

MULTIPLE INFECTION WITH **TOXOCARA CANIS**. INFLUENCE OF ANTIHISTAMINES AND CORTICOSTEROIDS IN HEMATOLOGICAL COUNTS AND IMMUNODIAGNOSTIC TESTS

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RESUMO

Infestação múltipla por *Toxocara canis*. Influência de antihistamínicos e corticosteróides na fórmula leucocitária e testes imuno-diagnósticos.

O Autor descreve um modelo de infestação de camundongos com múltiplas doses de ovos embrionados de *Toxocara canis*. Foram realizadas contagem de fórmula leucocitária total e diferencial, intradermorreação, gel-difusão e testes de imunoaderência em camundongos-controles não infectados e em animais infectados desta forma, observando-se também grupos tratados com clorprofeniramina e betametasona. Todos os animais infestados desenvolveram leucocitose e eosinofilia, sendo esta menos intensa nos grupos tratados com corticos-

teróide ou antihistamínico. A intradermorreação foi altamente positiva em todos os grupos infestados, exceto aquele que foi medicado com clorprofeniramine, que exibiu reações menos intensas. Todos os animais infestados apresentaram anticorpos circulantes facilmente detectáveis pelos métodos empregados, independente do tratamento a que estavam sendo submetidos.

INTRODUCTION

The serological and hematological findings in visceral larva migrans have been described in several papers in which infection occurred naturally or as a result of experimental design^{1, 4}.

The way in which natural infection occurs is not known in detail, although it is probable that contaminated soil is ingested

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by affected children on several occasions 2, 3. On the other hand, most of the experimental work done on toxocariasis describe infections established in one or two single doses, and it is possible that the difference in the number of doses might determine some differences between natural and experimental infection, making extrapolation even more difficult.

This work will describe a form of multiple infection of mice with *T. canis* infective eggs, the hematological and immunological findings and the alterations imposed upon these parameters by corticosteroids and antihistamines.

MATERIALS AND METHODS

Adult white mice weighing 20-25 g. were used throughout the study. The obtainance and embryonation of infective eggs were described previously¹⁰. Infections were carried out by means of a blunted 18-gauge needle introduced as a stomach tube, with the animals unanesthetized, and each animal received 10 doses of 100 infective eggs with intervals of 4 days between consecutive doses. The precautions outlined by Oshima⁶ were followed to ensure accuracy in the number of eggs administered. One group of mice was given saline per os and served as a non-infected control. After the last dose of *T. canis* eggs, the infected mice were divided into three groups: the first one (non-treated) received intramuscular saline injections of 0.5 ml twice a day for 5 consecutive days. The second group (antihistamine-treated) received chlorpheniramine

(0.0085 mg/kg/day) in two intramuscular injections each day 5 consecutive days. The third group was given betamethasone (1 mg/Kg/day) in two intramuscular dally injections for 5 consecutive days. A fourth group consisted of non-infected, non-treater mice.

On the morning of the sixth day, all the animals were shaved on their back a skin test was performed with 0.1 ml of *T. canis* antigen 1:1,000 (Woodruff & Thacher⁹) and a control injection of saline. A positive result was recorded when the initial papule increased its size to more than twice the original size, doubtful-positive when the increase in size was of less than double, and negative, when a decrease of size was observed. After the reading of the test, the mice were bled from the retroorbital plexus to death. A small amount of blood was separated for leukocyte counts and blood smears, while the rest was allowed to clot for the separation of serum. White blood cells and differential leukocyte counts were done according to routine methods⁵. Microimmunodiffusion was carried out according to Ouchterlony⁷. Immune adherence experiments were performed in the following way: second stage. *T. canis* larvae were obtained from infective eggs by treatment with 5% sodium hypochlorite for 30 minutes followed by three washings with distilled water and strong straining with clean sand of homogeneous particle size. Decantation and repeated washing with water were the following steps. The

clean larvae were then concentrated by centrifugation and incubated at 37° C for 30 minutes with the inactivated serum of the test animals. As the test was not meant to be quantitative, all serum samples were diluted to a final dilution of 1:50. The larvae were washed three times with PBS pH 7.2 and again incubated at 37° C for 30 minutes with guinea-pig serum diluted at 1:30 in PBS. After washing three times with PBS pH 7.2, the larvae were exposed to a 3% suspension of human red cells (AB Rh +) and transferred to specially designed glass slides, where the sample could be sealed with paraffin, according to Soulsby⁸. Reading was done after 10 minutes and the results were scored as either positive or not.

