Journal of Helminthology (2006) **80,** 417–423 DOI: 10.1017/JHL2006369

Eosinophil cationic protein, specific IgE and IgG4 in human toxocariasis

J.-F. Magnaval*, J.-H. Faufingue, B. Morassin and R. Fabre

Department of Parasitology, CH Rangueil, University Hospitals, 31059 Toulouse 9, France

Abstract

Among 67 French patients presenting a toxocaral infection, various demographic, environmental, clinical and laboratory parameters (blood eosinophil count, eosinophil cationic protein (ECP), serum total IgE, specific IgE against common inhalant allergens, specific IgE and IgG4 against *Toxocara* excretory-secretory antigens) were investigated. Correlation studies and logistic regression analyses were conducted, testing elevated levels of ECP, specific anti-*Toxocara* IgE or IgG4 as outcome variables An elevated ECP level was significantly associated with both cough and rhinitis, a high level of specific anti-*Toxocara* IgE with itchy rashes and possible atopic status, and an increase of specific anti-*Toxocara* IgG4 with rural residence.

Introduction

Toxocariasis is a worldwide helminthic zoonosis due to the migration in humans of infective larvae of *Toxocara canis,* an intestinal roundworm of dogs (Soulsby, 1987), or *T. cati*, in cats (Nagakura *et al.*, 1990). Common toxocariasis, primarily described in France (Glickman *et al.*, 1987; Magnaval *et al.*, 2001b), and covert toxocariasis in Ireland (Taylor *et al.*, 1988), are the most frequently encountered syndromes in westernized countries. In French adults, toxocariasis has been characterized by chronic weakness, *pruritus simplex*, itchy rashes, difficult breathing and abdominal pain, along with moderate blood eosinophilia and increased serum total IgE level. In Irish children, the most frequent clinical findings have included fever, anorexia, headache, abdominal pain, sleep and behaviour disorders, pneumonia, cough, wheeze and hepatomegaly.

Toxocaral infection induces a complex immunological response, including several humoral and cellular changes. Among non-specific laboratory disturbances that are usually observed, both increase in the blood eosinophil count (Beaver, 1956) and that of serum total IgE (Magnaval & Baixench, 1993) are the most prominent.

The measurement of eosinophil cationic protein (ECP), that is released by activated eosinophils, has been proposed for the monitoring of asthma, a disease in which elevated levels of this protein have been reported (Motojima *et al.*, 1997). Serum ECP level has been studied in very few helminthic infections, and has been found to be elevated in filariasis and schistosomiasis patients (Tischendorf *et al.*, 2000) and also in common toxocariasis patients (Magnaval *et al.*, 2001a).

The presence of specific IgE has been previously established in various helminthiases, including toxocariasis (Genchi *et al.*, 1988; Magnaval *et al.*, 1992). The kinetics of this parameter have been studied during the post-treatment follow-up of toxocariasis patients (Magnaval *et al.*, 1992; Magnaval, 1995), but the correlation with patients' status (atopy) or with other laboratory parameters has only been partially investigated thus far (Obwaller *et al.*, 1998).

Specific IgG4 has been detected in sera from patients suffering from helminth diseases such as cystic hydatidosis (Daeki *et al.*, 2000), lymphatic filariasis (Addiss *et al.*, 1995) and schistosomiasis (Li *et al.*, 2001). An increased level of this subclass of immunoglobulins has correlated with more serious features of these infections, such as cyst proliferation for hydatidosis or a lack of resistance to reinfection in japonicum schistosomiasis. The relationship between specific IgE and IgG4 is intriguing and, e.g. in lymphatic filariasis, a remarkable correlation exists between these isotypes, according to the clinical presentation. In areas endemic for bancroftosis, asymptomatic individuals displayed high titres of specific IgG4, whereas elephantiasis cases tended to have significantly more elevated levels of specific IgE (Kurniawan *et al.*, 1993). Moreover, the mean value of specific IgG4 was

* Fax: 33 5 61 14 59 72
E-mail: magnaval@cict.fr

found to be significantly increased, and that of specific IgE lowered, in microfilaraemic vs. amicrofilaraemic subjects (Simonsen & Meyrowitsch, 1998). Concerning toxocariasis, an ELISA detecting IgG4 directed against larval excretory–secretory antigens was found to be more specific than in conventional assays (Noordin *et al.*, 2005).

