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Immune Response of *Aspicularis tetraptera* infected Mice

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Abstract: The study was undertaken to evaluate the immune response of *Aspicularis tetraptera* infected mice, immunized with different somatic antigens. Total 15 mice were used. Five mice were used for positive control, 5 mice used for negative control and 5 mice used for experiment. The immune response parameters were studied viz. adult worm recovery, PCA response and IgE response. The result showed that the infected and non-immunized mice have more worm burden 21st day post infection, low PCA response on 14th day post infection and high IgE response on 21st day post infection but the immunized mice showed less worm burden 21st day post infection, high PCA response on 14th day post infection and less IgE response on 21st day post infection. The level of immune response was assessed based on above studied parameters in infected and immunized mice with different somatic antigens of *Aspiculuris tetraptera.* The decreased in worm burden, IgE response and increased in PCA response in infected and immunized mice compared to infected and non immunized suggested the involvement of above studied parameters in immune response. This study also proves that somatic antigen of *A. tetraptera* was effective in imparting immunity in mice.

Key words: *Aspiculuris tetraptera* · PCA response · IgE response · Immune Response and Somatic antigens

INTRODUCTION

The *Aspiculuris tetraptera* mouse pinworm is useful parasite model of the human parasite *Entero biusvermicularis.* Behnke reported the immune expulsion of the nematode *Aspiculuris tetraptera* from mice given primary and challenge infections [1-3]. Some scientist reported the analysis of somatic antigens extracted from *Aspiculuriste traptera* (Oxyuridae) and their role in eliciting immune response in laboratory mice [4-5]. The expulsion of some nematode parasites from the host during the second and third weeks of primary infection is well documented in the literature [6-10] and it is invariably accompanied by an acquired immunity to further infection manifest by a more rapid expulsion of challenge infection and a smaller residual worm burden persisting after the events of immunity [11-13].

Parasitism and gastrointestinal nematode parasitism in particular, is arguably the most serious constraint affecting small ruminant production worldwide. Economic losses are caused by decreased production, cost of prevention, cost of treatment and the death of infected animals [14-18]. Chronic intestinal nematode infections pose a significance threat to the health of man and domestic animals. The mechanisms employed to evade host immunity are still poorly understood but there is growing consensus that a number of diver's species exert an immune modulatory influence, causing potentially host-protective effecter responses to be down regulated [19-22] although most of these studies have been carried out on nematodes. *A. tetraptera* nematodes also provide a good model for studying the mechanisms of innate and acquired immunity to nematode infection. In order to ascertain whether the expulsion of *A. tetraptera* was mediated by immunological phenomena, it was important to know whether a primary infection initiated a state of acquired immunity. Therefore, the present study was under taken to find out the role of antibody in active immunization against *A. tetraptera* in mice.

MATERIALS AND METHODS

Experimental Animal: The Inbred female Swiss albino mice, *Musmusculus albinus* of 6-8 weeks old and 15-20 gm in weight were selected as an experimental animals. Total 15 mice were used. Five mice were used for positive control, 5 mice used for negative control and 5 mice used for experiment.

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Experimental Parasite: For the present investigation *A. tetraptera* was selected as an experimental parasite and it being routinely maintained in the laboratory by serial passage

Preparation of Somatic Antigen: Somatic antigens were prepared by homogenization and lyophilization. The eggs, the larvae and the adult stages of the worms, were washed thoroughly and homogenized separately in the protein free culture medium (Hopkins serum free culture medium). The homogenate were lyophilized and kept at 4 °C.

Immunization of Mice: An initial dose of 0.4 ml of the suspension with 0.2 ml of antigenic sample containing the required content Protein content of antigenic sample was estimated by method of Lowry [23] and 0.2 ml of Freund's complete adjuvant(FCA) was injected subcutaneously (SC) for immunization. The protein content of the antigenic sample varied according to the experiments, however, the booster dose was of 0.2ml, containing required amount of the of protein without FCA.

