

Early stages of *Ascaris suum* induce airway inflammation and hyperreactivity in a mouse model

C. S. ENOBE,¹ C. A. ARAÚJO,¹ A. PERINI,² M. A. MARTINS,² M. S. MACEDO³ & M. F. MACEDO-SOARES¹

¹Laboratory of Immunopathology, Butantan Institute, ²Department of Medicine and ³Department of Immunology, University of São Paulo, São Paulo, Brazil

SUMMARY

The inflammatory and functional changes that occur in murine lung after infection with 2500 infective Ascaris suum eggs were studied in this work. A sequential influx of neutrophils, mononuclear cells and eosinophils occurred into airways concomitantly with migration of larvae from liver to the lungs. Histological analysis of the lung showed a severe intra-alveolar haemorrhage at the peak of larval migration (day 8) and the most intense inflammatory cell infiltrate on day 14. Ascaris L3 were found in alveolar spaces and inside bronchioles on day 8. The number of eosinophils was elevated in the blood on days 8 and 14. The peak of eosinophil influx into the lung was at day 14, as indicated by the high levels of eosinophil peroxidase activity, followed by their migration into the airways. The antibody response against egg and larval antigens consisted mainly of IgG1 and IgM, and also of IgE and anaphylactic IgG1, that cross-reacted with adult worm antigens. Total IgE levels were substantially elevated during the infection. Measurement of lung mechanical parameters showed airway hyperreactivity in infected mice. In conclusion, the murine model of A. suum infection mimics the Th2-induced parameters observed in pigs and humans and can be used to analyse the immunoregulatory properties of this helminth.

Keywords airway hyperresponsiveness, antibody, *Ascaris suum*, helminth, inflammation

INTRODUCTION

Parasitic infections may be the commonest cause of chronic infection in humans. In many low income countries, it is more common to be infected than non-infected (1). Gastrointestinal helminth infections are extremely widespread and contribute significantly to both morbidity and mortality among humans and livestock in developing countries (2). Among parasitic helminths, the most prevalent in humans is *Ascaris lumbricoides*. It is estimated that around 1·5 billion people are infected in the world, mainly in tropical regions, and although this infection is generally chronic and not fatal, it is associated with significant morbidity (3). Besides, other *Ascaris* species, such as *Ascaris suum*, also have an important role in economic loss in infected animals (4). Studies with *A. suum* are believed to provide important information about the biology of other ascarid nematodes, especially human ascarids.

Ascaris suum is an intestinal roundworm of pigs and its life cycle is identical to that of *A. lumbricoides*. Therefore, it is a useful model for this important human parasite. In the mouse, the early larval migration behaviour mimics their behaviour in pigs (5). Briefly, the eggs hatch in the intestine and larvae penetrate the wall of caecum and upper large intestine; then, they move to the liver, most likely via the venous blood stream. After that, the larvae advance to the lungs where they develop, penetrate the alveolar space and migrate up the trachea to be swallowed once again, being eliminated in the faeces.

Recently, some authors provided new evidences that *A. suum* frequently infects humans (6–8). These findings, together with the longevity of infective *Ascaris* eggs, represent an important public health issue because of the widespread use of pig manure as a fertilizer, especially in the United States and Europe (9). It is also important to note that the pathogenesis of the disease and economic losses in livestock are to a large extent the result of the effects caused by migrating larvae (10).

Correspondence: Dr Maria Fernanda de Macedo Soares, Laboratory of Immunopathology, Butantan Institute, Av Vital Brasil, 1500, 05503-900 São Paulo SP Brasil (e-mail: fmacedo@usp.br).

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Ascaris suum infections, as well as other helminth infections, are characterized by mast cell hyperplasia, eosinophilia, and markedly elevated levels of circulating IgE (11–13). Elevated levels of anti-parasite IgE and eosinophilia have been associated with increased resistance to helminth infections and their pathogenesis (14–16). In addition to the increased number of circulating eosinophils, an infiltration of these cells can be noted at the site of the parasite migration and/or in local tissue fluids.

Since the migratory larvae go through the lungs, we decided to analyse the alterations provoked by them in this organ. The present work describes the inflammatory and functional changes that occur in murine lung after infection with *A. suum* infective eggs. For this, we determined the influx of inflammatory cells into BAL and the number of larvae present in lungs at different days after infection. We also measured the different isotypes as well as anaphylactic antibodies in the plasma of infected mice on days 8, 14 and 21. Furthermore, we verified the pulmonary function of these animals by measurement of conductance and dynamic compliance 2 weeks after infection.

MATERIALS AND METHODS

Experimental animals

BALB/c mice, weighing 18–22 g, were utilized for this study. All mice were housed in the COBEA (Brazilian Committee of Animal Experimentation)-approved animal facilities at the Butantan Institute. Three-month-old Wistar rats were used for passive cutaneous anaphylactic reactions. The animals were on a 12 h : 12 h light–dark cycle and temperature-controlled environment. They received food and water *ad libitum*.

Infection protocol

Ascaris suum adults worms were obtained at a slaughterhouse (FISA, Itapequerica da Serra, SP, Brazil) and eggs were collected from the uteri of females as previously described (17). The eggs were isolated from the worm's uterus and cultured to the infective stage in 0.1 N H₂SO₄ for 4–5 weeks, at room temperature, under light protection. Groups of 4–5 mice were inoculated by the intragastric route (with the help of a plastic cannula) with 0.2 mL of egg suspension containing 2500 infective eggs, and afterwards with 0.1 mL of H₂O to remove the remaining eggs out of the syringe and needle.

