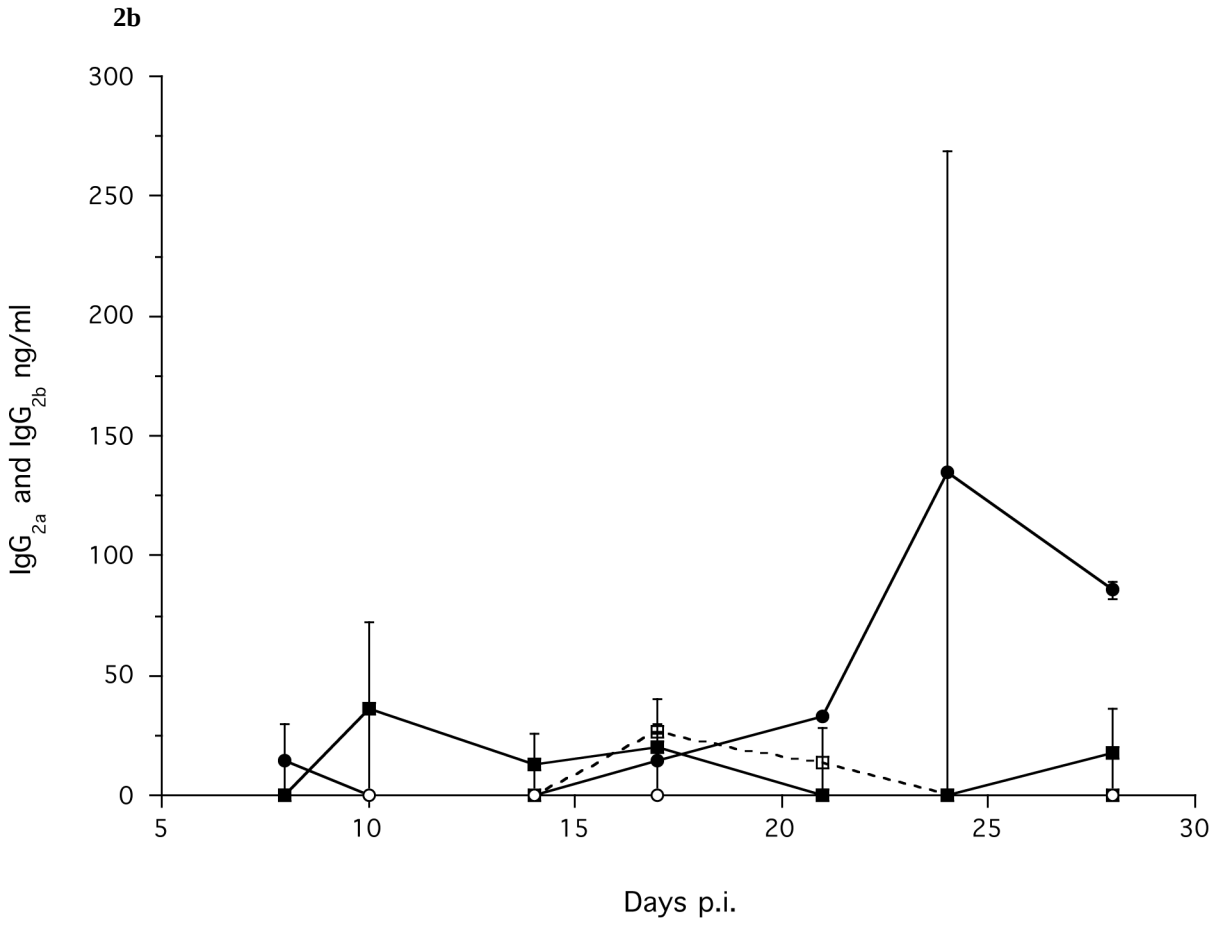



**Figure 2.**

The mean serum concentration of parasite specific (a) IgG (●) and $IgG_1$ (■), (b) $IgG_{2a}$ (●) and $IgG_{2b}$ (■) in infected (solid symbols, solid line) and non-infected control (hollow symbols, dashed line) animals during an infection. Error bars are ±1 S.E. Note the different scales. The mean values of proportion of hyperimmune serum in (a) were calculated from transformed data, but back transformed values are shown here. The minimum models are: $IgG_1 = -10.20 + 6.21 \times T_{REATMENT} + 0.270 \times T_{IME\ P.I.}$, $\chi_2^2 = 45.21$, $p < 0.0001$; $IgG_{2a} = = -6.52 + 5.49 \times T_{REATMENT} + 0.74 \times T_{IME\ P.I.}$, $\chi_2^2 = 18.11$, $p = 0.00012$.