Preparation of Inoculums for Infection: The 100 viable eggs were fed to each mouse. After inoculation, mice were kept in cages, labeled according to the design of experiments, were fed routinely with the same standard diet.

Collection of the Blood Samples, Separation of Serum: Blood from experimental and control mice were collected by cardiac puncture under mild ether anesthesia, before incision each mouse were swabbed with 90% alcohol, heart ex-posed, blood collected from the ventricle by a 2 ml sterilized dry glass syringe fitted with a suitable in cold overnight for clotting after which serum carefully pipetted out in to clean sterilized serum collecting tubes and stored at-20 °C Until required.

Immediate Type Hypersensitivity (PCA): Passive cutaneous anaphylaxis (PCA) was done by Ovary method [24] method.

Elisa Test: ELISA test was performed by the steps suggested by Voller [25].

Recovery of Adult Worms from Caecum of Mice: Animals from different groups were sacrificed under mild ether anesthesia at various intervals according to the experimental design. The caecum was cut open longitudinally adult mature worm was collected in luke warm physiological saline solution and counted.

RESULTS

The level of immune response was assessed on the basis of adult worm recovery, PCA and IgE antibody response in experimental group immunized with different somatic antigen for active immunization. Results of PCA and IgE antibody response in control and vaccinated mice are summarized in Tables 1 and presented by Figure 1-3.

Adult Worm Recovery: In control i.e. infected and non-immunized mice (INI) adult worm recovery was 48 on 21st days post infection. In experimental groups the worm recovery were 6, 11 and16 in infected and immunized with eggs somatic antigen group (IIESAg), larval somatic antigen group (IILSAg) and adult somatic antigen group (IIASAg) respectively on 21st days post infection at the dose of 100 µg. Thus a remarkable decrease in adult worm recovery was observed in experimental group compare to control group.

Percent protection was 52% in control i.e. infected and non-immunized mice (INI). Percent protection in experimental groups were 94%, 89% and 84% in (IIESAg), (IILSAg) and (IIASAg) respectively on 21st days post infection at the dose of 100 µg concentration. The maximum protection (94%) was provided to the mice vaccinated with eggs somatic antigen and minimum protection (84%) was provided to the mice vaccinated with adult somatic antigen. Eggs somatic antigen was found to be more potent in providing protection as compared to larval and adult somatic antigens. The level of adult worm recovery and protection was found to be directly proportional to the quality of antigen. Statistically significant differences occurred in various groups.

PCA Response: PCA reactions were found to be directly proportional to the quality of antigens. In INI group PCA reaction was 6.5mm. PCA responses were found to be 9.3 mm in IIESAg, 8.5 mm in IILSAg and 7.2 mm in IIASAg at the dose of 100 µg concentration on 14th days post infection.

Over all PCA reaction was observed maximum (9.3 mm) in the group IIESAg and minimum (7.2 mm) in the group IIASAg. The PCA reaction was increased in experimental groups as compared to control group. The maximum PCA reaction was observed in mice vaccinated with eggs somatic antigen and minimum PCA reaction was observed in mice vaccinated with adult somatic antigen. Eggs somatic antigen was found to be more potent in providing protection as compared to larval and adult somatic antigens. All the values obtained in the various experimental groups were statistically found significant.

Table 1: Adult worm recovery, PCA and IgE response in infected and immunized mice with different somatic antigens of *A. tetraptera*

Group No.	Group Name	Dose	Adult worm recovery on 21st day's p.i. S.E.M.	Percent infection	Percent Protection	PCA Response in mm On 14th day's p.i. S.E.M.	IgE Antibody Response On 21st day's p.i. IgE(KIU/ml) S.E.M.
1.	NINI	-	-	-	-	-	132.50± 0.502
2.	INI	-	48 ±1.00	48	52	6.5 ± 0.252	213.4±0.351
3.	IIESAg	100 µg	06 ±2.00	06	94	9.3 ±0.300	130.2±0.200
4.	IILSAg	100 µg	11±1.00	11	89	8.5±0.200	166.8±0.400
5.	IIASAg	100 µg	16±3.00	16	84	7.2±0.153	186.2±0.252