Recovery of larvae from lungs

The recovery of *A. suum* larvae was performed individually based on a protocol previously described (18). Briefly, the

tissue sample immersed in 3 mL of saline (38°C) was mixed with 3 mL of 2% agar solution (Difco, Bacto-Agar, Detroit, MI) and poured equally onto horizontal trays (lid of a microtitre plate, 8.0 cm × 12.0 cm × 0.8 cm) with disposable cloths. After the agar gel had solidified, the cloths with the adhering agar gels were placed in a vertical position in a conic tube filled with 45 mL of 0.9% warm saline and incubated at 37°C, overnight. After this incubation, the material was further incubated at 4°C for 24 h. The cloths with agar were discarded, the suspension was centrifuged at 200 *g* for 15 min, at 4°C, and the volume reduced by aspiration to 3–5 mL (depending on the amount of sediment). The number of larvae was counted with an inverted microscope at 10× magnification.

Bronchoalveolar lavage (BAL)

Mice were killed by an overdose of chloral hydrate. The tracheas were cannulated and the airway lumen was washed with 2 × 0.5 mL of PBS. The resulting BAL fluids were centrifuged at 170 *g*, for 10 min. The cell pellet was resuspended in 300 µL of PBS for cell counts. Total cell counts were performed in a haemocytometer using Trypan blue solution and differential cell counts in cytocentrifuge preparations were stained with HEMA-3 (Biochemical Sciences Inc., NJ, USA).

Eosinophil peroxidase assay

The eosinophil peroxidase (EPO) activity in lung homogenates was measured according to a method described earlier (19). Briefly, after perfusion with 10 mL of PBS, lungs were removed, weighed and homogenized to give a 5% (wt/vol) suspension in Hank's balanced salt solution (HBSS) and centrifuged (1500 *g*, 10 min). After lysis of red blood cells, the pellet was homogenized in HBSS + 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical Co, St. Louis, MO, USA) and frozen/thawed three times using liquid nitrogen. The lysate was centrifuged (1500 *g*, 4°C, 10 min) and the supernatant was used for EPO assay. To measure the enzymatic activity, the supernatant was placed in a 96-well plate (150 µL/well) followed by the addition of 75 µL of substrate (1.5 mM OPD and 6.6 mM H₂O₂ in 0.05 M Tris-HCl, pH 8.0). After 30 min at room temperature, the reaction was stopped by the addition of 75 µL of sulphuric acid and absorbance of the samples was determined at 492 nm.

Histology of lungs

Infected BALB/c mice were sacrificed on day 8 or 14 and the lungs were removed. The lungs were fixed in formaldehyde

and dehydrated gradually in ethanol (70%, 90%, 100%). After the dehydration, they were immersed in paraffin, sliced and haematoxylin-eosin stained. Non-infected mice were used as control groups.

Preparation of *Ascaris* antigens

The *A. suum* crude extract (ASC) was prepared as previously described (20). In summary, live worms mixed with borate-buffered saline pH 8.0 were homogenized in an Ultra Turrax apparatus and stirred overnight at 4°C. After centrifugation and dialysis of the supernatant against distilled water for 24 h at 4°C, it was centrifuged again, aliquoted and lyophilized. Egg and larval extracts were obtained by sonication for 10 min on ice, at 100 Hz⁻¹ and 6 pulses/sec (Sonics & Materials Inc., Danbury, CT). The eggs were previously treated with 5% sodium hydrochloride. After sonication, the suspension was spun at 15 000 *g* for 30 min at 4°C. The supernatant was harvested and kept at -20°C until use.

Measurement of antibody isotypes

Antibodies of different isotypes were measured by ELISA in plasma from infected and naïve mice. ELISA plates (Nalgene Nunc Int. Co, Rochester, NY, USA) were coated with 1 µg of antigen extracts from different stages of the *A. suum* life cycle in 0.05 M carbonate buffer, pH 9.5. The plates were incubated at 4°C overnight, and washed three times with PBS containing 0.05% Tween 20 (PBS/T). The wells were blocked with 200 µL of PBS containing 5% skimmed milk for 2 h at 37°C. After a new cycle of washing with PBS/T, serial dilutions of the plasma were added and incubated for 1 h at 37°C. After the incubation, the wells were washed three times with PBS/T and 100 µL of peroxidase-conjugated rat monoclonal antibodies (mAb) against mouse µ, γ1, γ2a, γ2b or γ3 chains were added. The reactions were developed with 0.5 M sodium citrate, pH 5.0, containing 5.5 mM o-phenylenediamine (OPD – Sigma Chemical Co, St. Louis, MO, USA) and 0.6 mM H₂O₂. The plates were read at 492 nm on an automated ELISA reader (Titertek-Multiskan, Flow Laboratories, Helsinki, Finland). The results were expressed as the difference obtained between the absorbance of infected and naïve plasma. A similar procedure was followed to measure total serum IgE except for the use of 1 µg of rat anti-mouse IgE mAb to coat the plates and peroxidase-conjugated rat anti-mouse ε chain mAb. The amount of IgE was determined by referring to a standard curve of mouse IgE. All the mAb against mouse Ig were developed in the Experimental Immunology Unit (UCL, Brussels) and are commercially available (Serotec, Kidlington, UK or Zymed Laboratory, San Francisco, CA).

