Rev. Inst. Med. trop. São Paulo
37 (3): 187-190, maio/junho, 1995.

PERSISTENCE OF SPECIFIC ANTIBODY RESPONSE IN DIFFERENT EXPERIMENTAL INFECTIONS OF MICE WITH *TOXOCARA CANIS* LARVAE

Pedro Paulo CHIEFFI (1), Benedito A. PERES (2), Elisabete O. de MELLO (2), Hermínia KANAMURA (3) & Marcelo M. BRANDÃO (4)

SUMMARY

Anti-*Toxocara* antibody production and persistence were studied in experimental infections of BALB/c mice, according to three different schedules: Group I (GI) - 25 mice infected with 200 *T. canis* eggs in a single dose; Group II (GII) 25 mice infected with 150 *T. canis* eggs given in three occasions, 50 in the 1st, 50 in the 5th and 50 in the 8th days; Group III (GIII) - 25 mice also infected with 150 *T. canis* eggs, in three 50 eggs portions given in the 1st, 14th and 28th days. A 15 mice control group (GIV) was maintained without infection. In the 30th, 50th, 60th, 75th, 105th and 180th post-infection days three mice of the GI, GII and GIII groups and two mice of the control group had been sacrificed and exsanguinated for sera obtention. In the 360th day the remainder mice of the four groups were, in the same way, killed and processed. The obtained sera were searched for the presence of anti-*Toxocara* antibodies by an ELISA technique, using *T. canis* larvae excretion-secretion antigen. In the GI and GII, but not in the GIII, anti-*Toxocara* antibodies had been found, at least, up to the 180th post-infection day. The GIII only showed anti-*Toxocara* antibodies, at significant level, in the 30th post-infection day.

KEYWORDS: . *Toxocara canis*; Antibodies anti-*Toxocara*; Experimental toxocariasis.

INTRODUCTION

In the last 20 years, the importance of infection by *Toxocara canis* larvae, as causative agent of visceral larva migrans syndrome (VLM) in humans, has been emphasized by investigators from different countries [1, 18]. In Brazil, a seroepidemiological survey carried out in 2,025 individuals from five municipalities of São Paulo State found 3.7% of them with anti-*Toxocara* antibodies at significative levels [7].

The pattern of anti-*Toxocara* antibody production, as well as, its persistence in serum of *T. canis* infected humans is not totally clear. CYPESS et al. [8] and FENOY et al. [12] suggest a long persistence of anti-*Toxocara* antibodies in infected humans and similar results had been showed in mice [4, 16]. To know about this subject is, however, mandatory to the clear understanding of finding anti-*Toxocara* antibodies among human beings without any VLM symptoms, as well as to adequately appraise the persistence and level of these antibodies in patients with overt disease.

Supported by grants of Laboratório de Investigação Médica (LIM-06 and LIM-48).

(1) Instituto de Medicina Tropical and Departamento de Medicina Preventiva (FMUSP), São Paulo, S.P., Brasil.
(2) Instituto de Medicina Tropical and Departamento de Doenças Infecciosas e Parasitárias (FMUSP). São Paulo, S.P., Brasil.
(3) Departamento de Análises Clínicas (FCFUSP). São Paulo, S.P., Brasil.
(4) Centro de Controle de Zoonoses da Prefeitura Municipal de São Paulo. São Paulo, S.P., Brasil.
Correspondence to: P. P. Chieffi, Inst. Med. Tropical de São Paulo, Av. Dr. Enéas Carvalho Aguiar, 470, 05403-000 São Paulo, S.P., Brasil.

In this paper the presence and one-year long persistence of anti-*Toxocara* antibodies in three schedule *T. canis* infected mice had been studied, simulating a situation that could be actually common, i.e., the frequent occurrence of reinfection in natural conditions.

MATERIAL AND METHODS

1. Obtention of T. canis *infective eggs*

T. canis eggs were obtained by dissecting female worms from dog necropsies at the São Paulo Zoonosis Control Centre. The eggs collected in 2.0% formalin were kept at 28°C. After reaching the infective stage the eggs were washed with destilled water.

2. Production of T. canis *excretion-secretion antigen (TES)*

T. canis larvae were hatched from embryonated eggs and cultivated according to the DE SAVIGNY [9] method. TES was obtained from this culture according to the technique described by GLICKMAN et al. [13].