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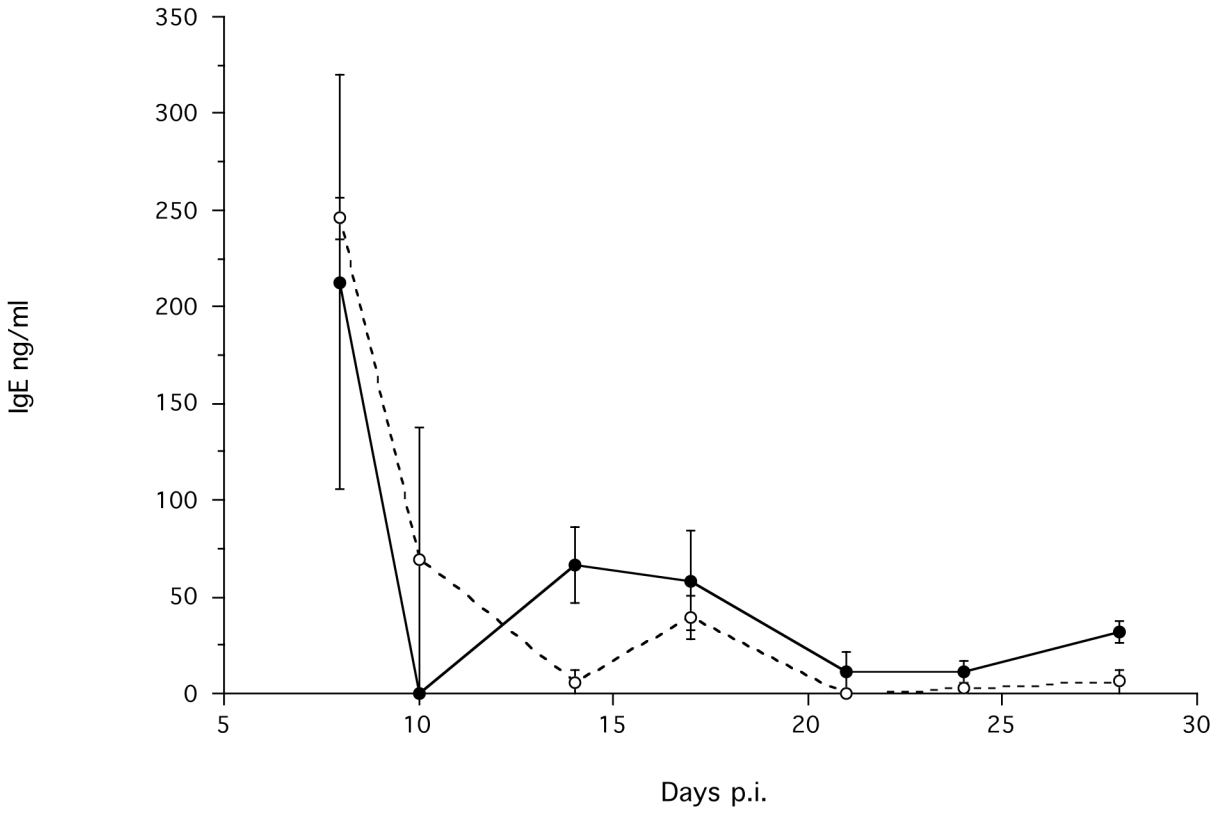


**Figure 3.**

The mean serum concentration of total IgE in infected (–•–) and non-infected control (--○--) animals during an infection. Error bars are ±1 S.E. The minimum model is IgE = −5.48 - 0.14×$T_{IME\ P.I.}$, $\chi_1^2 = 4.24$, p = 0.039.