Passive cutaneous anaphylaxis (PCA)

Plasma levels of anti-ASC IgE and anaphylactic IgG1 were measured by passive cutaneous anaphylaxis (PCA), as previously described (21). PCA reactions were performed in mice (for IgG1) or rats (for IgE), using a sensitization period of 2 or 24 h, respectively. Each animal was shaved on the back and received intradermal injections of serial dilutions of pooled plasma from each group. After the sensitization period, challenge was performed intravenously by injection of 250–500 µg of ASC in 0.25% Evans blue solution. The PCA titre was expressed as the reciprocal of the highest dilution that produced a lesion of more than 5 mm in diameter in triplicate tests. Variation of the PCA titre for the same sample between different triplicate tests was two-fold or less, as previously reported (22,23). The detection threshold of the technique was established at 1 : 5 dilution to avoid positive reactions due to a non-specific degranulation of mast cells.

Measurement of airway responsiveness

Conductance of the respiratory system (Gr_s) and dynamic compliance (Cr_s) were monitored in anaesthetized, tracheostomized, and ventilated mice in a Harvard 683 apparatus (Harvard Apparatus, South Natick, MA). Different doses of methacholine (0.01–10 mg/kg) were administered via a silastic catheter tied into the jugular vein and data were stored at 0.5, 1, 2, 3 and 4 min after agonist injection. Whole body plethysmograph pressure (from which changes in lung volume were derived) and tracheal pressure were transduced by differential pressure transducers. The outputs were amplified and transferred to a computer in which 16 consecutive breaths were stored to average one data-point. The Gr_s and Cr_s response to each agonist dose was expressed as percentage of baseline value obtained before the next dose of agonist. The results were analysed over a range of 40–80% of reduction in Cr_s and Gr_s. The percentage of reduction chosen to compare the groups depended on the sensitivity of each parameter (that represented the response of different compartments of the lung) to the agonist and the slope of the log dose–peak response curve to methacholine varied for each mechanical parameter according to the experimental model. The dose of methacholine required to reduce Gr_s to 70% (ED 70%) and Cr_s to 40% (ED 40%) of their control values was chosen as our data-point.

Statistical analysis

Values were expressed as means ± standard deviation. Parametric data were evaluated using one-way analysis of variance, followed by the Tukey test for multiple comparisons.

Nonparametric data were analysed by the Mann–Whitney *U*-test (PRISM 3.0). The differences were accepted as significant for $P < 0.05$.

RESULTS

Ascaris suum infection augments the influx of inflammatory cells into lungs and airways

In order to determine how *A. suum* infection influences pulmonary inflammation, we characterized the kinetics of cell migration into lungs and airways during 49 days after infection. These results were compared with the kinetics of larval migration into the lungs. In BALB/c mice infected with 2500 eggs, the number of larvae (L3) found in the lungs was 79.44 ± 25.56 on day 8 and declined rapidly to almost zero until day 14 (data not shown). The peak of larval migration was followed by an intense local inflammation. The total number of cells in BAL fluid (Figure 1a) was significantly

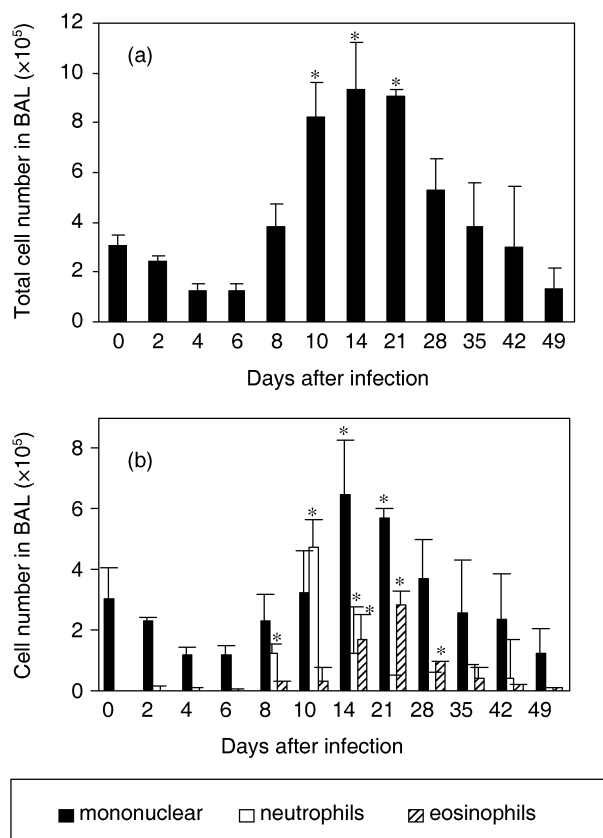


Figure 1 Kinetics of airway inflammation in *Ascaris suum*-infected mice. BALB/c mice were infected with 2500 infective eggs, and total (a) and differential (b) cell migration were analysed in the BAL fluid during the infection. Data represent the mean $\pm$ SEM for four mice/group. The results are representative of two repeats. * $P < 0.05$ compared with non-infected group (day 0).

