Journal of Helminthology (2001) **75,** 137–140 DOI: 10.1079/JOH200171

A comparative study of toxocariasis and allergic asthma in murine models

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Abstract

Histopathology of the lung and total IgE in serum were compared in toxocariasis and allergic asthma murine models using BALB/c and C57BL/6 mice. Infection with *Toxocara canis* resulted in both strains of mice in marked histological changes and increased levels of total serum IgE. The ovalbumin (OVA) sensitization/challenge treatment for the induction of allergic asthma resulted in similar histological changes in BALB/c and, to a less extent, in C57BL/6 mice. Serum IgE levels of OVA-treated C57BL/6 mice were low. Histological changes observed included perivascular infiltration with eosinophils and mononuclear cells, peribronchiolitis, alveolitis and mucus production. Although these changes in addition to increased IgE production did occur in *T. canis*-infected C57BL/6 mice they were more pronounced in BALB/c mice. Thus, BALB/c mice appear to be the most appropriate strain of mice to perform studies on the possible connection between infection with *T. canis* and allergic asthma.

Introduction

The roundworm *Toxocara canis* is a parasite of dogs. Infection of paratenic hosts such as man and small rodents with this nematode results in toxocariasis (visceral larva migrans) (Kayes, 1997). After ingestion of eggs present in soil contaminated with dog faeces, larvae hatch in the intestine of the host and migrate to different tissues in the body inducing inflammatory responses. In paratenic hosts larvae do not develop beyond the migrating phase. Features of toxocariasis include eosinophilia and increased serum IgE levels (Sugane & Ohshima, 1984; Obwaller *et al.*, 1998). In man, infection with *Toxocara* spp. has been associated with asthmatic manifestations (Feldman & Parker, 1992; Taylor *et al.*, 1988; Varga *et al.*, 1998). Allergic asthma is a clinical syndrome that is characterized also by increased levels of serum IgE as well as infiltration of inflammatory cells, such as eosinophils and mast cells in to the airways of these patients (Hendrick *et al.*, 1975; De Monchy *et al.*, 1985; Bousquet *et al.*, 1990). Clinical symptoms such as wheezing, coughing and episodic airflow obstruction have been described for patients with toxocariasis and allergic asthma.

We are interested in studying the mechanisms involved in the immunopathology of toxocariasis and in particular, to elucidate the possible link between *Toxocara* infection and allergic asthma. We initiate our studies by comparing two murine models, one for toxocariasis and another for allergic asthma. In the present study we compare data on total serum IgE and histopathology of *T. canis*-infected or ovalbumin-sensitized/challenged BALB/c and C57BL/6 mice in addition to untreated control animals.

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Materials and methods

Parasites and experimental infection

Toxocara canis adult worms were taken from naturally infected dogs. Eggs were collected from the uteri of female worms and were allowed to embryonate in 0.1 M

H_2SO_4 in the dark at room temperature for 4–6 weeks. Embryonated eggs were stored in 0.1 M H_2SO_4 at 4°C until use.

Specified pathogen-free female BALB/c and C57BL/6 mice (5–6 weeks old) were obtained from Harlan (The Netherlands) and Charles River Laboratories (France) respectively. Mice were housed in Macrolon III cages with food and water *ad libitum*. Infection was performed by oral administration of 1000 embryonated eggs per mouse in 0.3 ml sterile phosphate buffered saline (PBS). The egg suspension was administered orally to mice by using a syringe fitted with a blunt needle. On day 8 after infection, mice were anaesthetized and bled as described below. Uninfected mice were also included in this study. Infected and uninfected groups consisted of eight animals.

Allergic asthma model

Sensitization was performed by seven intraperitoneal injections of 0.5 ml ovalbumin solution (OVA, chicken egg albumin, grade V, Sigma Chemical Company, St Louis, USA) (20 μg ml^{-1} PBS) every other day starting at day 0. At days 33–40, mice were challenged by OVA aerosol (2 mg ml^{-1} in PBS) during 5 min for eight consecutive days. On day 42 mice were anaesthetized and bled as described below. OVA-treated and untreated (or uninfected) groups consisted of eight animals.