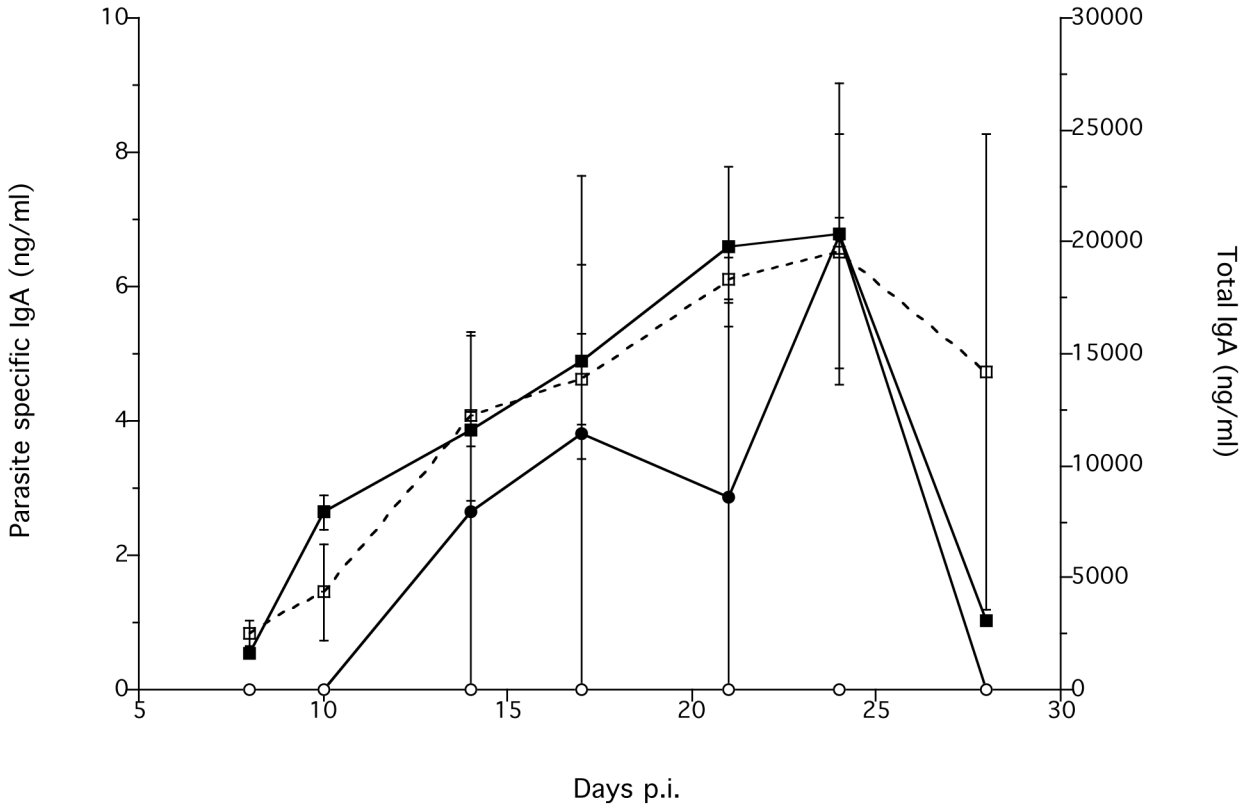


**Figure 4.**
The mean intestinal tissue concentration of parasite specific (●) and total IgA (■) in infected (solid symbols, solid line) and non-infected control (hollow symbols, dashed line) animals during an infection. Error bars are ±1 S.E. Note the different scales. The minimum model is parasite specific IgA = $-1.9\times10^{-18} + 0.70\times T_{REATMENT}$, $\chi_1^2 = 6.83$, p = 0.0089.