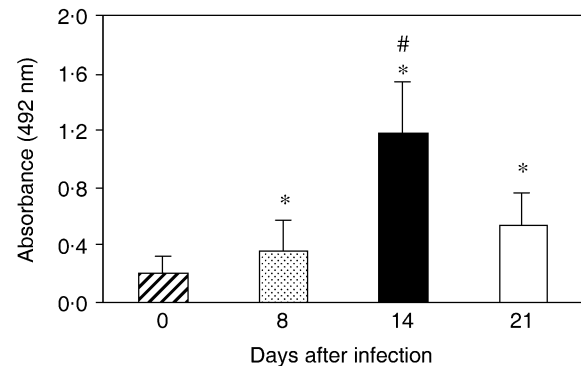


Figure 2 Measurement of EPO activity in lung tissue from *Ascaris suum*-infected mice. BALB/c mice were infected orally with 2500 infective eggs and sacrificed 8, 14 or 21 days later. The EPO activity was determined in the homogenate of the lung tissue. The results represent the mean $\pm$ SEM absorbance for five animals/group. * $P < 0.05$ compared with non-infected group (day 0). # $P < 0.05$ compared with other experimental groups. The results are representative of two repeats.

increased after day 10, reached a maximum on day 14 and declined to the control levels after 4 weeks. The differential cell counts (Figure 1b) showed a first influx of neutrophils, followed by mononuclear cells, and then by eosinophils into BAL. The greatest numbers of each of these cell types were found on days 10, 14 and 21, respectively.

To investigate the accumulation of eosinophils in the lung tissue, the presence of EPO was evaluated on days 8, 14 and 21 (Figure 2). EPO activity was increased in infected lungs from day 8 until day 21, but on day 14 was significantly higher than at the other time-points.

Table 1 also shows more circulating eosinophils in blood samples on days 8 and 14 after infection, being correlated with the peak of eosinophils in lung tissue and in BAL fluid. Mononuclear cells and neutrophils remained unchanged in the blood at the same time-points.

Detailed histological analysis of the lung from infected mice showed a severe intra-alveolar haemorrhage and an inflammatory cell infiltrate around the bronchioles on day 8 post-infection (Figure 3b). After 2 weeks, the inflammatory cell infiltrate was much more intense (Figure 3c). *Ascaris* L3 were found in the alveolar spaces (Figure 3d) and inside the bronchioles only at 8 days of infection.

Specificity of antibodies stimulated in infected mice

The profile of antibody isotypes against adult worm, egg and larval antigens was determined on days 8, 14 and 21 in plasma from infected mice. As shown in Figure 4, IgG1 and IgM antibodies reacted with egg and larval antigens, IgG1 being more reactive against L3. The same isotypes reacted

Table 1 Percentage of leucocytes in the blood of *Ascaris suum*-infected mice

Cell type	Non-infected mice	Infected mice ^a (8th day)	Infected mice (14th day)
Mononuclear	76.6%	66.2%	67.8%
Neutrophil	23.0%	28.8%	25.8%
Eosinophil	0.4%	5.0%*	6.4%*

^aBALB/c mice infected with 2500 embryonated eggs of *Ascaris suum* were bled at different time-points after infection and leucocytes were counted in blood smears stained with H/E ($n = 5$ mice/group). * $P < 0.001$ compared with control group (non-infected mice).

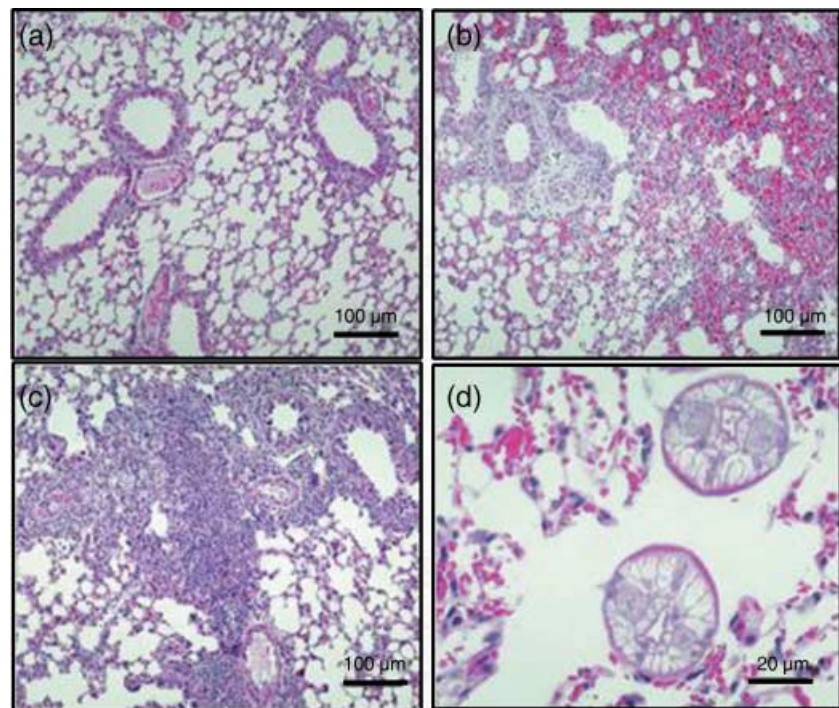


Figure 3 Histological alterations of lung tissue after *Ascaris suum* infection. Representative lung section from normal (a) or infected mice after 8 (b) or 14 days (c), showing severe intra-alveolar haemorrhage (b) and intense inflammatory cell infiltration (c). Larvae in the alveolar space on day 8 of infection (d). H/E staining.

also with adult worm extract, indicating a cross-reactivity among the antigens of all stages of the *A. suum* life cycle. A robust IgM response was observed during the whole infection. When plasma from naïve mice were added to the ELISA plates an absorbance of < 0.1 was obtained in the IgM assay and of < 0.05 in the assays for the other isotypes, indicating the antigen specificity of the antibodies detected in infected plasma.