Histology

Mice were anaesthetized on day 42 for the OVA-treated mice and on day 8 post-infection for the *Toxocara*-infected mice, by intraperitoneal injection of a mixture of Ketalar (Park and Davis, USA) and Rompun (Bayer, Germany). After anaesthesia lungs were perfused via the right heart ventricle by injection of 10 ml PBS. Lungs were removed and fixed intratracheally with 10% neutral-buffered formalin and embedded in paraplast. Five μm sections were stained with haematoxylin–eosin and PAS (Periodic Acid Schiff) for mucus staining. Different lung lesions were scored semiquantitatively from absent (0), minimal (1), slight (2), moderate (3), marked (4) to severe (5).

Total IgE

Total serum IgE antibodies were determined in sera of mice by using a modified capture ELISA described by Van Halteren *et al.* (1997). Briefly, plates were coated overnight at 4°C with EM-95 (Baniyash & Eshhar, 1984), a monoclonal rat anti-mouse IgE. As the detecting antibody a biotinylated rat-anti-mouse IgE (Pharmingen, San Diego, USA) was used. Plates were then incubated with peroxidase-conjugated streptavidin (Pharmingen). Colour development was performed by incubating with O-phenylenediamine dihydrochloride (OPD) (Sigma), substrate solution and stopped by adding H_2SO_4 (2 M). The absorbance was determined at 490 nm and expressed as optical density (OD).

Statistical analysis

Differences between groups were analysed by using the Student's t test after logarithmic ($\log(x+1)$) transformation of the data. A probability value $P < 0.05$ was considered statistically significant.

Results

Histology

All histological changes and scores of the different treatment groups are shown in table 1. After a *T. canis* infection a marked perivascular infiltration with eosinophilic granulocytes and mononuclear cells (histiocytes and lymphocytes) was observed in lungs of both strains of mice. Peribronchiolitis with the same pattern of inflammatory cells was also found but to a lesser extent. In addition, foci with interstitial pneumonia with moderate to marked alveolitis containing large macrophages, eosinophils and haemorhages were observed. Marked to severe hypertrophy of the bronchiolar goblet cells was seen with PAS-positive staining indicating mucus production.

The OVA sensitized BALB/c mice showed a slight to moderate peribronchiolar and perivascular infiltrate of mononuclear cells. However, less eosinophils were observed compared to *T. canis*-infected mice. A moderate hypertrophy of the PAS-positive bronchiolar goblet cells was observed. The C57BL/6 mice showed a slight perivascular inflammation and only in four out of the eight mice, a minimal peribronchiolar response was found. Hypertrophy of mucous bronchiolar cells was present in only three out of eight mice, mostly very

Table 1. Summary of histological lung lesions in BALB/c and C57BL/6 mice after OVA-treatment (OVA), *Toxocara canis* infection (TOX) and in untreated/uninfected controls (Ctrl).

	BALB/c			C57BL/6		
Treatment	OVA	TOX	Ctrl	OVA	TOX	Ctrl
Number of mice examined	8	8	8	8	8	8
Peribronchiolitis						
Minimal	2	3	0	4	0	0
Slight	1	5	0	0	1	0
Moderate	5	0	0	0	7	0
Perivascular infiltrate						
Minimal	0	0	0	3	0	1
Slight	5	0	0	4	0	0
Moderate	3	1	0	1	0	0
Marked	0	5	0	0	2	0
Severe	0	2	0	0	6	0
Hypertrophy bronchiolar goblet cells						
Minimal	0	0	0	1	0	0
Slight	0	0	0	1	0	0
Moderate	6	0	0	0	1	0
Marked	2	4	0	0	1	0
Severe	0	4	0	1	6	0
Alveolitis						
Minimal	0	0	0	2	0	1
Slight	0	0	0	1	0	0
Moderate	0	7	0	0	1	0
Marked	0	1	0	0	4	0
Severe	0	0	0	0	3	0