The aim of the present study is therefore to investigate in toxocariasis patients the level of these three parameters, then to assess any correlation with demographic, epidemiological and clinical features of the disease, and also with the possible presence of an atopic status. The design of this study was approved by the Board of Clinical Research of Toulouse University Hospitals.

Materials and methods

Patients' group (group 1)

Sixty-seven patients who attended consultations in the Department of Parasitology, and who were diagnosed as having an active common toxocaral infection were enrolled in the study. All these patients were from the regional catchment of the Department of Parasitology. They had to present a positive toxocariasis immunodiagnosis, to be symptomatic, to display a blood eosinophil count greater than 500 cells mm^{-3} (Simmons *et al.*, 1974), and to be free of any concurrent microbial, viral, fungal or parasitic infections. Especially, autochthonous parasitic diseases were ruled out after stool examination and serological investigations.

On the first visit, a detailed questionnaire inquired about demographic (age and sex) and epidemiological (place of residence: urban or rural) data. The medical history was recorded, and special attention was paid to any previous report of clinical atopy, e.g. asthma, hives, itchy rashes, hay fever, seasonal conjunctivitis. The period of time which elapsed between the disease onset and the consultation, termed as 'duration of the disease (DD) before consultation' was recorded in months. The presence of 14 clinical items, that were the most frequently encountered in these toxocariasis patients, was evaluated on general examination and these included arthralgias, chronic weakness, conjunctivitis or 'itchy eye', chronic cough or laryngeal irritability, digestive pain, oedemas, headache, loss of appetite, loss of weight, myalgias, *pruritus simplex*, rhinitis, sneezing, and chronic urticaria or any itchy rash. In order to quantify the clinical impact of toxocariasis, a score system was used: before treatment, the 14 clinical items were rated as '0' when absent and '1' when present. The total represented the 'clinical score' value. All consultations were done and the questionnaire was approved by the same investigator (JFM).

A blood sample was collected for the following laboratory tests: total and differential blood counts, the detection of specific IgE against 14 common inhalant allergens, and measurement of the levels of ECP, serum total IgE, specific anti-*Toxocara* IgE and specific anti-*Toxocara* IgG4, respectively. Serological detection of autochthonous diseases other than toxocariasis was also carried out using this blood sample.

Reference group (group 2)

To assess the normal level of laboratory tests, 27 apparently healthy subjects (residents or technicians) consented to be included in the study, but they had to display negative results for stool examination, serological check-up for autochthonous helminthiases including toxocariasis, and for the detection of specific IgE to common inhalant allergens. These subjects also had a blood eosinophil count <500 cells mm^{-3}, and a serum total IgE <150 kilo International Units (kIU) l^{-1}. Results of laboratory investigations are displayed in table 1.

Table 1. Comparison of continuous variables including age, duration of disease, clinical score, eosinophil count result, eosinophil cationic protein level, serum total IgE level, and specific anti-*Toxocara* IgE and IgG4 titres between patients (group 1) and reference subjects (group 2).

						Mann-Whitney *U*	
Variable	Group	Geometric mean	95% CI		Median	Z-statistic	*P*
Age	1	37.2	31.9	43.4	44.0		
	2	28.1	26.4	29.9	27.0	3.98	0.0001
DD	1	8.25	6.2	11.1	8	NA	NA
Clinical score	1	3.75	3.25	4.34	4.0	NA	NA
Eosinophils (cells mm^{-3})	1	919.0	790.0	1068	850		
	2	103.0	67.0	158.0	124.0	7.55	<0.0001
ECP ($\mu g\,l^{-1}$)	1	31.2	25.9	37.7	30.0		
	2	8.7	6.7	11.3	8.0	5.97	<0.0001
Serum total IgE (kIU l^{-1})	1	546.1	364.7	817.6	547.0		
	2	29.4	20.0	43.1	31.0	6.60	<0.0001
Specific IgE (TU l^{-1})	1	7.4	4.1	13.4	4.0		
	2	0	0	0	0	5.19	<0.0001
Specific IgG4 (FU l^{-1})	1	414.7	341.2	504.1	335.0		
	2	118.2	114.5	121.9	116.0	7.24	<0.0001