***Ascaris suum* infection up-regulates the secretion of anaphylactic antibodies**

In the above tested plasma, the levels of anaphylactic antibodies were measured by ELISA (total IgE) and by PCA (antigen-specific IgE and anaphylactic IgG1). The amount of total IgE rose from < 0.04 µg/mL in non-infected mice to 6.87 ± 0.49 µg/mL in infected mice on day 8, 8.59 ± 1.01 µg/mL on day 14, and 21.66 ± 0.11 µg/mL on day 21. The same

kinetics was observed in antigen-specific IgE (Figure 5a) and anaphylactic IgG1 (Figure 5b) antibody production detected by PCA after ASC challenge.

Airway hyperreactivity is increased in infected mice

To measure the influence of *A. suum* infection on pulmonary function, airway hyperreactivity was evaluated regarding conductance (Grs) and dynamic compliance (Crs). The effect of the nematode infection on the mechanical parameters of the respiratory tract was evaluated by a dose-response curve to methacholine, 14 days after infection with 2500 infective eggs of *A. suum*. Values of Crs, which represents peripheral airway responsiveness, and Grs, which represents central airway responsiveness, were obtained by volume, airflow and pressure data. The results presented in Figure 6 are expressed as the log of the dose of methacholine that reduced to 40% and 70% the baseline values of

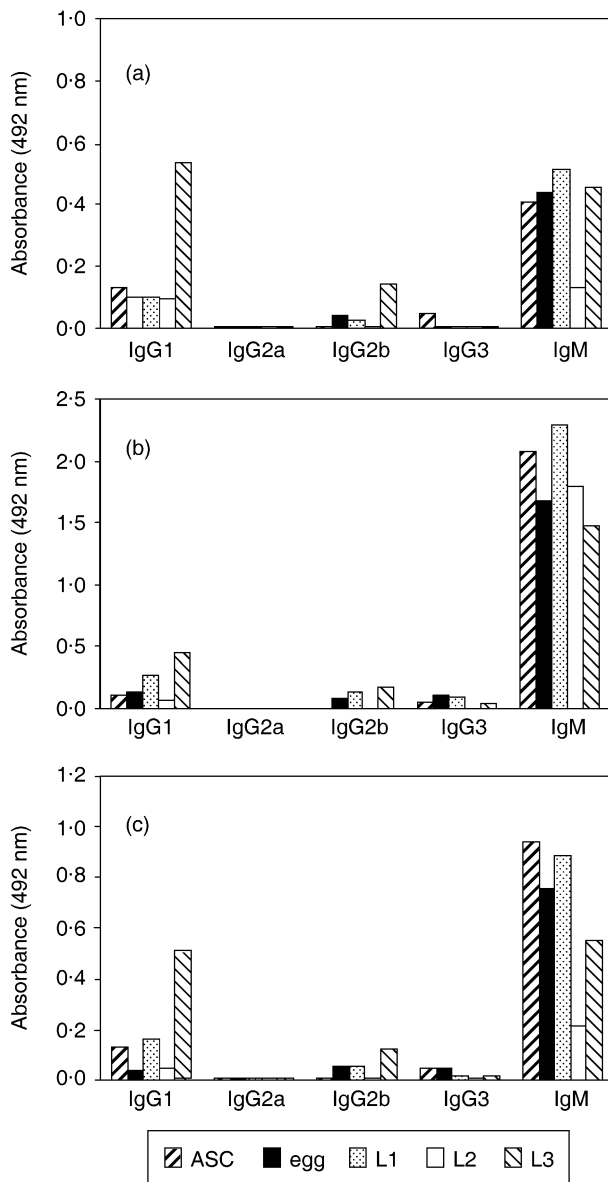


Figure 4 Profile of antibody production in *Ascaris suum*-infected mice. ELISA plates were coated with extracts from adult worms (ASC), eggs, L1, L2 and L3 larvae, and incubated with plasma from infected mice obtained after 8 (a), 14 (b) or 21 (c) days, followed by rat mAb against mouse isotypes. Data represent the increase in absorbance of pooled infected plasma above naive plasma at 1 : 20 dilution (five animals/group). The results are representative of three repeats.

Crs and Grs, respectively. In infected BALB/c mice lower doses of the agonist were required to reduce Crs (Figure 6a) and Grs (Figure 6b) compared with control group (non-infected mice), indicating an acute response when the agonist was administered.

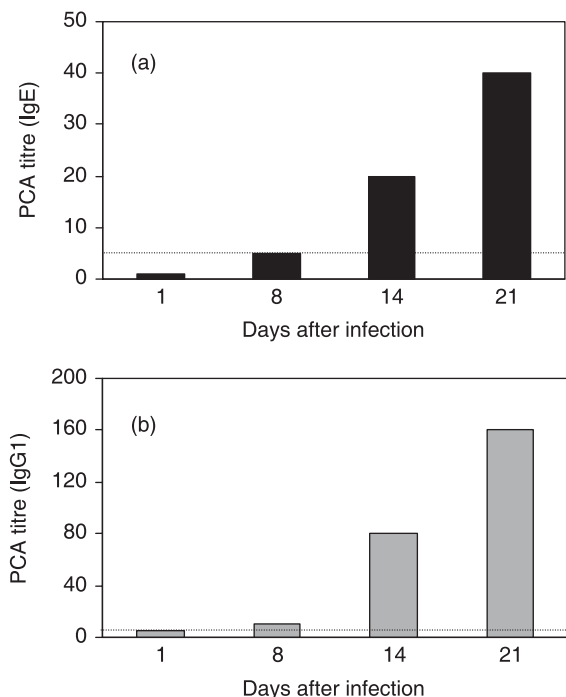


Figure 5 Anaphylactic antibody production in *Ascaris suum*-infected mice. IgE (a) and anaphylactic IgG1 (b) antibody titres were obtained by PCA in rats and mice, respectively, sensitized with plasma from infected mice and challenged with ASC. The PCA titre represents the highest dilution of pooled plasma that induced a positive reaction (> 5 mm of diameter) in a triplicate test (dotted line, detection limit of the assay). The results are representative of three repeats.