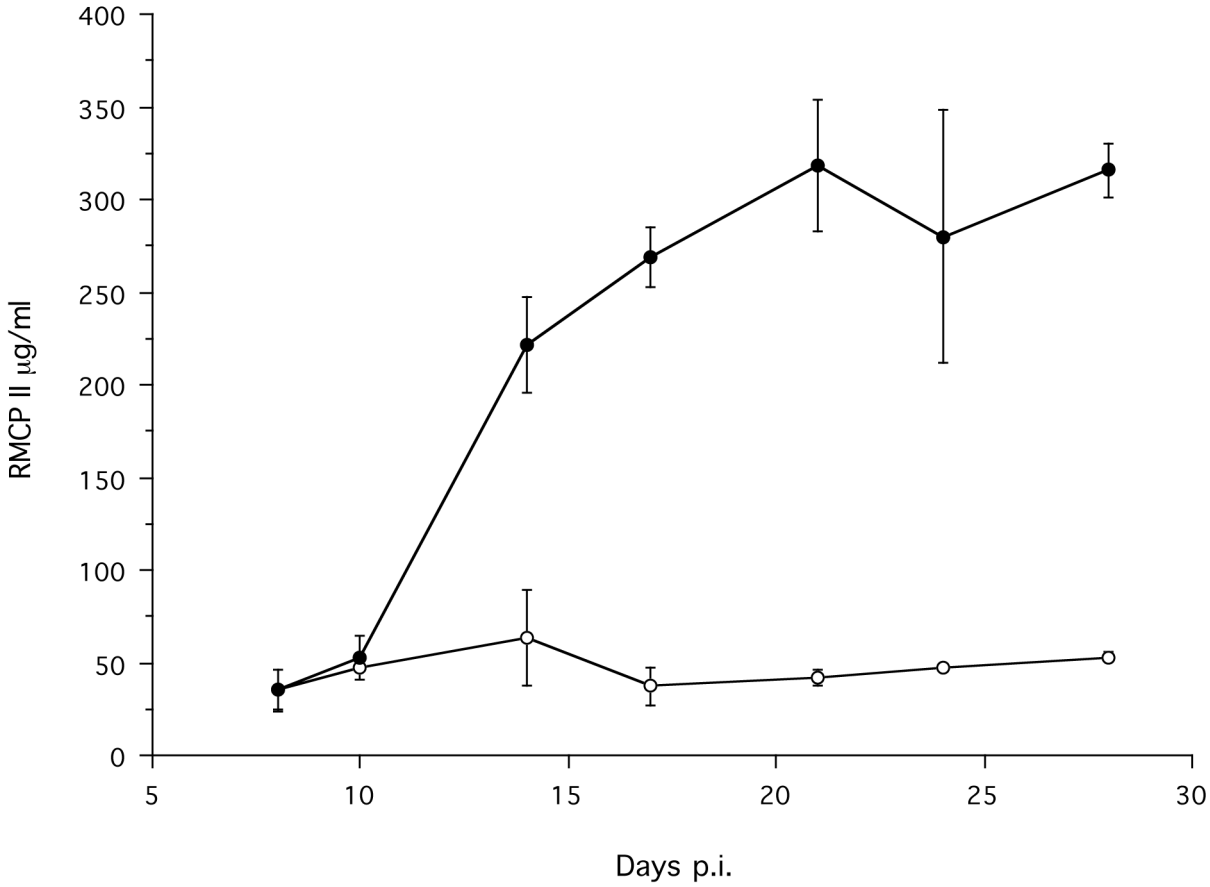


**Figure 5.**
The mean intestinal tissue concentration of RMCPII in infected (●) and non-infected control (○) animals during an infection. Error bars are ±1 S.E. The minimum model is $RMCPII = 64327 - 96814 \times_{TREATMENT} - 1391 \times_{TIME\ P.I.} + 16292 \times_{TREATMENT} *_{TIME\ P.I.}$, $\chi_3^2 = 53.6$, $p < 0.0001$.

