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Study on lymphocyte migration inhibition factors in mice experimentally infected and vaccinated with *Aspicularis tetraptera*

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Abstract
Present study deals with the immune response and effectors mechanism which was confirmed by MIF (Migration Inhibition Factor)**.** For MIF assay, lymphocytes were separated from heparinized blood of experimental and control mice. MIF reaction was maximum (16.4) mm in the group IVEgSoAg5 and minimum (10.2) mm in the group IVEgSoAg1. So the maximum and minimum MIF reaction was shown by Egg Somatic antigen Ag_5 (100 µg) and Ag_1 (20 µg) respectively. The results showed that the Migration Inhibition Factor is directly proportional to the vaccination dose, in other words the reaction was dose dependent. Somatic antigens were highly potent in stimulating an effective cell mediated response as evident by greater migration inhibition of lymphocytes in response of somatic antigen of eggs stage. Increased MIF value playing an important role in imparting effective cell mediated immunity.

Keywords: *Aspicularis tetraptera*, migration, lymphocytes

Introduction
Helminths are generally known as disease causing, multicellular, eukaryotic organisms. The word helminth is originated from the Greek meaning "worms" (Faust *et al.*, 1970). They are responsible for the reduction of productivity of useful animals. They are also supposed to be reducing the level of meat, milk and manure output and asset value through increased mortality, especially of young stock (Cabaret *et al.*, 2002; Githiori 2004 and Sorobetea *et al.*, 2018) [6, 10, 21].
According to World Health Organization (WHO, 2015) [26] estimates, 870 million childrens are living in area of high prevalence. Africa, South Asia and South America are most affected regions of the world (Lobo *et al.*, 2011) [15]. With 220.6 million cases, India contributes 25% to the total global cases of children infected with helminths which are in need of preventive chemotherapy (Slam and Azam, 2017) [18].
Helminths are recognized as a major constraint to livestock production (Githori *et al.*, 2004). They are also responsible for:

- Retarded growth (Ashraf, 1985 and Kochapakdee *et al.*, 1995) [3, 13].
- Low-down productivity (Perry and Randolph, 1999) [17].
- Mortality (Sykes, 2001 and FAO, 2002) [22, 7].
- High economic losses (Irfan, 1984 and Iqbal *et al.*, 1993) [12, 11].

In group helminth nematodes, are known as roundworms. This group is considered as second largest phylum in the animal kingdom, comprising up to 500,000 species. The pinworms are (a kind of round worm) nematode parasites. *Syphcia obelvata* and *Aspicularis tetraptera* are belongs to family Oxyuridae, which have simple and direct life cycles and are frequent contaminants colonies of laboratory mice (Anya, 1966 and Gaherwal, 2014) [6, 9].
Nematodes generally inhabit the gastrointestinal tract of animals including humans. Larvae emerge from eggs, after ingestion by the host in the lower intestine and enter the crypts of Lieberkuhn in the mid-colon within 24 h of infection (Anya, 1966; Behnke, 1974) [1, 4]. Host-parasite contact on 6-7-day-long crypt phase, become closer and some larvae penetrate the lamina propria and causing little damage to the host epithelium. At this stage, host inflammatory response is evident. The larvae arrived back to the lumen of the colon and tried resettle in the anterior colon after seven days and then they live in between the colonic rugae in close opposition to the host epithelium (Behnke, 1974) [4]. The infection becomes vigorous on day 24 (Anya, 1966) [1].
This multicellular pathogen has a challenge to host immune system because individuals are continually exposed to the infective stages (Sorobetea *et al.*, 2018) [21]. These multicellular organisms are rarely causing death (Savioli and Albonico, 2004; Slam and Azam, 2017) [18].