DISCUSSION

The ability of nematodes to modify the host's immune response has been an area of considerable research interest. An attempt to understand the relationship between these parasites and their hosts could elucidate the mechanisms of immune regulation involved in this interaction, contributing to the development of vaccines and novel therapeutic approaches in areas where helminth infections are endemic. In this study, we analysed the antibody immune response and pulmonary damage induced during *Ascaris suum* infection with regard to the influx of inflammatory cells into lungs and the changes to pulmonary function.

Our data showed that concomitantly with migration of L3 larvae to the lungs, infiltrating leucocytes, which consisted of mononuclear and polymorphonuclear cells, were found in lung tissue and in BAL fluid. The rise in neutrophil and eosinophil numbers in BAL fluid of infected mice was dramatic (more than 2800-fold) compared with non-infected mice, whereas mononuclear cell numbers increased only 2-fold. Circulating eosinophils were the only type of

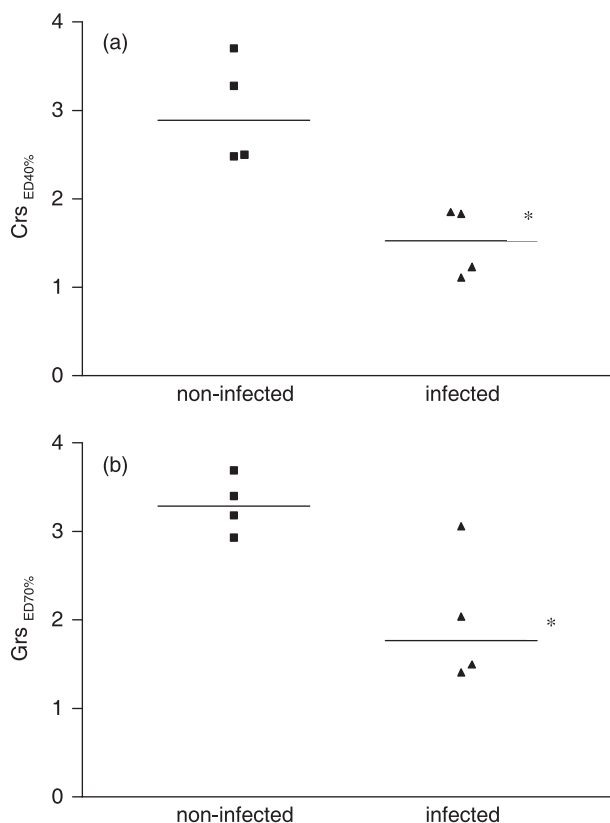


Figure 6 Effect of *Ascaris suum* infection on airway responsiveness. Two weeks after infection, mice were intravenously injected with different doses of methacholine and results expressed as doses required to reduce dynamic compliance (Crs) (a) to 40%, and conductance (Grs) (b) to 70% of their control values. Horizontal bars represent the mean values. * $P < 0.01$ compared with non-infected mice. The results are representative of two repeats.

leucocytes that increased substantially in the blood during the second week of infection. In addition, histology confirmed the extent of haemorrhage grossly evident that accompanied the peak of larval migration on day 8. The same pattern of cell infiltration and focal haemorrhage was observed in the lungs of rats infected with embryonated *A. suum* eggs (24). Haemorrhage was also detected in the lungs of dogs, guinea pigs, mice and humans infected with other types of helminths (25–28).

In our model, the peak of eosinophils in the infected lung tissue was at day 14, as demonstrated by the highest levels of EPO present at this time-point, followed by eosinophil migration into the airway lumen (peak at day 21 in BAL fluid). EPO is one of main components of eosinophil granules, a cationic protein representing approximately 25% of total granular content, and a specific component to these cells (29). The eosinophilia commonly observed in blood and tissues is a major hallmark of helminth infections,

especially during the invasive stages of the parasite (30). Such eosinophilia is also observed in *Ascaris suum* infections, as documented by this and other studies in the native host (31,32). The presence of eosinophils in helminth-infected individuals is associated with IgE production and mast cell-mediated hypersensitivity, indicating the activation of the Th2 system (33). In our model of *Ascaris*-infected mice, serum IgE levels as well as ASC-specific IgE and anaphylactic IgG1 also increased substantially until day 21. These results indicate that the early larval stages of the worm developed in the murine model contain allergens capable of inducing anaphylactic antibodies that cross-react with allergens of adult worms. In this sense, we have previously purified and characterized an allergenic protein from adult worms which is also present in eggs and in L2 and L3 larvae (34).

