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SERUM IgE LEVEL IN MICE INFECTED WITH SINGLE DOSES OF *ANCYLOSTOMA CANINUM* LARVAE

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ABSTRACT
The level of IgE in the serum of singly infected mice (group A, 500 dose; B, 1000 dose; group C, 2000 dose) was studied during murine ancylostomiasis in all the 3 groups of singly infected animals, IgE level began to decrease on 1st day of infection, reached the lowest value on day 30 and progressively declined from day 1 to 30 of experimental period. Statistical analysis indicated a significant decrease in all the 3 groups when compared with controls. These studies demonstrated that the larval antigens provoked an immune response resulting into a low level of serum IgE in the mouse system.

INTRODUCTION

Gastrointestinal nematode parasites infect a large proportion of the world's human and domestic/companion animals. The canine hookworm, *A. caninum* is capable of causing anemia and death in severely infected puppies, and is a useful model for the human hookworm, *A. duodenale* and *Necator americanus*. Canine hookworms are of great importance for humans because of their ability to cause zoonosis (Menelaos and Smaragda, 2006). Hookworms suck large amounts of blood from infected hosts leading to fatal anemia (Bowman, 1999). Humans can get hookworm infection through ingestion or direct penetration of hookworm larvae (Little *et al*., 1983; Prociv and Croese, 1990; Landman and Prociv, 2003). Human enteric infections with infective larvae of *A. caninum* were reported in North eastern Austria and Southern USA and infected humans are being suffered due to eosinophilic enteritis (Glickman and Schantz, 1981). Hookworm anemia could be induced from hemolysis or from dysfunction of bone marrow; a decrease in circulating RBC was reported in abnormal host (mouse) (Vardhani, 1976). Hypercholesterolemia was reported in female Swiss albino mice during A. *caninum* infection (Vardhani and Krishna Rao, 1995). Hookworm infection retards growth and development in millions of children (Hotez and Pritchard, 1995) and is a major parasitic cause of morbidity in the developing nations of the tropics (Hotez *et al.,* 1999). The school age children in a rural area of the tropics showed highest level of IgE during active infection with *A. duodenale* (Cooper *et al.,* 2003).

Studies in northeastern Australia reveal that enteric infection with *A. caninum* is a leading cause of human eosinophilic enteritis; enteric infection of humans with *A. caninum* excretory and secretory antigens was associated with an increase of IgG and IgE levels (Prociv and Croese, 1996). Sera of humans with *Ancylostoma* infection showed considerable antibody activity against antigens (Correa-Oliveira *et al*., 1988; Loukas *et al.,* 2005). Development of antigen specific immunoglobulin E antibody response was found in vaccinated dogs against subcutaneous infection of 500 L3 of canine hookworm, *Ancylostoma caninum* (Hotez *et al.,* 2003). Viveka Vardhani and Sakunthala (2012) reported no correlation between serum IgG level and worm load in mice during *A. caninum* infection. The main objective of the study is to determine the level of serum immunoglobulin IgE from male Swiss albino mice infected with various single doses of *A. caninum* larvae.

MATERIALS AND METHODS

Culture of *A. caninum* larvae

A pure strain of *A. caninum* is maintained in an experimentally injected pup, where the infection had been maintained in dogs for several years. Faeces from the infected pup, collected from the floor of the kennel, was cultured in the dark at 26ºC for 8 days using the petridish method of Sen *et al*. (1965).

Acquisition and preparation of experimental animals

Male Swiss albino mice (6-8 weeks old; 26-28g. wt.) were purchased from dealers and were divided into 4 groups. Three experimental groups A, B, and C with 10 in each were infected with a single dose of 500, 1000 and 2000 larvae of *A. caninum* respectively. Another group D, with 10 mice, was kept as uninfected control for comparison. Two mice from each group

were sacrificed and serum samples were separated from the blood collected by cardiac puncture on day 1, 4, 9, 16 and 30 of infection; two mice from the control group (D) were also necropsied on the same designated days for the collection of blood. Serum was separated and processed for the estimation of IgE using ELISA technique.

RESULTS

Infected mice from all the 3 groups survived for 30 days experimental period. The mean values of IgE are shown in Table 1.

The results pertaining to the 3 singly dosed groups (group A, 500 larvae ; group B, 1000 larvae ; group C, 2000) showed a marked decrease in the level of IgE when compared with the uninfected controls (group D). Group A showed highest serum IgE level on day 1 (152.9 g/L) (still lower than control) and decreased gradually till day 30 (25.0 g/L). Mice received a single dose of 1000 larvae (group B) showed gradual decline of IgE level from day 1 (142.2 g/L) to 30 (28.0). Mice (group C) infected with a heavy dose (2000 larvae) showed fairly high IgE level on day 1 (111.1g/L) and decreased gradually by day 30 (28.0 g/L); all the decreased serum IgE levels were not exceeded the IgE level of controls (group D). Statistical analysis of these results indicates that there was a significant decrease in the level of IgE in experimental and compared with controls (Table 2). Also, there was a statistically significant difference (in the decreased value of IgE) when the level of serum IgE in experimentals (A, B and C) were compared with one another.

