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SERUM LEVEL OF IgG AND WORM LOAD IN MALE SWISS ALBINO MICE INOCULATED WITH L3 LARVAE OF *ANCYLOSTOMA CANINUM*

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ABSTRACT
Experiments were conducted to study the level of serum IgG and the effect of infective, filariform larvae of *Ancylostoma caninum* in male swiss albino mice (6 to 8 wks old, 23-26 g wt). Three groups of mice (group A, 500 doses; group B, 1000 dose; group C, 2000 dose) were infected orally with a single dose of infected larvae. The decrease of serum IgG level in all the experimental groups of mice was influenced by the retained worm load in the host system. No correlation was found between the level of serum IgG and retained worm burden in the host system.

INTRODUCTION

Ancylostomiasis (hookworm disease) is reported throughout the world. Off all the hookworm species, *Ancylostoma caninum* (the dog hookworm) is found in dogs throughout the tropical and sub tropical countries. *A. caninum* has received greater attention in the field of veterinary as well as public health research due to its zoonotic importance. Migration and survival pattern of male swiss albino mice has been studied by several researchers (Banerjee *et al.,* 1970; Vardhani and Johri, 1981). Human enteric infection with *Ancylostoma caninum* confirmed the presence of IgG and IgE in patients of northeastern Australia (Prociv and Croese, 1996). Hookworm infection inhibits growth and intellectual development in millions of children (Hotez and Pritchard, 1995). Recombinant polypeptides of *Ancylostoma* secreted protein (ASP) (genetically engineered vaccine) have been shown to reduce hookworm burdens after larval infection in mice (Hotez *et al.,*1999) School age children living in a rural area of the tropics showed high levels of serum IgE (>/=3564 IU/L) during the infection of *Ancylostoma duodenale* (Cooper *et al.,* 2003). Following adult worm transfer, hamsters acquired vigorous serum IgG responses to soluble whole hookworm extract and ES products (Bungiro Jr. *et al.,* 2003). Hamsters immunized by *Ancylostoma caninum* infection showed protective, mucosal response against secondary infection (Alkazmi and Behnke, 2010). Liver may serve as immunological barrier in preventing the onward migration in mice infected with infective larvae of *Ancylostoma caninum* (Viveka Vardhani and Sakunthala, 2011). No information is available on serum antibody level during experimental ancylostomiasis in mice. Therefore, the present investigation is undertaken to estimate the level of serum IgG at various periods of infection and to assess their activity with regard to the retained /expelled worm burden in mice.

MATERIALS AND METHODS

Infective *Ancylostoma caninum* larvae were cultured following the method of Sen *et al.,* (1965). Six weeks old male swiss albino mice (23-26g) were used as experimental animals. Thirty mice were infected orally with 500 larvae (group A), 30 with 1000 (group B) and 30 with 2000 (group C). Ninety mice were kept as uninfected control (group D). Five mice from each infected group were sacrificed on day 1,4,9,16,23 and 30 after infection for the collection of blood and larvae. Five mice from control group were also sacrificed on the same designated days of each experiment for the collection of blood. Serum was separated and analyzed for IgG using ELISA and the worm load in different organs was assessed from larvae recovering through Baermann's technique.

RESULTS

The mean values of serum IgG from the infected (groups A, B and C) and control (group D) mice and larval recoveries for the 30 days experimental period are shown in Table 1. In group A (500 dose), there was a considerable decrease of IgG

Table 1: Serum IgG value (g/L) and larval recoveries from experimental (group A, B and C) and control (group D, IgG value) mice at different period of infection. (Values are expressed in mean derived from five observations)

Period of infection	Experimental groups						Control group D IgG
	A		B		C		
	IgG	WL	IgG	WL	IgG	WL	
1	4.8	63.0	6.2	71.O	11.31	56.0	8.5
4	1.0	55.4	2.8	68.2	1.0	45.4	8.48
9	1.0	48.0	1,0	67.0	1.0	45.0	8.5
16	7.2	46.0	5.12	63.5	0.15	36.0	8.47
23	7.1	36.0	5.11	60.0	0.18	34.0	8.5
30	8.5	24.0	2.0	57.0	2.0	32.0	8.5

A = 500 dose; B = 1000 dose; C = 2000 dose; D = uninfected; W L = worm load

Table 2: "T" values obtained for different groups of mice infected with 500, 1000 and 2000 dose of *Ancylostoma caninum* larvae

Groups	A	B	C	D
IgG				
Mean	4.9	3.7	2.6	8.49
T value	A D /_____/ t = 2.68* (p > 0.05)	B D /_____/ t = 6.33* (p > 0.05)	C D /_____/ t = 3.65* (p > 0.05)	
	A B /_____/ t = 0.83** (p < 0.05)	A C /_____/ t = 1.12** (p < 0.05)	B C /_____/ t = 0.60** (p < 0.05)	
Worm burden:				
Mean	45.4	41.4	64.45	
T value	A B /_____/ t = 3.425* (p > 0.05)	A C /_____/ t = 0.484** (p < 0.05)	B C /_____/ t = 5.864* (p > 0.05)	

p value at 5% level of significance is 2.306;*- statistically significant value; ** -Non significant value

on day 1, 4 and 9 after primary infection as compared to control mice. The level of IgG again increased on day 16 and 23 (below normal level); by day 30 IgG increased to normal level.

In group B (1000 dose), there was a marked decrease of IgG on day 1,4,9,16,23 and 30 of infection when compared to controls. In group C (2000 dose), a marked increase of IgG was found on day 1 (11.31g/L) of infection. Interestingly, there was a steep fall in the level of IgG on day 4 and 9 of infection (1.0g/L). IgG level decreased further by day 16 (0.15g/L) and 23 (0.18g/L) of infection. There was a slight increase on day 30 of infection (2.0g/L) but it was still below normal value. The total yield of *A. caninum* larvae declined from day 1 to 30 in the all groups (Table 1). The mean values of serum IgG and larval recoveries with their t values are shown in Table 2. Differences in IgG levels were statistically significant in all the three experimental groups when compared with controls, but there was no significant difference when compared among them. There was a significant difference in larval recoveries in groups A, B and C when compared with each other.

DISCUSSION

The IgG level decreased significantly and remained lower than those of controls throughout the experimental period (day 1 to 30 of infection) in all these experimental groups except the marked rise of IgG on day 1 of infection in group C (infected with 2000 dose of larvae). The decreased values of serum IgG level in all the groups (except on day 1 in group C) indicate the correlation with the pathogenic effect of the infective larvae of *A. caninum* (Vardhani, 2006). The low values of serum IgG in infected mice confirm the observations of Correa – Oliveira *et al*. (1988) who found low activity of antibodies in humans against *A. caninum* infection. Bungiro Jr. *et al*. (2003) also reported no significant differences in antibody titer in hamsters treated with ES products and in those infected with A. *caninum* hookworm on day 14 of experiment. Though the decreased level of IgG is not dose dependent in experimental animals, the retained larval infection triggered considerable immunogenicity in lowering the level of IgG. Complete resolution of anemia and weight loss by day 9 of infection suggested by Vardhani (2003) during ancylostomiasis in mice.

In the present investigations, it is clear that the protective role of the major immunoglobulin, IgG is reduced in all the experimental groups of mice (infected with single doses). Bungiro Jr. *et al*. (2003) suggested that mice may fail to produce appreciable hookworm specific antibody responses following a single exposure to infection during ancylostomiasis.

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