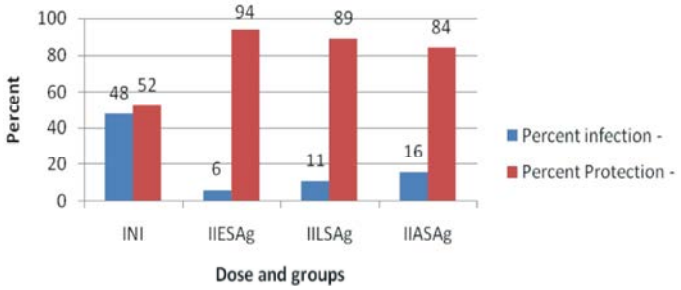

Fig. 1: Percent infection and protection in infected and immunized mice with different somatic antigens of *A. tetraptera*

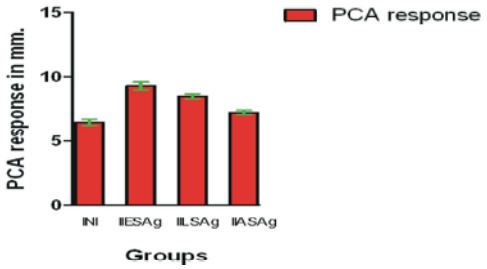

Fig. 2: PCA response in infected and immunized mice with different somatic antigens of *A. tetraptera*

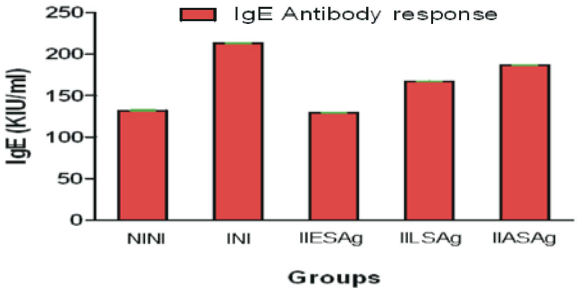

Fig. 3: IgE response oin infected and immunized mice with different somatic antigens of *A. tetraptera*

The order of PCA response was observed as- IIESAg>IILSAg>IIASAg>INI

IgE Antibody Response: IgE antibody response was found to be inversely proportional to the quality of antigens. In NINI group IgE antibody response was 132.5 KIU/ml and INI group the IgE antibody response was 213.4 KIU/ml on 21st days post infection. In experimental groups the IgE antibody response were found to be 130.2 KIU/ml in IIESAg, 166.8 KIU/ml in IILSAg and 186.2 KIU/ml in IIASAg at the dose of 100 µg concentration on 21st days post infection.

Over all IgE antibody response was observed maximum (186.2KIU/ml) in the group IIASAg and minimum (130.2 KIU/ml) in the group IIESAg. The IgE antibody response was decreased in experimental groups as compared to control group. The maximum IgE antibody response was observed in mice vaccinated with Adult somatic antigen and minimum IgE antibody response was observed in mice vaccinated with eggs somatic antigen. Eggs somatic antigen was found to be more potent in providing protection as compared to larval and adult somatic antigens. All the values obtained in the various experimental groups were statistically found significant.

The order of IgE response was observed as:

IIESAg< NINI<IIASAg<IILSAg< INI

NINI	Non Infected Non Immunized
INI	Infected Non Immunized
IIESAg	Infected and Immunized with Egg somatic Antigen
IILSAg	Infected and Immunized with larval somatic Antigen
IIASAg	Infected and Immunized with Adult somatic Antigen
PCA	Passive cutaneous Anaphylaxis
KIU	Kilo International Unit
S.E.M.	Standard Error of Mean
P.I.	Post Infection Days

DISCUSSION

Helminthic infections occur in situations where invasion by the parasite either exceeds the defense capacity of the host or where the host defense capacity is

below normal. Boosting of the host defense back to normal level (immuno-restoration) or above normal (immuno-stimulation) could be a method of treatment of helminthic infection [26]. The immune response to GIN infection develops gradually, but cattle exposed repeatedly to infections become highly resistant.

