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

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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, 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. 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. The pattern of anti-Toxocara antibody production, as well as, its persistence in serum of T. canis infected humans is not totally clear. CYFESS et al. and FENGY et al. suggest a long persistence of anti-Toxocara antibodies in infected humans and similar results had been showed in mice. 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.
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 * method. TES was obtained from this culture according to the technique described by GLICKMAN et al. 11.

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 8 μg/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 block 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 wells 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 8H H2SO4 solution. The optical density at 492 nm was measured in each well using an automatic microplate reader (Titertek Multispec 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 1. Reinforcing this trend JACOB et al. 12 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, 14.

The enzyme-linked immunosorbent assay (ELISA) performed with excretory-secretory antigens delivered by T. canis larvae 1, is the most frequently used technique for the diagnosis of Toxocara human and animal infections 15, by demonstration of anti-Toxocara antibodies. Although some investigations
had shown the long time persistence of anti-Toxocara antibodies in human and animal sera 5, 8, 12, 19, 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 paratonic hosts for T. canis 3, 4, 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 paratonic 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 paratonic 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.

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**TABLE I**

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

<table>
<thead>
<tr>
<th>Days</th>
<th>G1 OD</th>
<th>G1 T</th>
<th>GII OD</th>
<th>GII T</th>
<th>GIII OD</th>
<th>GIII T</th>
<th>GIV OD</th>
<th>GIV T</th>
</tr>
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<tbody>
<tr>
<td>30</td>
<td>1.23</td>
<td>&gt;1280</td>
<td>1.33</td>
<td>&gt;1280</td>
<td>0.95</td>
<td>160</td>
<td>0.36</td>
<td>160</td>
</tr>
<tr>
<td>50</td>
<td>1.54</td>
<td>&gt;1280</td>
<td>1.56</td>
<td>&gt;1280</td>
<td>0.44</td>
<td>40</td>
<td>0.37</td>
<td>40</td>
</tr>
<tr>
<td>60</td>
<td>1.59</td>
<td>&gt;1280</td>
<td>1.54</td>
<td>&gt;1280</td>
<td>0.41</td>
<td>40</td>
<td>0.37</td>
<td>20</td>
</tr>
<tr>
<td>75</td>
<td>1.44</td>
<td>&gt;1280</td>
<td>1.56</td>
<td>&gt;1280</td>
<td>0.32</td>
<td>40</td>
<td>0.29</td>
<td>20</td>
</tr>
<tr>
<td>105</td>
<td>1.56</td>
<td>&gt;1280</td>
<td>1.56</td>
<td>&gt;1280</td>
<td>0.32</td>
<td>40</td>
<td>0.29</td>
<td>20</td>
</tr>
<tr>
<td>180</td>
<td>1.47</td>
<td>&gt;1280</td>
<td>1.56</td>
<td>&gt;1280</td>
<td>0.42</td>
<td>40</td>
<td>0.29</td>
<td>20</td>
</tr>
<tr>
<td>360</td>
<td>0.19</td>
<td>20</td>
<td>0.14</td>
<td>20</td>
<td>0.14</td>
<td>20</td>
<td>0.29</td>
<td>20</td>
</tr>
</tbody>
</table>

*In each day G1, 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.
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-infeção 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 excréção-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-infeção foi possível detectar anticorpos anti-*Toxocara*.

REFERENCES


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