Material and Method
Maintenance of mice
The swiss Albino mice, *Mus Musculus albinus* was selected for the present study. These animals were obtained from IPS Academy, Indore and were kept in the animal house under laboratory condition of light, temperature and ventilation. Only healthy helminths infection free male mice of 7-8 weeks old and 30-50 gms were chosen as the experimental animals. It was confirmed by examining the stool. Finally these mice were kept in sterilized cages with dry husk padding and were fed daily with standard balanced diet for further study.

Maintenance of *Aspicularis tetraptera* strain
Aspicularis tetraptera strain was obtained from Parasitology Laboratory, department of Zoology, Govt. Holkar Science

College, Indore (M.P). *Aspicularis tetraptera* were routinely maintained in the laboratory by serial passage in healthy mice, after every 31st days post infection with a dose of 100 variable embryonated eggs. The infected animals provided the various stages of the parasite for experimental purposes. The methods employed for maintenance, infection and recovery stages of *Aspicularis tetraptera* was described by Wakelin, (1967) [24].

Preparation of somatic antigens

Somatic antigens of *Aspicularis tetraptera* were prepared as described by Artis *et al.* (1999) [2]. Somatic antigens were prepared by homogenization and lyophilisation. The eggs, the larvae and the adult stages of the worms, were washed thoroughly and homogenized separately in the protein free culture medium. The homogenate were lyophilized and kept at 4° C.

Immunization of mice, challenge infection

The method adopted for immunization of mice was described by Wakelin, (1975) [25]. An initial dose of 0.4 ml of the suspension with 0.2 ml of antigenic sample containing the required protein content (determined earlier) 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 0.2 ml, containing required amount of protein without FCA. The different doses of somatic antigens used were containing 20, 40, 60, 80 and 100 µg proteins. A challenge oral infection of a single dose of 100 embryonated eggs of *Aspicularis tetraptera* was uniformly given after two weeks to each mouse of all experiments.

Collection of blood samples and separation of serum

Blood from experimental and control mice were collected by cardiac puncture under mild ether anaesthesia. Before incision each mouse was swabbed with 90% alcohol. Heart exposed to collect blood from ventricle by 2ml sterilized dry glass syringe, fitted with a suitable needle. Blood sample were kept in 15ml centrifuge tube and kept 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.

Analysis of lymphocyte migration inhibition factor (MIF)

The lymphocytes were separated by employing the method of density gradient separation using Ficoll Hypaque Gradient (Talwar, 1983) [23]. Lymphocytes were separated from heparinized blood of experimental and control mice. Aliquots of cell suspension were placed in four wells cut in a preparation of agarose in a petridish (15x90mm). Agarose was prepared according to the method of Noel *et al.* (1986) [16]. Two well were filled with medium (control well). Petridish was incubated overnight at 37°C in a humidified environment. Cells migrated were fixed and stained. Diameter of migrated area was measured with ocular micrometer. Finally following formula was used to calculate MI:

$$\text{Migration index (MI)} = \frac{\text{Mean Area of Miration in presence of Antigen}}{\text{Mean Area of Migration in Absence of Antigen}}$$

Result and Discussion

Results of MIF reactions in mice infected and vaccinated with somatic antigens are summarized in the tables (14, 16 and 18) and presented through figures (10, 11 and 12). MIF reaction greater than 20% was considered significant.

In the present study MIF reactions were found directly proportional to the concentrations of antigens. In infected non vaccinated control, MIF was 8.4 mm whereas, in experimental group vaccinated with somatic antigens, it was higher reaching to maximum in group $IVEgSoAg_5$ (16.4 mm) and minimum in group $IVEgSoAg_1$ (10.2 mm).