CI, confidence interval; DD, duration (months) of the disease between onset and consultation; ECP, eosinophil cationic protein; FU, fluorescence units, kIU, kilo International Units; NA, not applicable; TU, *Toxocara* units.

https://doi.org/10.1017/JOH2006369 Published online by Cambridge University Press

Stool examination

For every patient or control, stool examination was based upon a zinc sulphate flotation technique along with a merthiolate-iode-formol diphasic method. Moreover, detection of *Strongyloides stercoralis* larvae was carried out using Baermann's method.

Blood count and serum total IgE measurement

Total and differential blood counts were performed in the Department of Haematology using a SHT 330™ blood analyser (TOA Sysmex, Roche Diagnostics, Neuilly-sur-Seine, France). Eosinophilia level was expressed in cells mm^{-3}. The total IgE assay was carried out using the IMX™ system (Abbott Laboratories, Rungis, France), and results were expressed in kilo International Units (kIU) per litre.

Detection of specific IgE against airborne allergens

The IgE against 14 inhalant allergens were detected on a Matrix Aero™ (Abbott Laboratories, Rungis, France). Only subjects exhibiting a titre greater than 0.70 kIU l^{-1} (class 2) against at least one aeroallergen were classified as positive.

ECP assay

ECP titration was performed using the CAP™ system (Pharmacia, Guyancourt, France). Blood samples were taken according to the manufacturer's instructions, with special attention paid to the clotting temperature (always between 20 and 24°C). Values were given in $\mu g\,l^{-1}$.

Immunodiagnosis of toxocariasis

The serodiagnosis was performed by Western-blot (Magnaval *et al.*, 1991) using *Toxocara canis* excretory-secretory antigens (TES Ag) that were produced according to de Savigny's prototype method (De Savigny, 1975) modified by Bowman *et al.* (1987). With Western blotting, animal and human reference sera from toxocariasis cases showed a typical pattern where seven bands were split into two groups, the first group including four lower molecular weight bands, the second three higher molecular weight bands. Statistical analysis of results from all sera has previously demonstrated that this seven-band pattern was significantly correlated with a toxocaral infection.

*Specific anti-*Toxocara *IgE*

Specific IgE raised against TES Ag were detected and their level quantified according to the method previously described by Magnaval *et al.* (1992). This technique was subsequently adapted to the CAP ™ FEIA Pharmacia system. Briefly, 96-well microtitration polystyrene plates (Immunoplate™ Nunc, Poly-Labo Block, Strasbourg, France) were loaded with a TES-Ag solution (50 μl per well) containing 5.0 μg protein ml^{-1} in a 0.1 M pH 9.6 carbonate-bicarbonate buffer. The plates were maintained for 18 h at 25°C in a moist chamber. Then the antigenic solution was discarded and the plates were washed three times with PBS Tween (Tween 20: 0.05%) pH 7.2 buffer. They were then dried and finally stored at −70°C. Sera from patients were tested undiluted, according to Pharmacia's procedure. Results were expressed in *Toxocara* units (TU) per litre.

*Specific anti-*Toxocara *IgG4*

The CAP™ FEIA Pharmacia system was also used to detect and quantify the level of this subclass of immunoglobulin. The same 96-well plates coated with TES-Ag were utilized. Sera were also tested undiluted, according to instructions from the CAP™ specific IgG4 FEIA Pharmacia kit. Results were expressed in fluorescence units (FU) l^{-1}.

Serological tests for other autochthonous diseases

Specific antibodies were searched using ELISA only (anisakiasis, strongyloidiasis and trichinellosis) or a combination of ELISA and indirect haemagglutination (fascioliasis and hydatidosis).