https://doi.org/10.1079/JOH200171 Published online by Cambridge University Press

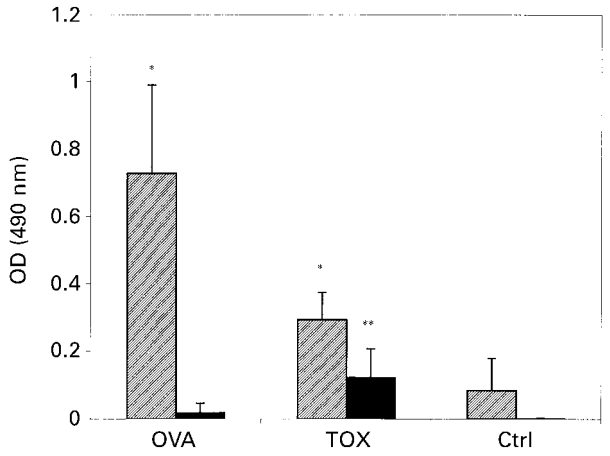

Fig. 1. Total IgE serum levels after *Toxocara canis* infection or OVA-treatment. BALB/c mice (shaded) or C57BL/6 (black) were ovalbumin (OVA) sensitized/challenged, *T. canis*-infected (TOX) or untreated/uninfected (Ctrl). Serum samples were diluted 1:40 and a capture ELISA to determine total IgE was performed. Mean serum levels of total IgE of eight mice per group were expressed as mean OD ± SD. $*P < 0.05$ compared with control BALB/c mice. $**P < 0.05$ compared with control C57BL/6 mice.

slight. The uninfected control mice of both strains showed no histopathological abnormalities.

Total serum IgE levels

Total serum IgE levels of the different experimental groups are shown in fig. 1. The sensitization and challenge protocol with OVA resulted in increased total serum IgE in BALB/c and not in C57BL mice. In contrast, infection with *T. canis* resulted in a significant increased total serum IgE in both BALB/c and in C57BL/6 mice. Total serum IgE levels were lower in *T. canis*-infected mice compared to OVA-sensitized/challenged BALB/c mice.

Discussion

In the present study BALB/c and C57BL/6 mice were either infected with *T. canis*, treated with ovalbumin for the induction of allergic asthma or untreated/uninfected. The histopathology of lung and serum IgE levels were compared between these groups of animals.

Comparison of histological lesions between the two strains of mice showed that after a *T. canis* infection all mice reacted to the same extent. A moderate, mainly perivascular inflammatory response and large inflammatory foci with a strong alveolitis were present in lungs of both strains of mice.

After treatment of mice with OVA a moderate to severe peribronchiolar and also a perivascular inflammatory response were observed. However, this response was clearly found in BALB/c and to a lesser extent in C57BL/6 mice. The allergic asthma murine model here described has been shown to display immunological and pathological features similar to those observed in patients with allergic asthma (Hessel *et al.*, 1995). This model was originally described using BALB/c mice.

In an infected host *T. canis* larvae migrate via blood vessels to most parts of the body including the lungs. This could explain why we observed mainly a perivascular inflammatory response in lungs of *T. canis*-infected mice compared to OVA-treated mice. The infiltrating cells for both *T. canis* and OVA-treated mice included eosinophils and lymphocytes. Similar cell infiltration has been previously described for other strains of mice infected with *T. canis* (Kayes, 1986). A possible role of these cells in increasing permeability of the microvasculature has been suggested (Kayes, 1986; Buijs *et al.*, 1994).

Comparing the levels of IgE we found that total IgE increased in serum of both strains of mice after *T. canis* infection. Levels of IgE in these mice were lower compared to OVA-treated BALB/c mice. The difference is most probably due to the time the mice were exposed either to OVA or *T. canis*. In this experiment OVA sensitization took place on days 0 to 12. Challenge with OVA took place on days 33 to 40. Serum samples were taken 9 days after the OVA-challenge started and 2 days after it finished. For *T. canis*-infected mice, serum samples were taken 8 days post-infection. Buijs *et al.* (1994) have reported that IgE levels peaked at 14 days after infection of BALB/c mice with 1000 eggs of *T. canis*.

Epidemiological studies suggesting a link between *T. canis* infection and asthma has been reported (Hakim *et al.*, 1997). The occurrence of asthma and hospitalization due to asthma were significantly related to *Toxocara* seroprevalence in schoolchildren around 5 years of age (Buijs *et al.*, 1997). *Toxocara* seroprevalence was associated with an increased total inhalation allergen-specific IgE, eosinophil number and asthma.

We intend to study the possible link between *T. canis* infection and asthma and in the present study we report common features such as IgE production and lung histopathology in murine toxocariasis and allergic asthma. Our results indicate that in BALB/c mice both *T. canis* infection and OVA-treatment result in similar histological changes and an increased IgE production. Although C57BL/6 *T. canis*-infected mice did show histopathological changes and IgE production, these changes were less compared to BALB/c mice. Moreover, IgE responses in the OVA-treated C57BL/6 mice were not significantly higher compared to control mice. Therefore the BALB/c strain would be the most appropriate to use to further investigate the mechanisms involved in the possible connection between *T. canis* infection and asthma.