DISCUSSION

Canine hookworm infection results in the decrease of serum IgE levels in mouse host. Comparatively, the level of IgE was greater in mice infected with 500 dose (group A) than in those infected with 1000 (group B) and 2000 (group C) dose on day 1 of infection ; this may be because of greater larval retention in group A than in group B and group C. The decreased level of IgE is attributed to the primary immune response mounted by the retained worm load in the host system. These observations confirm that of Bhopale and Johri (1975) who also reported increased level of serum albumin and globulin on day 1 of infection during ancylostomiasis in mice. The peak rise of serum IgE on day 1in all the 3 infected animals coincide with the maximum immune response of the host – these results confirm those of Vardhani and Johri (1979), Gowri and Vardhani (1992) and Nirmala and Vardhani (2007) who reported significant increase of mast cells, eosinophilia and neutrophilia in the gastrointestinal tract during *A. caninum* infection in female mice. Increase of both circulating and intestinal eosinophils during the third week of infection and their sustenance upto 30th day was attributed to gut anaphylaxis during ancylostomiasis in mice (Vardhani, 2002 and 2003).

The gradual decreased level of serum IgE from day 1 to 30 in all the 3 singly infected groups can be attributed to the poor larval yield as reported by Vardhani and Johri (1981). It is interesting to find that the synthesis of IgE is lowered by the inhibition of essential stimulus, since that agency can thus be excluded from the immune mechanism. The stimulus is a direct action on the synthesis of IgE (reagimic) (allergic) antibody by larval antigens is more probable. The constant association of eosinophil leucocytes with the presence of tissue foreign antigens (during helminthic infection) is well established. There can be two possibilities for the decreased level of serum IgE. Either the synthesis of IgE is inhibited or the retained larval burden is incapable producing anaphylaxis. Alkazmi and Behnke (2011) reported increase of mast cells, eosinophils and goblet cells in the gut of hamsters infected with *A. ceylanicum*; the cellular reactions in the mucosa are responsible for the production of immunoglobulins.

Another interesting observation which needs consideration is the lowest level of IgE on day 30 of infection in all the 3single infected groups. This observation suggests that the stimulus of infection was insufficient to synthesize IgE although the mechanism is not known. Suffon and Gould (1993) suggested that IgE is present only in mammals and reaches lowest

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

Period of infection	Experimental groups			Control group D IgE
	A IgE	B IgE	C IgE	
1	152.9	142.2	111.1	165.20
4	102.9	96.7	100.5	165.23
9	78.1	44.8	87.3	165.25
16	37.5	47.1	49.4	165.21
30	25.0	28.0	28.0	165.23

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	79.28	71.76	75.26	165.22
Tvalue	A D	B D	C D	
	_____\	_____\	_____\	
	t=4.62*	t =3.08*	t =5.27*	
	(p>0.05)	(p>0.05)	(p>0.05)	
	A B	A C	B C	
	_____\	_____\	_____\	
	t = 5.09*	t =4.93*	t = 3.86*	
	(p>0.05)	(p>0.05)	(p>0.05)	

Note: P value at 5% level of significance is 2.306; * statistically significant value

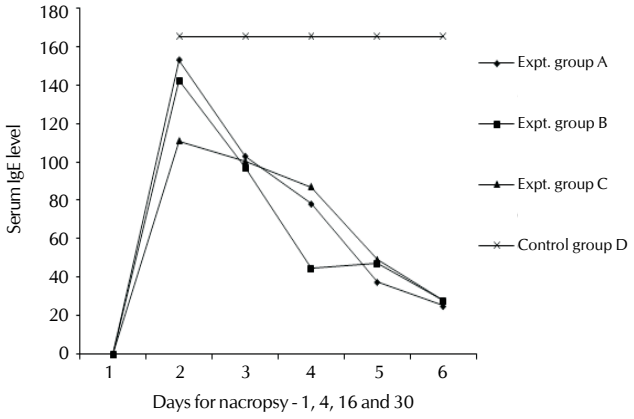

Figure 1:

A = 500 dose; B = 1000 dose; C = 2000 dose; D = Uninfected;

concentration of all antibody classes. Our results compare well with that of Turner *et al*. (1979), Negrao-Correa *et al*. (1999) and Negrao-Correa (2001) who also found increase of serum IgE level in *Nippostrongylus brasiliensis* and *Trichinella spiralis* infections respectively. Although the role of TH2 cytokines in immunity to intestinal nematodes is well documented (Schopf *et al*., 2002), the direct role of peripheral antibody is less conclusive inspite of protection with the passive transfer of immune cells in mice during ancylostomiasis (Vardhani and Johri, 1987).

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