Statistical analysis

The distribution of values was compared between groups using the Mann-Whitney *U* test, as implemented with the SPSS 11.0 statistical software (SPSS, Chicago, Illinois, USA). Correlation studies were based upon the Spearman's rank test, also from SPSS. Multivariate unconditional regression analyses were implemented with Egret™ (Cytel Corp., Cambridge, Massachusetts, USA). The set of variables included the following: age, sex, place of residence, clinical score value and results from blood eosinophil counts, measurements of ECP, detection of specific IgE for inhalant allergens, levels of serum total IgE and those of specific anti-*Toxocara* IgE and IgG4. In order to be used as outcome variable, ECP, specific anti-*Toxocara* IgE and IgG4 results were sorted in two classes (elevated or not) using the upper limit of 95% CI of the geometric mean as a cut-off limit.

Each of these three parameters was tested as outcome variables by forwards and backwards stepwise regressions in three different logistic models. Egret™ started any analysis by a screening procedure of the variable set, and only the parameters displaying in the score test a P-value ≤ 0.05 (forwards stepwise) or ≤ 0.15 (backwards stepwise) were tested by regression. Odds ratio estimates were adjusted for other variables by logistic regression analysis; approximate 95% confidence limits were based on maximum likelihood estimates of logistic parameters.

Results

Table 1 displays the geometric means, 95% confidence intervals and medians for the continuous laboratory variables, and the results of the comparison between groups 1 and 2.

Correlation studies and logistic regression analyses

Table 2 shows the results from the Spearman's rank test between eight continuous variables (age, DD, clinical

https://doi.org/10.1017/JOH2006369 Published online by Cambridge University Press

Table 2. Results of the correlation study between continuous variables including age, duration of disease, clinical score, eosinophil count, eosinophil cationic protein level, serum total IgE level, and specific anti-*Toxocara* IgE and IgG4 titres.

							Specific anti-*Toxocara*	
Variable	Age	DD	Clinical score	Eosinophil count	ECP	Total IgE	IgE	IgG4
Age	NA	NS	NS	NS	NS	$r = 0.290$ $P = 0.017$	NS	NS
DD		NA	NS	NS	NS	NS	NS	NS
Clinical score			NA	NS	NS	NS	NS	NS
Eosinophil count				NA	$r = 0.442$ $P < 0.001$	$r = 0.246$ $P = 0.045$	NS	$r = 0.353$ $P = 0.003$
ECP					NA	NS	NS	$r = 0.347$ $P = 0.004$
Total IgE						NA	$r = 0.537$ $P < 0.001$	NS
Specific anti-*Toxocara* IgE							NA	NS
Specific anti-*Toxocara* IgG4								NA

DD, duration of disease before consultation; ECP, eosinophil cationic protein level; NA, not applicable; NS, non-significant; *r*, Spearman's correlation coefficient.

score, eosinophil count, ECP, serum total IgE, and specific anti-*Toxocara* IgE or IgG4). The most important finding was a significant correlation between specific anti-*Toxocara* IgG4 level and eosinophil count value or ECP level, respectively.

For two binary variables, namely the type of residence (rural or urban) and the presence of a possible atopic status, as suspected by a positive detection of specific IgE against common airborne allergens (Aalberse, 2000), the correlation with the eight above-cited continuous parameters was studied using a comparison of distribution (Mann-Whitney test). Between 32 rural and 35 urban patients, only eosinophil count levels differed significantly (Mann-Whitney test: $U = 403.8$; $Z = -1.982$; $P = .049$). Geometric means of this variable were 786 cells mm^{-3} (95% CI: 657, 942) in patients from cities having 2500 inhabitants or more, and 1087 (95% CI: 852, 1387) in those from rural areas. Between 31 possibly atopic patients and 37 negative subjects, only the level of specific anti-*Toxocara* IgE was significantly different (Mann-Whitney test: $U = 328$; $Z = -2.932$; $P = .003$). Geometric means were 20.4 TU l^{-1} (95% CI: 6.8, 62) in 31 possibly atopic cases, and 3.1 TU l^{-1} (95%CI: 2.0, 4.75) in 36 patients without any sensitization to common allergens.

The results of the logistic regression analyses are displayed in table 3.