Acknowledgements

The authors thank G. van Leuveren and C.G.H. de Rond for their expert technical assistance. We are also grateful to D. Kegler, D. Elbers, P. van Schaaik and C. Moolenbeek for performing the experimental infections, OVA treatment and autopsies of mice.

References

Baniyash, M. & Eshhar, Z. (1984) Inhibition of IgE binding to mast cells and basophils by monoclonal

antibodies to murine IgE. *European Journal of Immunology* **14**, 799–807.

Bousquet, J., Chanez, P., Lacoste, J.Y., Barneon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony-Lafontaine, J. & Godard, P. (1990) Eosinophilic inflammation in asthma. *New England Journal of Medicine* **323**, 1033–1039.

Buijs, J., Lokhorst, W.H., Robinson, J. & Nijkamp, F.P. (1994) *Toxocara canis*-induced murine pulmonary inflammation: analysis of cells and proteins in lung tissue and bronchoalveolar lavage fluid. *Parasite Immunology* **16**, 1–9.

Buijs, J., Borsboom, G., Renting, M., Hilgersom, W.J., van Wieringen, J.C., Jansen, G. & Neijens, J. (1997) Relationship between allergic manifestations and *Toxocara* seropositivity: a cross-sectional study among elementary school children. *European Respiratory Journal* **10**, 1467–1475.

De Monchy, J.G., Kauffman, H.F., Venge, P., Koeter, G.H., Jansen, H.M., Sluiter, H.J. & De Vries, K. (1985) Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *American Review of Respiratory Disease* **131**, 373–376.

Feldman, G.J. & Parker, H.W. (1992) Visceral larva migrans associated with the hypereosinophilic syndrome and the onset of severe asthma. *Annals of Internal Medicine* **116**, 838–840.

Hakim, S.L., Thadasavanth, M., Shamilah, R.H. & Yogeswari, S. (1997) Prevalence of *Toxocara canis* antibody among children with bronchial asthma in Klang Hospital, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 528.

Hendrick, D.J., Davies, R.J., D'Souza, M.F. & Pepys, J. (1975) An analysis of skin prick test reactions in 656 asthmatic patients. *Thorax* **30**, 2–8.

Hessel, E.M., Van Oosterhout, A.J., Hofstra, C.L., De Bie, J.J., Garssen, J., Van Loveren, H., Verheyen, A.K., Savelkoul, H.F. & Nijkamp, F.P. (1995) Bronchoconstriction and airway hyperresponsiveness after ovalbumin inhalation in sensitized mice. *European Journal of Pharmacology* **293**, 401–412.

Kayes, S.G. (1986) Nonspecific allergic granulomatosis in the lungs of mice infected with large but not small inocula of the canine ascarid, *Toxocara canis*. *Clinical Immunology and Immunopathology* **41**, 55–65.

Kayes, S.G. (1997) Human toxocariasis and the visceral larva migrans syndrome: correlative immunopathology. *Chemical Immunology* **66**, 99–124.

Obwaller, A., Jensen-Jarolim, E., Auer, H., Huber, A., Kraft, D. & Aspock, H. (1998) *Toxocara* infestations in humans: symptomatic course of toxocarosis correlates significantly with levels of IgE/anti-IgE immune complexes. *Parasite Immunology* **20**, 311–317.

Sugane, K. & Ohshima, T. (1984) Interrelationship of eosinophilia and IgE antibody production to larval ES antigen in *Toxocara canis*-infected mice. *Parasite Immunology* **6**, 409–420.

Taylor, M.R.H., O'Connor, P., Keane, C.T., Mulvihill, E. & Holland, C. (1988) The expanded spectrum of toxocaral disease. *The Lancet* **26**, 692–695.

van Halteren, A.G., van der Cammen, M.J., Biewenga, J., Savelkoul, H.F. & Kraal, G. (1997) IgE and mast cell response on intestinal allergen exposure: a murine model to study the onset of food allergy. *Journal of Allergy and Clinical Immunology* **99**, 94–99.

Varga, E.M., Auer, H. & Zach, M. (1998) Toxocariasis in a 5-year-old boy manifesting as bronchial asthma and behavioral disorder. *Klinische Paediatrie* **210**, 128–131.

(Accepted 30 January 2001)