This resistance is associated with an immune response in the gut mucosa involving (IgE, IgG and IgA) and cellular CD 4 + T-cells, mast cells and eosinophil leucocytes effector mechanisms [27].

The protective immune responses to parasitic infections include both 'cell mediated immunity' and 'humoral immunity'. Cytotoxic T (Tc) cells, natural killer (NK) cells and activate macrophages are important in activation of 'cell mediated immunity', while IgM, IgG, IgE immunoglobulins have a role in humoral immunity. The first antibody to appear is IgM and is present in acute infection. IgG antibodies are present in helminthic infections. In most of the parasitic infections, immunity lasts till infection remains active. This type of immunity is known as concomitant immunity and was previously called premonition or infection immunity [28]. The interactions between the various components of the innate and acquired immune systems are extremely complex and have been studied for gastrointestinal nematode infections of sheep and cattle [29-31].

In the present investigation the level of immune response was assessed on the basis of adult worm recovery, PCA and IgE antibody response in experimental group immunized with different somatic antigen for active immunization. The adult worm recovery was decreased in experimental groups as compared to control group on 21st days post infection.

The expulsion of some nematode parasites from the host during the second and third weeks of a primary infection is well documented in the literature [1-3, 7, 13, 17, 32, 33] and it is invariably accompanied by an acquired immunity to further infection manifest by a more rapid expulsion of challenge infection and a smaller residual worm burden persisting after the events of immunity [11-12].

The results of the present study also correlate with those of the observations of the above mentioned authors. Results of the present study indicate that the host may acquire immune response even after an oral egg-infections and this acquired immune response helps in the killing or expulsion of eggs as well as the adult worm in secondary infections.

In the present investigation assay of immediate type of hypersensitivity (ITH) reactions was performed by skin testing for passive cutaneous anaphylaxis (PCA). Immediate type hypersensitivity is an allergic reaction induced by specific antigen provoked by re-exposure to the same antigen mediated by specific IgE antibodies and produced by the cellular release of histamine and other vasoactive mediators, resulting in an immediate local or system i.e. reaction, IgE antibodies that constitutively express high affinity surface receptors for the Fc component of IgE. Binding and cross linking of the allergen to surface receptor bound IgE triggers the immediate release from cytoplasmic granules of mast cells and basophils performed vasoactive mediators of immediate hypersensitivity and also release the bio-chemically active mediators [34].

In the present investigation the PCA reactions were found to be directly proportional to the quality of antigens. The PCA reaction was increased in experimental groups as compared to control group on 14th days post infection. Increase in PCA reactions indicates the stimulation of reaginic (IgE) response by the antigen as these are the only type of antibodies which are involved in anaphylactic reactions. Increased levels of IgE are responsible for mortality of the parasites. The role of reaginic antibodies in killing/expulsion of helminthic parasites conferring protection to the host is well known [16-17, 35-36]. The results PCA reaction of the present study also correlates with those of the observations of the above mentioned authors. Results of the present study indicate that the host may acquire immune response even after oral egg-infections.

In the present investigation IgE antibody response was found to be inversely proportional to the quality of antigens. The IgE antibody response was decreased in experimental groups as compared to control group on 21st days post infection. The maximum IgE antibody response was observed in mice vaccinated with Adult somatic antigen and minimum IgE antibody response was observed in mice vaccinated with eggs somatic antigen. Eggs somatic antigen was found to be more potent in providing protection as compared to larval and adult somatic antigens.

IgE antibodies are known to play a central role in mediating type I hypersensitivity reactions. The production of IgE tends to increase during parasite infections, but the ultimate effects of IgE vary considerably, depending on the host-parasite

relationships. Hyper immune allergic reactions have been closely associated with IgE production [36].The role of IgE antibodies in killing/ expulsion of allergen and helminth parasites [9-10, 37] are well known. The decline in the IgE levels of infected rats after expulsion/killing of parasite observed by [38]. The same result obtained in the present investigation. Thus the results of present study supported by above mentioned researchers.

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