RESULTS

The results are shown in Table I.

Leukocytosis and eosinophilia were observed in all infected animals, being more striking in infected and non-treated animals. Eosinophilia was of a lower degree in antihistamine and corticosteroid-treated animals. The skin test was negative in non-infected animals and strongly positive in infected, non-treated mice and in those treated with betamethasone. Somehow smaller papules (less than twice the original size) were seen in antihistamine-treated mice. Immunodiffusion and immune adherence tests showed positive results in all infected animals, independent

of whatever treatment they had undergone.

DISCUSSION

This work was designed with the intention of simulating the natural conditions in which infections take place, so that experimental infections with multiple doses of eggs yield results more readily comparable to human clinical material.

The blood cell counts are in agreement with previous findings in the literature 1, 4, and although the number of experiments was small, corticosteroids and antihistamines seemed to have reduced the eosinophilic response in blood without changing considerably the number of total leukocytes.

The skin test was quite sensitive and was partly diminished by the systemic administration of antihistamines. This might be explained on the basis of a biochemical block of the action of histamine, which was not complete since in a mouse other substances are important in the genesis of local anaphylaxis. Further experiments using anti-serotonin agents would be an interesting development.

All infected animals showed significant antibody production as demonstrated by immunodiffusion and immune adherence results, and it is apparent that antihistamines and betamethasone did not interfere in this aspect, at least considering the present scheme of administration and doses of the drugs. This is in keeping with

T A B L E I

EXPERIMENTAL RESULTS OF MULTIPLE INFECTION OF MICE WITH *TOXOCARA CANIS*

Groups	Non-infected controls			Infected controls			Antihistamine treated mice			Corticosteroid treated mice		
	B L O O D C E L L C O U N T S											
Leukocytes §	7.2	6.4	7.5	12.5	11.5	10.8	9.7	9.3	10.5	10.3	11.0	9.5
Eosinophis *	2	1	5	28	16	19	14	12	10	15	14	13
Polymorphs *	30	28	37	16	24	13	20	22	34	28	13	23
Lymphocytes *	60	59	50	47	52	56	57	59	47	50	66	55
Monocytes *	8	12	8	7	8	12	9	7	9	7	7	8
	S K I N T E S T +											
	-	-	-	+	+	+	D	D	D	+	+	+
	M I C R O - I M M U N O D I F F U S I O N											
N. of bands	-	-	-	1	2	2	2	1	1	1	2	2
	I M M U N E A D H E R E N C E T E S T											
	-	-	-	+	+	+	+	+	+	+	+	+

(§) = x 1,000; (*) = in percentage; (+) = positive; (-) = negative; D = doubtful positive (see text).

Table I — Experimental results of multiple infection of mice with *Toxocara canis*. (§) = x 1,000; (*) = in percentage; (-) = negative; D = doubtful positive (see text).

finding of Aljeboori & Ivey¹ and Fernando⁴, among others.

It is our intention to use the multiple-dose infection model in subsequent works.

SUMMARY

MULTIPLE INFECTION WITH *TOXOCARA CANIS*. INFLUENCE OF ANTIHISTAMINES AND CORTICOSTEROIDS IN HEMATOLOGICAL COUNTS AND IMMUNODIAGNOSTIC TESTS.

The Author describes a multiple-dose model of infection of mice with *Toxocara canis* embryonated eggs. Blood cell counts, skin test, immunodiffusion and immune adherence tests are performed in mice infected according to this model. The effect of betamethasone and chlorpheniramine are observed on these parameters. Leukocytosis and eosinophilia were observed in all infected mice, the latter being less striking in groups treated with both drugs. The skin test was strongly positive in all infected animals, except the antihistamine, treated group, which showed smaller reactions. Circulating antibody response was similar in all infected groups, independent of whatever treatment they had undergone.

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