Discussion

ECP values correlated with eosinophil count results, a fact previously reported in toxocaral disease (Magnaval *et al.*, 2001b). More surprisingly, another correlation was observed with specific IgG4 level whereas a linkage with specific IgE was expected, since antibody-dependent cell cytolysis (ADCC) following eosinophil activation by these homocytotropic immunoglobulins has been claimed to be the killing mechanism in some helminth infections such as mansoni schistosomiasis (Capron & Capron, 1992).

Table 3. Logistic regression analysis of demographic, epidemiological, clinical and laboratory findings associated with elevated values of eosinophil cationic protein, specific anti-*Toxocara* IgE and IgG4.

Variable	Coefficient	*P*	Odds ratio	95% CI	
A. Associated with elevated ECP result ($\geq 30\,\mu g\,l^{-1}$)					
Cough	1.7	0.004	5.4	1.7	16.7
Rhinitis	2.9	<0.0001	18.0	6.7	48.1
Likelihood ratio statistic on 3 DF = 287.5, $P < .0001$					
B. Associated with elevated specific anti- *Toxocara* IgE result ($\geq$ 4 TU l^{-1})					
Itchy rashes	2.6	<0.001	13.3	3.6	49.1
Presence of sp. IgE IA	3.1	<0.001	22.6	7.9	65.25
Likelihood ratio statistic on 3 DF = 308.1, $P < 0.001$					
C. Associated with elevated specific anti-*Toxocara* IgG4 result ($\geq$ 504 FU l^{-1})					
Rural residence	3.7	<0.001	41.0	16.2	104.6
Likelihood ratio statistic on 2 DF = 280.5, $P < 0.001$					

CI, confidence interval; ECP, eosinophil cationic protein level; FU, fluorescence units; sp. IgE IA, specific IgE for common inhalant allergens; TU, *Toxocara* units.

https://doi.org/10.1017/JOH2006369 Published online by Cambridge University Press

Specific IgG4 is not known to induce the release of eosinophil granule proteins.

With logistic regression analysis (model A), higher ECP levels were explained by both chronic cough and rhinitis. There was no association with laboratory abnormalities usually evocative of allergic disease, such as elevated total IgE levels and the presence of specific IgE against common inhalant allergens. The present association of higher ECP titres with cough and rhinitis was similar to that described in asthma (Fujisawa *et al.*, 1998) or common allergic rhinitis (Tomassini *et al.*, 1996). Hence the infection by *Toxocara canis* larvae appears to also induce – or boost in atopic patients – inflammation of the respiratory tract, by release of cationic proteins inside bronchial and nasal mucosae. Such an effect was probably systemic, but the transient presence of *Toxocara sp.* larvae in the airways cannot be excluded.

Titres of specific anti-*Toxocara* IgE correlated classically with serum total IgE levels. Following logistic regression analysis (model B), higher values of specific anti-*Toxocara* IgE were explained by the likely presence of atopy and chronic urticaria or itchy rashes. These clinical symptoms of allergy are commonly observed in patients sensitized to common food or inhalant allergens. The finding of the association of high specific anti-*Toxocara* IgE with atopy and skin allergy signs therefore strengthens the hypothesis of allergy symptoms caused by sensitization to TES-Ag, that are probably close to the so-called TBA-1 (Yahiro *et al.*, 1998). It also suggests that an atopy status could be a worsening factor among toxocariasis patients.

Specific IgG4 levels significantly correlated with both blood eosinophil levels and ECP values, and this is related to the results of the regression analysis (model C). Higher levels of specific anti-*Toxocara* IgG4 were then explained only by rural residence, namely by the risk of having repeated and/or stronger infections, where chronic blood eosinophilia is common. IgG4 antibodies are known to become prominent later on when a chronic antigenic stimulation is encountered, since IgG4 switched B-cells are more likely to proliferate than IgE switched B-cells (Brinkman & Heusser, 1993).