3. Infection of mice

One hundred and five BALB/c mice were divided into four groups (GI, GII, GIII and GIV). Fifteen had been maintained uninfected as control group (GIV). The remainder three groups were infected by stomach tube according to the following schedule:

GI - 25 mice received a single dose of 200 embryonated *T. canis* eggs.

GII - 25 mice received three doses of 50 embryonated *T. canis* eggs each in the 1st, 5th and 8th day.

GIII - 25 mice received three doses of 50 *T. canis* embryonated eggs in the 1st, 14th and 28th day.

In the 30th, 50th, 60th, 75th, 105th and 180th days post-infection 3 mice of the GI, GII and GIII groups and 2 mice of the control group (GIV) were heavily anesthetized and exsanguinated via brachial artery. In the 360th day post-infection the remaining mice of all groups were sacrificed in the same way.

Serum was obtained from the blood of each sacrificed mouse, and stored at - 70°C.

4. Enzyme Immunoassay (ELISA)

Plastic microplates were sensitized with 200 µL of antigen, after adjusting the concentration to 8µg/mL protein in 0.1M carbonate buffer (pH=9.6). The plates were incubated at 37°C for two hours and, after, overnight at 4°C. Afterward the plates were washed twice with pH 7.2 buffered NaCl 0.15M solution with Tween 0.05% (PBS-T). To bloch the free binding sites 200 µL of 0.1% bovine serum albumine diluted in PBS (BSA-PBS) were added.

After one hour incubation at 37°C the plates were washed with PBS-T and 200 µL of serial two fold dilutions of mouse serum (1:20 to 1:1250) were added to each well and incubated at 37°C for 30 minutes. Then, 200 µL of anti-mouse IgG conjugated with peroxidase (CAPEL laboratories, U.S.A) were added and after incubating for 30 minutes the plates were washed twice with PBS-T. The substrate solution (OPD, SIGMA) was placed and after 30 minutes the reaction was stopped with 25 µL of 8N H2SO4 solution. The optical density at 492 nm was measured in each well using an automatic microplate reader (Titertek Multiscan MCC/340). All serum samples were previously absorbed with *Ascaris suum* antigenic extract obtained from adult worms.

RESULTS

Anti-*Toxocara* antibodies were detected by ELISA in all collected sera, showing high optical density values in mice of the GI and GII groups, at least, from the 30th up to the 180th day post-infection. The GIII mice only showed high optical density values in the sera collected 30 days after the first infection (Table 1).

DISCUSSION

The concern about VLM has been improved in Brazil after the finding of significative levels of anti-*Toxocara* antibodies in 3.7% of the examined sera belonging to 2,025 individuals living in five municipalities of the State of São Paulo [7]. Reinforcing this trend JACOB et al. [15] have recently described several cases of symptomatic VLM in children of the municipality of São Paulo. Similar situation had previously occurred in other countries [11, 13, 18].

The enzyme-linked immunosorbent assay (ELISA) performed with excretory-secretory antigens delivered by *T. canis* larvae [9], is the most frequently used technique for the diagnosis of *Toxocara* human and animal infections [13], by demonstration of anti-*Toxocara* antibodies. Although some investigations

TABLE 1

Anti-*Toxocara* antibody levels in sera from experimentally infected mice, during 360 days of infection*.

Days	GI		GII		GIII		GIV	
	OD	T	OD	T	OD	T	OD	T
30	1.23	>1280	1.33	>1280	0.95	160	0.36	160
50	1.54	>1280	1.56	>1280	0.44	40	0.37	40
60	1.59	>1280	1.54	>1280	0.41	40	0.37	20
75	1.44	>1280	1.56	>1280	0.32	40	0.29	20
105	1.56	>1280	1.56	>1280	0.32	40	0.29	20
180	1.47	>1280	1.56	>1280	0.42	40	0.29	20
360	0.19	20	0.14	20	0.14	20	0.29	20

* In each day GI, GII and GIII mice results correspond to the mean of 3 mice; the GIV mice results correspond to the mean of 2 mice. OD = Optical density corresponding to the dilution of 1:160. T = Titre.

had shown the long time persistence of anti-*Toxocara* antibodies in human and animal sera [4, 8, 12, 16], the kinetics of production and persistence of these antibodies after *Toxocara* infections haven't been totally known yet.