Th2 cells, eosinophilic inflammation and elevated levels of IgE are mostly associated with airway hyperresponsiveness to specific and nonspecific stimuli (35). As we demonstrated here, *Ascaris*-infected mice were hyperreactive to a bronchoconstrictor (methacholine), showing a reduction of mechanical lung parameters such as conductance and dynamic compliance. This reduction may well be correlated with the presence of anaphylactic antibodies induced by the larvae, since we have previously demonstrated that IgE and anaphylactic IgG1 enhance eosinophilic inflammation and airway hyperresponsiveness (36,37). In children living in a highly endemic area for ascariasis, ongoing inflammatory processes and high IgE levels specific for an *Ascaris* allergen (ABA-1) appear to associate with natural immunity to this helminth (38).

Besides IgE and IgG1, the predominant isotype produced in the antibody response against *A. suum* was IgM. According to Crandall and Crandall (39), there is a 10- to 20-fold increase in serum concentrations of IgM during the second week of *A. suum* infection in BALB/c mice, 50% being precipitated by *Ascaris* extract. Inhibition studies showed that this IgM has a phosphorylcholine specificity. The protective role of IgM in defence mechanisms against larval *Strongyloides stercoralis* and *Brugia pahangi* has been demonstrated in mice lacking all B cells, B1 cells or circulating IgM (40,41). But, at least in the filarial infection, this protective IgM does not seem to be directed against phosphorylcholine (41). Nothing has been published with regard to other nematodes.

Other interesting results that we obtained in our *Ascaris*-infected mice (CS Enobe *et al.*, manuscript in preparation) were the suppression of the antibody response to a non-related antigen and of the inflammatory leucocyte migration induced by bacterial lipopolysaccharide. It has been proposed that helminth infections can protect from allergic diseases (42,43), eliciting in some experimental models a regulatory ($CD4^+CD25^+Foxp3^+$) T cell population that

down-regulates allergen-induced lung pathology (44, our unpublished results). Epidemiological studies, however, have yielded conflicting results (45,46). Lynch and collaborators (47) reported that low-level intestinal helminth infections can contribute to the clinical symptoms of asthma in an endemic situation via a response to the parasite and/or a potentiation of the response to environmental allergens. Our data, taken together, show that the murine model of *Ascaris* infection is an adequate tool to further analyse the immunomodulatory capacities of helminths.

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REFERENCES

- Awasthi S, Bundy DA & Savioli L. Helminthic infections. *BMJ* 2003; **327**: 431–433.
- Bundy DA. Immunoepidemiology of intestinal helminthic infections. I. The global burden of intestinal nematode disease. *Trans R Soc Trop Med Hyg* 1994; **88**: 259–261.
- Chan MS. The global burden of intestinal nematode infections – fifty years on. *Parasitol Today* 1997; **13**: 438–443.
- Crompton DW. *Ascaris* and ascariasis. *Adv Parasitol* 2001; **48**: 285–375.
- Slotved HC, Eriksen L, Murrell KD & Nansen P. Early *Ascaris suum* migration in mice as a model for pigs. *J Parasitol* 1998; **84**: 16–18.
- Anderson TJ. *Ascaris* infections in humans from North America: molecular evidence for cross-infection. *Parasitology* 1995; **110**: 215–219.
- Maruyama H, Nawa Y, Noda S, Mimori T & Choi WY. An outbreak of visceral larva migrans due to *Ascaris suum* in Kyushu, Japan. *Lancet* 1996; **347**: 1766–1767.
- Inatomi Y, Murakami T, Tokunaga M, Ishiwata K, Nawa Y & Uchino M. Encephalopathy caused by visceral larva migrans due to *Ascaris suum*. *J Neurol Sci* 1999; **164**: 195–199.
- Murrell KD, Eriksen L, Nansen P, Slotved HC & Rasmussen T. *Ascaris suum*: a revision of its early migratory path and implications for human ascariasis. *J Parasitol* 1997; **83**: 255–260.
- Fetterer RH, Hill DE & Urban JF Jr. The cuticular biology in developmental stages of *Ascaris suum*. *Acta Trop* 1990; **47**: 289–295.
- Jarrett EEE & Miller HRP. Production and activities of IgE in helminth infection. *Prog Allergy* 1982; **31**: 178–233.
- King CL & Nutman TB. Biological role of helper T-cell subsets in helminth infections. *Chem Immunol* 1992; **54**: 136–165.
- Else KJ & Finkelman FD. Intestinal nematode parasites, cytokines and effector mechanisms. *Int J Parasitol* 1998; **28**: 1145–1158.
- Meeusen EN. Immunology of helminth infections, with special reference to immunopathology. *Vet Parasitol* 1999; **84**: 259–273.
- Kimani G, Chung CN, Butterworth AE *et al.* Eosinophilia and eosinophil helminthotoxicity in patients treated for *Schistosoma mansoni* infections. *Trans R Soc Trop Med Hyg* 1991; **85**: 489–492.
- Fattah DI, Maizels RM, McLaren DJ & Spry CJ. *Toxocara canis*: interaction of human blood eosinophils with the infective larvae. *Exp Parasitol* 1986; **61**: 421–431.
- Williams JF & Soulsby EJJ. Antigenic analysis of developmental stages of *Ascaris suum*. I. Comparison of eggs, larvae and adults. *Exp Parasitol* 1970; **27**: 150–162.
- Boes J, Eriksen L & Nansen P. Embryonation and infectivity of *Ascaris suum* eggs isolated from worms expelled by pigs treated with albendazole, pyrantel pamoate, ivermectin or piperazine dihydrochloride. *Vet Parasitol* 1998; **75**: 181–190.
- Strath M, Warren DJ & Sanderson CJ. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J Immunol Meth* 1985; **83**: 209–215.
- Soares MF, Mota I & Macedo MS. Isolation of *Ascaris suum* components which suppress IgE antibody responses. *Int Arch Allergy Appl Immunol* 1992; **97**: 37–43.
- Mota I & Wong D. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci* 1969; **8**: 813–820.
- Fox DA, Chiorazzi N & Katz DH. Hapten specific IgE antibody responses in mice. V. Differential resistance of IgE and IgG B lymphocytes to X-irradiation. *J Immunol* 1976; **117**: 1622–1628.
- Ishizaka K & Adachi T. Generation of specific helper cells and suppressor cells in vitro for the IgE and IgG antibody responses. *J Immunol* 1976; **117**: 40–47.
- Bradbury SM, Percy DH & Strejan GH. Immunology of *Ascaris suum* infection. I. Production of reaginic antibodies to worm components in rats. *Int Arch Allergy Appl Immunol* 1974; **46**: 498–511.
- Atwell RB, Sutton RH & Buoro IB. Early pulmonary lesions caused by dead *Dirofilaria immitis* in dogs exposed to homologous antigens. *Br J Exp Pathol* 1986; **67**: 395–405.
- Conboy GA, Hayden DW & Stromberg BE. Hepatic and pulmonary pathology of experimental *Fascioloides magna* infection in guinea pigs. *J Comp Pathol* 1991; **105**: 213–223.
- Girod N, Brown A, Pritchard DI & Billett EE. Successful vaccination of BALB/c mice against human hookworm (*Necator americanus*): the immunological phenotype of the protective response. *Int J Parasitol* 2003; **33**: 71–80.
- Sakamoto T & Gutierrez C. Pulmonary complications of cystic echinococcosis in children in Uruguay. *Pathol Int* 2005; **55**: 497–503.
- Carlson MG, Peterson CG & Venge P. Human eosinophil peroxidase: purification and characterization. *J Immunol* 1985; **134**: 1875–1879.
- Kay AB. Eosinophils as effector cells in immunity and hypersensitivity disorders. *Clin Exp Immunol* 1985; **62**: 1–12.
- Eriksen L, Andersen S, Nielsen K, Pedersen A & Nielsen J. Experimental *Ascaris suum* infection in pigs. Serological response, eosinophilia in peripheral blood, occurrence of white spots in the liver and worm recovery from the intestine. *Nord Vet Med* 1980; **32**: 233–242.
- Rhodes MB, Keralis MB & Staudinger LA. Immune responses of swine to oral inoculation with embryonated eggs of *Ascaris suum*. *Am J Vet Res* 1982; **43**: 1604–1607.
- Bell RG. IgE, allergies and helminth parasites: a new perspective on an old conundrum. *Immunol Cell Biol* 1996; **74**: 337–345.