Although the degranulation of eosinophil cells obviously occurred in toxocariasis patients, any ADCC killing mechanism involving both eosinophil and specific IgE does not seem to exist here, unlike that reported in mansoni schistosomiasis (Nutten *et al.*, 1997). No correlation was found between ECP and specific anti-*Toxocara* IgE levels, a point that supports two explanatory hypotheses. Firstly, the detection of specific anti-*Toxocara* IgE by ELISA would have been biased by a masking effect from more abundant specific IgG. However, in this event, specific anti-*Toxocara* IgE would not have significantly correlated with total IgE, the detection of which is based upon a sandwich assay not affected by the level of other immunoglobulin isotypes.

Secondly, *Toxocara canis* larvae have developed an escape mechanism, namely an abundant production of soluble antigens against which the mammal host preferentially mounts the immunological response (Kayes, 1997). These TES-Ag are located on the larval epicuticle, which permanently sheds (Maizels & Page, 1990). Within about 3 h, a complete turn-over of the protein outer larval surface is achieved (Smith *et al.*, 1983). Hence, specific IgE that would be fixed on the parasites peel off, and eosinophil degranulation occurs far away from the parasitic tissue targets. Subsequently, it can be inferred that the larval killing process relies upon another immunological mechanism than ADCC. Experimental data suggest that larval trapping inside granulomata could be a candidate. This phenomenon was discovered in a mouse model, and seemed to be genetically controlled (Parsons & Grieve, 1990). Moreover, in mice infected by *Toxocara canis* larvae, parasitic elimination occurred at the same rate in IL-5 transgenic animals as in normal individuals (Sugane *et al.*, 1996). In human toxocariasis, dying larvae in the centre of granulomata were reported in hepatic (Kaplan *et al.*, 2001), neurological (Hill *et al.*, 1985) and especially ophthalmological (Werner *et al.*, 1999) cases. Muscle granulomatous lesions were described in laboratory-infected rodents (Kayes & Oaks, 1978). Since the novel so-called Th3 lymphocyte subset, producing TGF-β, could be mainly involved in this kind of granulomatous response (Meeusen, 1999), further oriented studies should be considered.

Concerning the relationship between atopy and toxocariasis, data from the present study are consistent with results reported by Buijs *et al.* (1994) and suggest that, in subjects having a hypothetical 'atopic genotype' (Moffatt & Cookson, 1998), toxocariasis could boost preexistent allergy symptoms or, acting as a 'developer', elicit these signs in previously asymptomatic patients.

Acknowledgements

The authors gratefully acknowledge Marie-Josée Touchard and Eric Dubly for their technical assistance, and Professor Jean René Mathieu for his invaluable help with the statistical analysis.

References

Aalberse, R. (2000) Specific IgE and IgG responses in atopic versus non-atopic subjects. *American Journal of Respiratory and Critical Care Medicine* **162**, 5124–5127.

Addiss, D.G., Dimock, K.A., Eberhard, M.L. & Lammie, P.J. (1995) Clinical, parasitologic, and immunologic observations of patients with hydrocele and elephantiasis in an area with endemic lymphatic filariasis. *Journal of Infectious Diseases* **171**, 755–758.

Beaver, P.C. (1956) Larva migrans. *Experimental Parasitology* **5**, 587–621.

Bowman, D.D., Mika-Grieve, M. & Grieve, R.B. (1987) Circulating excretory antigens levels and specific antibody responses in mice infected with *Toxocara canis*. *American Journal of Tropical Medicine and Hygiene* **36**, 75–82.

Brinkman, V. & Heusser, C.H. (1993) T-cell dependent differentiation phenomenon of human B cells into IgM, IgG, IgA, or IgE plasma cells: high rate of antibody production by IgE plasma cells, but limited IgE expression of IgE precursors. *Cellular Immunology* **152**, 323–332.

https://doi.org/10.1017/JOH2006369 Published online by Cambridge University Press

Buijs, J., Borsboom, G., van Gemund, J.J., Hazebroek, A., van Dongen, P.A., van Knapen, F. & Neijens, H.J. (1994) *Toxocara* seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. *American Journal of Epidemiology* **140**, 839–847.

Capron, M. & Capron, A. (1992) Effector functions of eosinophils in schistosomiasis. *Memorias do Instituto Oswaldo Cruz* **87** (Suppl 4), 167–170.