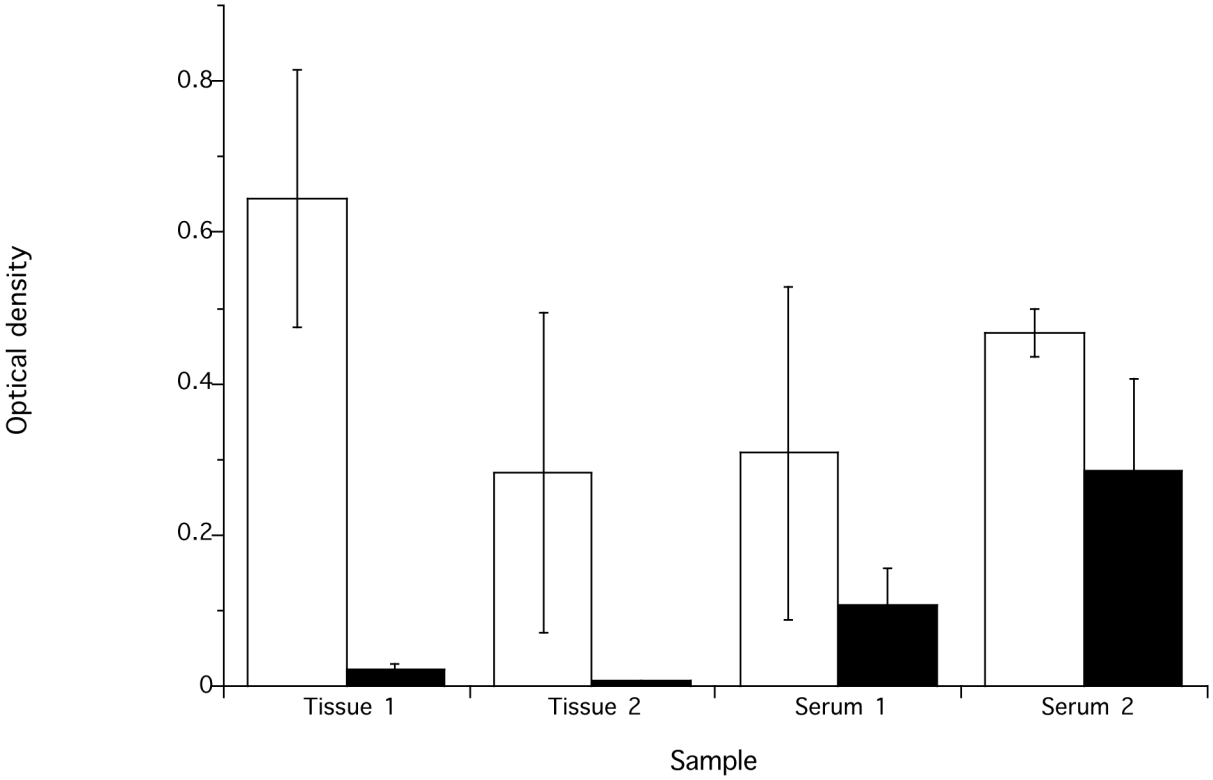


**Figure 6.**
The optical densities of 1/32 dilutions of two intestinal tissue homogenates and 1/50 dilutions of two serum samples with parasitic female (open bars) and iL3 antigen (black bars) both at a concentration of 5μg/ml. Error bars are ± 1 S.E.

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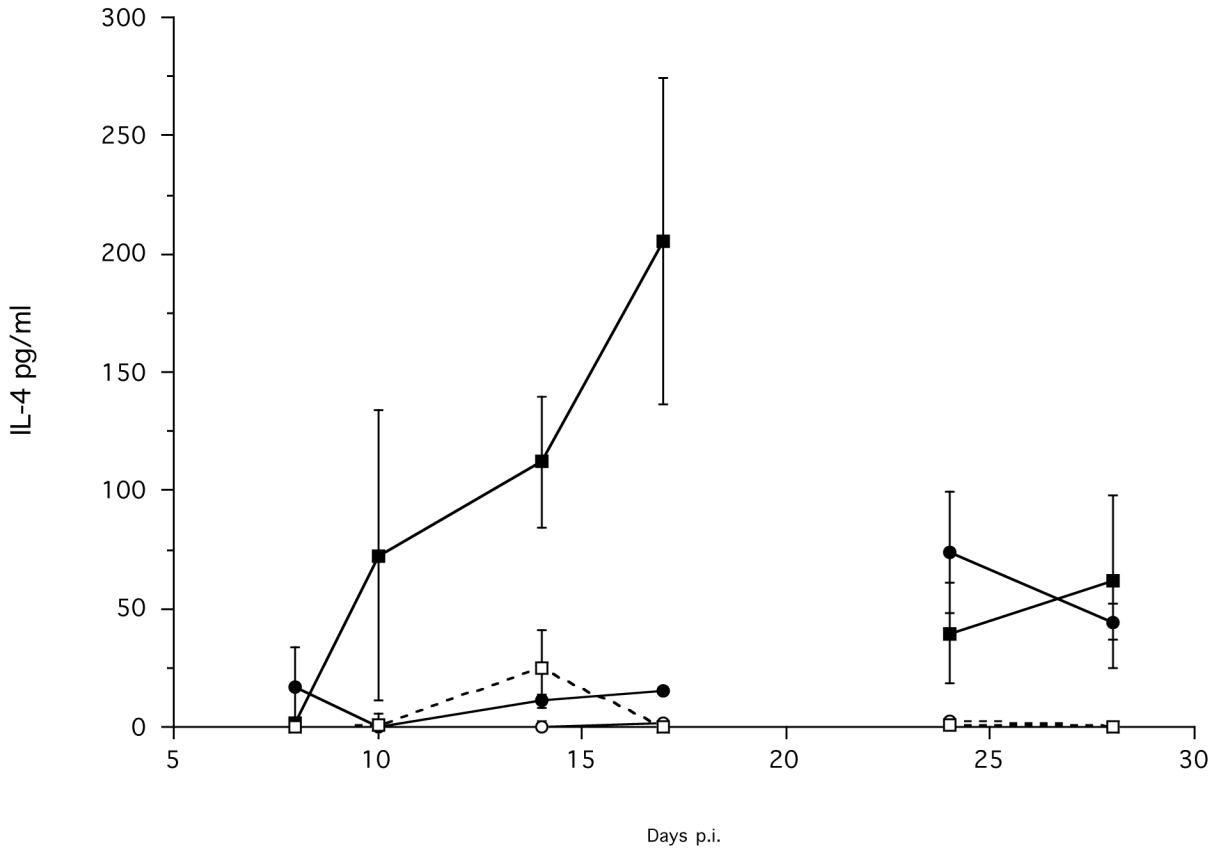
**7a**