- 34 Pires RR, Oshiro TM, Itami DM, Fernandes I & Macedo-Soares MF. Production and characterization of a monoclonal antibody against an *Ascaris suum* allergenic component. *Braz J Med Biol Res* 2001; **34**: 1033–1036.
- 35 Wills-Karp M. Immunological basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999; **17**: 255–281.
- 36 Itami DM, Latinne D, Bazin H *et al*. Immunoglobulin E is not required for but it enhances airway inflammation and hyperresponsiveness. *Allergy* 2003; **58**: 1117–1124.
- 37 Macedo-Soares MF, Itami DM, Lima C *et al*. Lung eosinophilic inflammation and airway hyperreactivity are enhanced by murine anaphylactic, but not nonanaphylactic, IgG1 antibodies. *J Allergy Clin Immunol* 2004; **114**: 97–114.
- 38 McSharry C, Xia Y, Holland CV & Kennedy MW. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infect Immun* 1999; **67**: 484–489.
- 39 Crandall CA & Crandall RB. *Ascaris suum*: immunoglobulin responses in mice. *Exp Parasitol* 1971; **30**: 426–437.
- 40 Herbert DR, Nolan TJ, Schad GA & Abraham D. The role of B cells in immunity against larval *Strongyloides stercoralis* in mice. *Parasite Immunol* 2002; **24**: 95–101.
- 41 Rajan B, Ramalingam T & Rajam T. Critical role for IgM in host protection in experimental filarial infection. *J Immunol* 2005; **175**: 1827–1833.
- 42 Lynch NR, Hagel I, Perez M, DiPrisco M, Lopez R & Alvarez N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993; **92**: 404–411.
- 43 Smits HH, Hartgers FC & Yazdanbakhsh M. Helminth infections: protection from atopic disorders. *Curr Allergy Asthma Rep* 2005; **5**: 42–50.
- 44 Wilson MS, Taylor MD, Balic A, Finney CAM, Lamb JR & Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory cells. *J Exp Med* 2005; **202**: 1199–1212.
- 45 Mao X-Q, Sun D-J, Miyoshi A *et al*. The link between helminthic infection and atopy. *Parasitol Today* 2000; **16**: 186–188.
- 46 Palmer LJ, Celedón JC, Weiss ST, Wang B, Fang Z & Xu X. *Ascaris lumbricoides* infection is associated with increased risk of childhood asthma and atopy in rural China. *Am J Respir Crit Care Med* 2002; **165**: 1489–1493.
- 47 Lynch NR, Palenque M, Hagel I & DiPrisco MC. Clinical improvement of asthma after anthelmintic treatment in a tropical situation. *Am J Respir Crit Care Med* 1997; **156**: 50–54.