Several wild animals, rodents in special, has been considered as paratenic hosts for *T. canis* [3, 6], showing some involvement in the maintenance of high levels of infection in dogs. There are some indications about the impairment of the behaviour in *Toxocara*-infected rodents, making easy their prey by dogs [5, 10, 14]. On the other hand, the experimental infection of mice by *T. canis* larvae apparently haven't modified their life span [17]. BEAVER [2] called attention for the long persistence of alive *T. canis* larvae in primate tissues. These data justify the interest in improving the knowledge about the persistence of *T. canis* larvae in the organism of paratenic hosts as mice, as well as in patients presenting the classical signs and symptoms of the VLM. On the other hand, would be worthwhile to follow the kinetics of anti-*Toxocara* antibody after specific treatment of these patients.

Usually in experimental infections it has been administered high doses of *Toxocara* infective eggs but, in natural conditions, probably paratenic hosts are exposed, many times, to small doses of embryonated eggs. In mice, experimentally infected with low doses of infective eggs, BOWMAN et al. [4] observed *T. canis* antigens in the serum, after treatment with EDTA and heat. They showed as well that *T. canis* serum antigenic concentration is proportional to the number of eggs administered to mice. Similar results were found by KAYES et al. [16], when they determined the levels of anti-*Toxocara* antibodies in experimentally infected mice.

In the present paper, we demonstrate the persistence of anti-*Toxocara* antibodies in high levels at least six months after the experimental infection of mice with low doses of *T. canis* embryonated eggs, when the infection was obtained by either single administration of 200 *T. canis* eggs or three doses of 50 eggs, given in the 1st, 5th and 8th days. Thirty days after the first dose of infective eggs the mice already had shown high levels of serum anti-*Toxocara* antibodies and the higher antibody levels were found in the 50th day after infection. KAYES et al. [16] had been found higher levels of anti-*Toxocara* antibodies in experimentally infected mice seven weeks after infection, as well.

In the GIII group it was possible to find significative levels of anti-*Toxocara* antibodies only 30 days after the first infective dose. In the other two groups, however, the presence of high levels of anti-*Toxocara* antibodies had been demonstrated at least six months after the first inoculation.

In fact, the three mice groups had received similar numbers of *T. canis* embryonated eggs, mainly the GII and GIII, and the different humoral responses could be explained by a hypothetic interference of the infective eggs given at the 14th and 28th day after the beginning of the experiment, when, theoretically, the level of anti-*Toxocara* antibody was growing up. However, the confirmation of this hypothesis needs further and more profound studies.

RESUMO

Persistência da resposta humoral em camundongos experimentalmente infectados com larvas de *Toxocara canis*.

Estudou-se a cinética de anticorpos anti-*Toxocara* em camundongos BALB/c infectados experimentalmente segundo três esquemas: Grupo I (GI): 25 camundongos infectados com dose única de 200 ovos embrionados de *T. canis*; grupo II (GII): 25 camundongos infectados com 150 ovos embrionados de *T. canis*, divididos em três doses de 50 ovos, administrados no 1º, 5º e 8º dias; Grupo III (GIII): 25 camundongos infectados com 150 ovos embrionados de *T. canis*, administrados em três doses de 50 ovos no 1º, 14º e 28º dias. Um grupo de 15 camundongos foi mantido nas mesmas condições, porém sem infecção, constituindo o grupo controle (GIV). No 30º, 50º, 60º, 75º, 105º e 180º dias pós-infecção três camundongos dos grupos GI, GII e GIII e dois do grupo controle foram sacrificados sob anestesia e sangrados para obtenção de soro. No 360º dia os animais restantes dos quatro grupos foram igualmente sacrificados. Pesquisou-se a presença de anticorpos anti-*Toxocara* nos soros obtidos utilizando-se teste imunoenzimático (ELISA) e empregando-se antígeno de excreção-secreção produzido por larvas de *T. canis* mantidas em cultura. Nos grupos GI e GII revelou-se a presença de anticorpos anti-*Toxocara*, em níveis elevados, pelo menos até o 180º dia após infecção. Nos camundongos do grupo GIII apenas no 30º dia pós-infecção foi possível detectar anticorpos anti-*Toxocara*.