Daeki, A.O., Craig, P.S. & Shambesh, M.K. (2000) IgG-subclass antibody responses and the natural history of hepatic cystic echinococcosis in asymptomatic patients. *Annals of Tropical Medicine and Parasitology* **94**, 319–328.

De Savigny, D.H. (1975) *In vitro* maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigens for use in serodiagnostic tests for visceral larva migrans. *Journal of Parasitology* **61**, 781–782.

Fujisawa, T., Terada, A., Atsuta, J., Iguchi, K., Kamiya, H. & Sakurai, M. (1998) Clinical utility of serum levels of eosinophil cationic protein (ECP) for monitoring and predicting clinical course in childhood asthma. *Clinical and Experimental Allergy* **28**, 19–25.

Genchi, C., Falagiani, P., Riva, G., Tinelli, M., Brunello, F., Boero, M. & Almaviva, M. (1988) IgE and IgG antibodies in *Toxocara canis* infection. A clinical evaluation. *Annals of Allergy* **61**, 43–46.

Glickman, L.T., Magnaval, J.-F., Domanski, L.M., Shofer, F.S., Lauria, S.S., Gottstein, B. & Brochier, B. (1987) Visceral larva migrans in French adults. A new disease syndrome? *American Journal of Epidemiology* **125**, 1019–1033.

Hill, I.R., Denham, D.A. & Scholtz, C.L. (1985) *Toxocara canis* larvae in the brain of a British child. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 351–354.

Kaplan, K.J., Goodman, Z.D. & Ishak, K.G. (2001) Eosinophilic granuloma of the liver: a characteristic lesion with relationship to visceral larva migrans. *American Journal of Surgical Pathology* **25**, 1316–1321.

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

Kayes, S.G. & Oaks, J.A. (1978) Development of the granulomatous response in murine toxocariasis. Initial events. *American Journal of Pathology* **93**, 277–294.

Kurniawan, A., Yazdanbakhsh, M., van Ree, R., Aalberse, R., Selkirk, M.E., Partono, F. & Maizels, R.M. (1993) Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. *Journal of Immunology* **150**, 3941–3950.

Li, Y., Sleigh, A.C., Ross, A.G., Li, Y., Zhang, X., Williams, G.M., Yu, X., Tanner, M. & McManus, D.P. (2001) Human susceptibility to *Schistosoma japonicum* in China correlates with antibody isotypes to native antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 441–448.

Magnaval, J.-F. (1995) Comparative efficacy of diethy lcarbamazine and mebendazole for the treatment of human toxocariasis. *Parasitology* **110**, 529–533.

Magnaval, J.-F. & Baixench, M.-T. (1993) Toxocariasis in Région Midi-Pyrénées, France. pp. 83–87 *in* Lewis, J.W. & Maizels, R.D. (*Eds*) *Toxocara and toxocariasis. Clinical, epidemiological, and molecular perspectives*. London, Institute of Biology and British Society for Parasitology.

Magnaval, J.-F., Fabre, R., Maurieres, P., Charlet, J.-P. & De Larrard, B. (1991) Application of the western-blotting procedure for the immunodiagnosis of human toxocariasis. *Parasitology Research* **77**, 697–702.

Magnaval, J.-F., Fabre, R., Maurieres, P., Charlet, J.-P. & De Larrard, B. (1992) Evaluation of an immunoenzymatic assay detecting specific anti-*Toxocara* immunoglobulin E for diagnosis and post-treatment follow-up of human toxocariasis. *Journal of Clinical Microbiology* **30**, 2269–2274.

Magnaval, J.-F., Berry, A., Fabre, R. & Morassin, B. (2001a) Eosinophil cationic protein as a possible marker of active human *Toxocara* infection. *Allergy* **56**, 1096–1099.

Magnaval, J.-F., Glickman, L.T., Dorchies, P. & Morassin, B. (2001b) Highlights of human toxocariasis. *Korean Journal of Parasitology* **39**, 1–11.

Maizels, R.M. & Page, A.P. (1990) Surface associated glycoproteins from *Toxocara canis* larval parasites. *Acta Tropica* **47**, 355–364.

Meeusen, E.N. (1999) Immunology of helminth infections, with special reference to immunopathology. *Veterinary Parasitology* **84**, 259–273.