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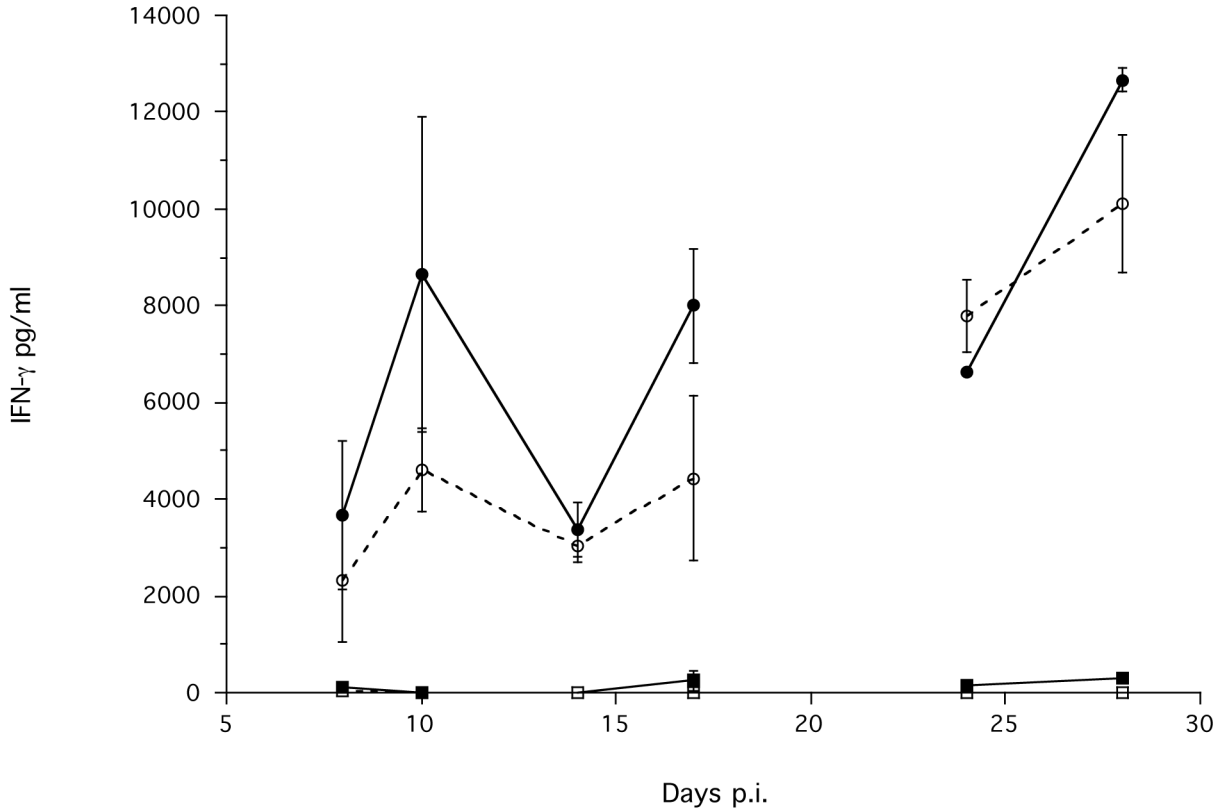
**7b**