REFERENCES

1. BARRIGA, O. - A critical look at the importance, prevalence and control of toxocariasis and the possibilities of immunological control. **Vet. Parasit., 29**: 195-234, 1988.

2. BEAVER, P.C. - Zoonoses, with particular reference to parasites of veterinary importance. **In**: SOULSBY, E.J.L., ed. *Biology of parasites. Emphasis on veterinary parasites.* New York, Academic Press, 1966. p. 215-227.

3. BEAVER, P.C. - The nature of visceral larva migrans. **J. Parasit., 55**: 3-12, 1969.

4. BOWMAN, D.D.; MIKA-GRIEVE, M. & GRIEVE, R.B. - Circulating excretory-secretory antigen levels and specific antibody responses in mice infected with *Toxocara canis*. **Amer. J. trop. Med. Hyg., 36**: 75-82, 1987.

5. BURRIGHT, R.G.; DONOVICK, P.J.; DOLINSKY, Z.S. & HURD, Y. - Behavioural changes in mice infected with *Toxocara canis*. **J. Toxicol. environ. Hlth., 10**: 621-626, 1982.

6. CHIEFFI, P.P.; DEL GUÉRCIO, V.M.F.; UEDA, M. & MELLO, L.B. - Importância de *Rattus norvegicus* capturados no município de São Paulo, SP, Brasil, como hospedeiros paratênicos de *Toxocara canis* (Ascaroidea, Nematoda). **Rev. Inst. Adolfo Lutz, 41**: 89-91, 1981.

7. CHIEFFI, P.P.; UEDA, M.; CAMARGO, E.D. et al. - Visceral larva migrans: a seroepidemiological survey in five municipalities of São Paulo State, Brazil. **Rev. Inst. Med. trop. S. Paulo, 32**: 204-210, 1990.

8. CYPESS, R.H.; KAROL, M.H.; ZIDIAN, J.L.; GLICKMAN, L.T. & GITLIN, D. - Larva-specific antibodies in patients with visceral larva migrans. **J. infect. Dis., 135**: 633-640, 1977.

9. DE SAVIGNY, D.H. - *In vitro* maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic tests for visceral larva migrans. **J. Parasit., 61**: 781-782, 1975.

10. DOLINSKY, Z.S.; BURRIGHT, R.G.; DONOVICK, P.J. et al. - Behavioral effects of lead and *Toxocara canis* in mice. **Science, 213**: 1142-1144, 1981.

11. EHRHARD, T. & KERNBAUM, S. - *Toxocara canis* et toxocarose humaine. **Bull. Inst. Pasteur, 77**: 225-287, 1979.

12. FENOY, S.; CUÉLLAR, C.; AGUILA, C. & GUILLÉN, J.L. - Persistence of immune response in human toxocariasis as measured by ELISA. **Int. J. Parasit., 22**: 1037-1038, 1992.

13. GLICKMAN, L.T.; SCHANTZ, P.M.; DOMBROSKE, R. & CYPESS, R. - Evaluation of serodiagnostic tests for visceral larva migrans. **Amer. J. trop. Med. Hyg., 27**: 492-498, 1978.

14. HAY, J. & AITKEN, P.P. - Experimental toxocariasis in mice and its effect on their behaviour. **Ann. trop. Med. Parasit., 78**: 145-155, 1984.

15. JACOB, C.M.A.; PASTORINO, A. C.; PERES, B.A. et al. - Clinical and laboratorial features of visceral toxocariasis in infancy. **Rev. Inst. Med. trop. S. Paulo, 36**: 19-26, 1994.

16. KAYES, S.G.; OMHOLT, P.E. & GRIEVE, R.B. - Immune responses of CBA/J mice to graded infections with *Toxocara canis*. **Infect. Immun., 48**: 697-703, 1985.

17. NICHOLAS, W.L.; STEWART, A.C. & MITCHELL, G.F. - Antibody responses to *Toxocara canis* using sera from parasite-infected mice and protection from toxocariasis by immunization with ES antigens. **Aust. J. exp. Biol. med. Sci., 62**: 619-626, 1984.

18. SCHANTZ, P.M. - *Toxocara* larva migrans now. **Amer. J. trop. Med. Hyg., 41** (suppl.): 21-34, 1989.

Recebido para publicação em 11/12/1994.
Aceito para publicação em 02/03/1995.