Moffatt, M.F. & Cookson, W.O. (1998) Gene identification in asthma and allergy. *International Archives of Allergy and Immunology* **116**, 247–252.

Motojima, S., Tateishi, K., Koseki, T., Makino, S. & Fukuda, T. (1997) Serum levels of eosinophil cationic protein and IL-5 in patients with asthma without systemic corticosteroids. *International Archives of Allergy and Immunology* **114**, 55–59.

Nagakura, K., Kanno, S., Tachibana, H., Kaneda, Y., Ohkido, M., Kondo, K. & Inoue, H. (1990) Serologic differentiation between *Toxocara canis* and *Toxocara cati*. *Journal of Infectious Diseases* **162**, 1418–1419.

Noordin, R., Smith, H.V., Mohamad, S., Maizels, R.M. & Fon, M.F. (2005) Comparison of IgG-ELISA and IgG4-ELISA for *Toxocara* serodiagnosis. *Acta Tropica* **93**, 57–62.

Nutten, S., Trottein, F., Gounni, A.S., Papin, J.P., Capron, A. & Capron, M. (1997) From allergy to schistosomes: role of Fc receptors and adhesion molecules in eosinophil effector function. *Memórias do Instituto Oswaldo Cruz* **92** (Suppl 2), 9–14.

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.

Parsons, J.C. & Grieve, R.B. (1990) Effect of egg dosage and host genotype on liver trapping in murine larval toxocariasis. *Journal of Parasitology* **76**, 53–58.

Simmons, A., Leaverton, P. & Elbert, G. (1974) Normal laboratory values for differential white cell counts established by manual and automated cytochemical methods (Hemalog D™). *Journal of Clinical Pathology* **27**, 55–58.

Simonsen, P.E. & Meyrowitsch, D.W. (1998) Bancroftian filariasis in Tanzania: specific antibody responses in

relation to long-term observations on microfilaremia. *American Journal of Tropical Medicine and Hygiene* **59**, 667–672.

Smith, H.V., Kusel, J.R. & Girdwood, R.W. (1983) The production of human A and B blood group like substances by in vitro maintained second stage *Toxocara canis* larvae: their presence on the outer larval surfaces and in their excretions/secretions. *Clinical and Experimental Immunology* **54**, 625–633.

Soulsby, E.J.L. (1987) Larva migrans in perspective. pp. 37–48 *in* Geerts, S., Kumar, V. & Brandt, J. (*Eds*) *Helminth zoonoses*. , Dordrecht, Martinus Nijhoff.

Sugane, K., Kusama, Y., Takamoto, M., Tominaga, A. & Takatsu, K. (1996) Eosinophilia, IL-5 level and recovery of larvae in IL-5 transgenic mice infected with *Toxocara canis*. *Journal of Helminthology* **70**, 153–158.

Taylor, M., Keane, C., O'Connor, P., Mulvihill, E. & Holland, C. (1988) The expanded spectrum of toxocaral disease. *Lancet* **i**, 692–695.

Tischendorf, F.W., Brattig, N.W., Lintzel, M., Buttner, D.W., Burchard, G.D., Bork, K. & Muller, M. (2000) Eosinophil granule proteins in serum and urine of patients with helminth infections and atopic dermatitis. *Tropical Medicine and International Health* **5**, 898–905.

Tomassini, M., Magrini, L., De Petrillo, G., Adriani, E., Bonini, S., Balsano, F. & Bonini, S. (1996) Serum levels of eosinophil cationic protein in allergic diseases and natural allergen exposure. *Journal of Allergy and Clinical Immunology* **97**, 1350–1355.

Werner, J.C., Ross, R.D., Green, W.R. & Watts, J.C. (1999) Pars plana vitrectomy and subretinal surgery for ocular toxocariasis. *Archives of Ophthalmology* **117**, 532–534.

Yahiro, S., Cain, G. & Butler, J.E. (1998) Identification, characterization and expression of *Toxocara canis* nematode polyprotein allergen TBA-1. *Parasite Immunology* **20**, 351–357.

(Accepted 25 May 2006)