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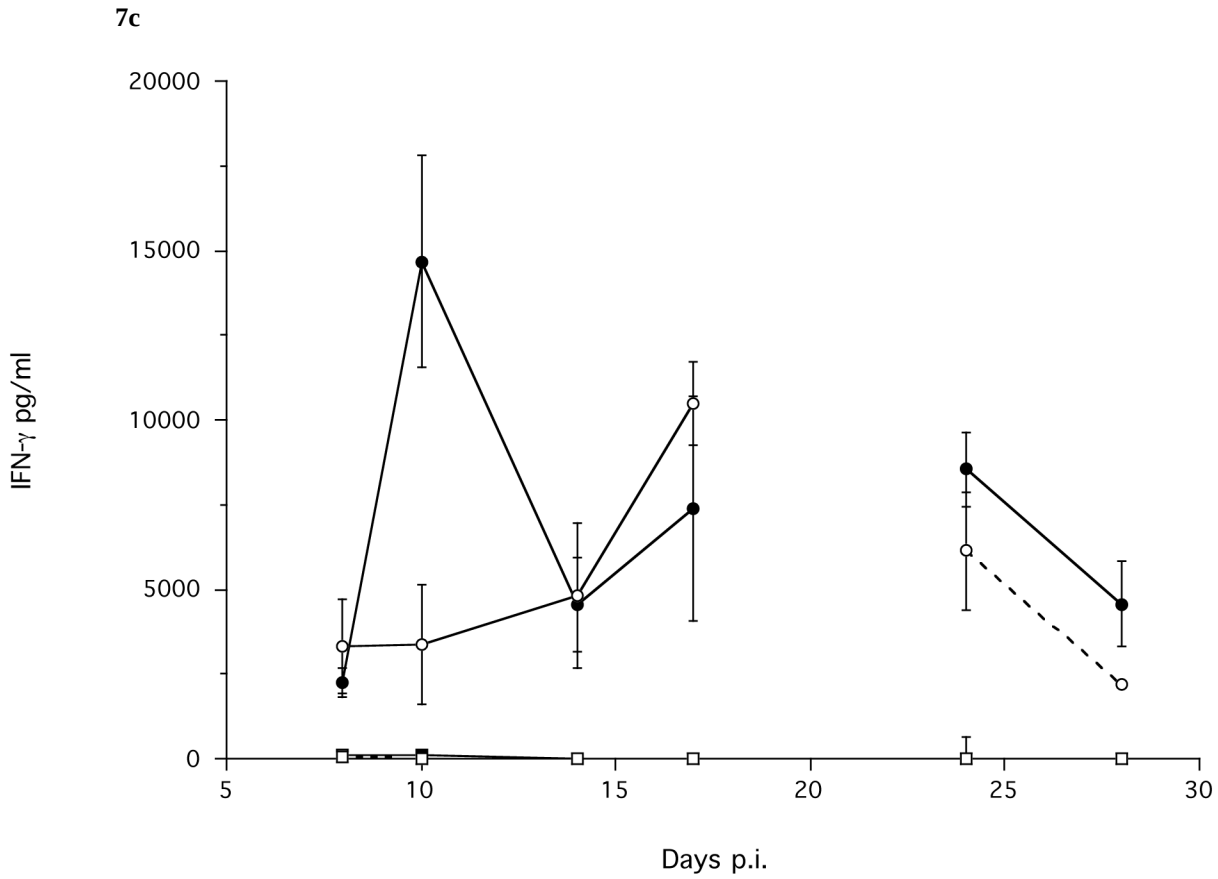


**Figure 7.**

The mean concentration of (a) IL-4 produced by MLN cells; IFN-γ produced by (b) MLN and by (c) spleen cells, stimulated with parasitic female antigen (■ or □) or ConA (● or ○) from infected (solid symbols, solid lines) and non-infected control (hollow symbols, dashed lines) animals during an infection. Error bars are ±1 S.E. Note the different scales. Data are not shown for day 21 p.i., due to the interruption of the carbon dioxide supply to these cells during their stimulation; see materials and methods. The minimum models are: IL-4 MLN stimulated with parasitic female antigen = $0.107 + 4.1\times_{\text{TREATMENT}}$, $\chi_1^2 = 16.02$, $p < 0.0001$; IL4 MLN stimulated with ConA = $-1.33 + 1.65\times_{\text{TREATMENT}} + 0.093\times_{\text{TIME P.I.}}$, $\chi_2^2 = 18.61$, $p < 0.0001$; IFN-γ MLN stimulated with ConA = $1195 + 320.8\times_{\text{TIME P.I.}}$, $\chi_1^2 = 14.9$, $p = 0.0001$; IFN-γ MLN stimulated with parasitic female antigen = $-0.31 + 0.95\times_{\text{TREATMENT}}$, $\chi_1^2 = 7.1$, $p = 0.0078$.