A. **MIF reactions in the group $IVEgSoAg_1$-Ag_5:** Migration inhibition (10.2 mm) was observed at 20 µg concentration, which increased to 12.6 mm in Ag_2, 13.5 mm in Ag_3 and 14.2 mm in Ag_4 and maximum 16.4 mm in Ag_5 on day 31st post infection.
B. **MIF reactions in the group $IVLSoAg_1$-Ag_5:** Migration inhibition (11.8 mm) was observed at 20 µg concentration, which increased to 12.5 mm in Ag_2, 13.2 mm in Ag_3 and 32.8 mm in Ag_4 and maximum 15.2 mm in Ag_5 on day 31st post infection.
C. **MIF reactions in the group $IVASoAg_1$-Ag_5:** Migration inhibition (12.2 mm) was observed at 20 µg concentration, which increased to 12.8 mm Ag_2, 13.5 mm in Ag_3 and 14.2 mm in Ag_4 and maximum (15.8 mm) in Ag_5 on day 31st post infection.

MIF reaction was maximum (16.4 mm) in the group $IVEgSoAg_5$ and minimum (10.2 mm) in the group $IVEgSoAg_1$. The maximum and minimum MIF reaction was shown by Egg Somatic antigen Ag_5 (100 µg) and Ag_1 (20 µg) respectively.

All obtained values in the various experimental groups were found statistically significant when compared to the control values (15, 17 and 19).

Table 1: MIF response in *A. tetraptera* infected mice vaccinated with different concentrations of somatic antigens of eggs.

Group no.	Group name	MIF reaction in mm
1	$NINVC_1$	-
2	$INVC_2$	8.4±0.141
3	$IVEgSoAg_1$	10.2±0.126
4	$IVEgSoAg_2$	12.6±0.219
5	$IVEgSoAg_3$	13.5±0.141
6	$IVEgSoAg_4$	14.2±0.301
7	$IVEgSoAg_5$	16.4±0.186

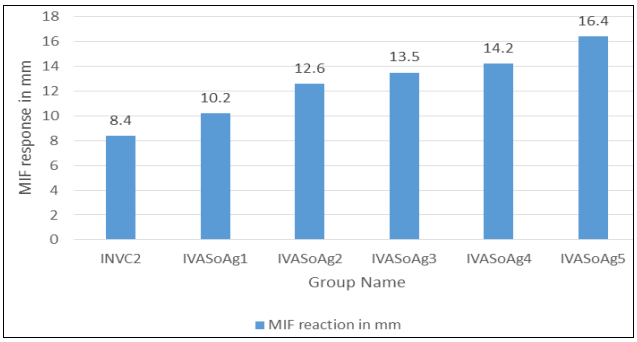

Fig 1: Showing MIF response in *Aspicularis tetraptera* infected mice vaccinated with somatic antigen of egg.

Table 2: MIF response in *A. tetraptera* infected mice vaccinated with different concentrations of somatic antigen of larvae.

Group no.	Group name	MIF reaction in mm
1	$NINVC_1$	-
2	$INVC_2$	8.4±0.141
3	$IVLSoAg_1$	11.8±0.754
4	$IVLSoAg_2$	12.5±0.222
5	$IVLSoAg_3$	13.2±0.222
6	$IVLSoAg_4$	13.6±0.546
7	$IVLSoAg_5$	15.2±0.216

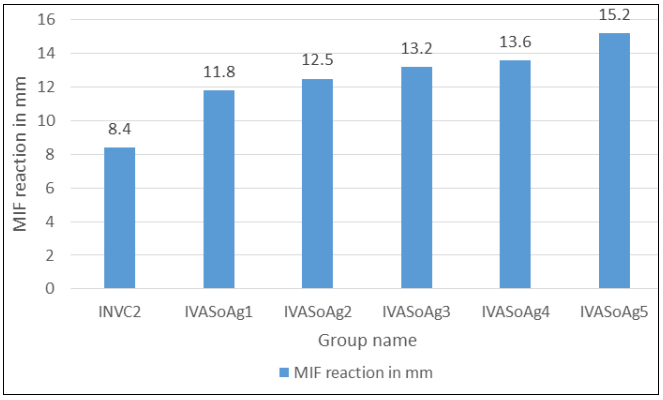

Fig 2: Showing MIF response in *Aspicularis tetraptera* infected mice vaccinated with somatic antigen of larvae.

Table 3: MIF response in *A. tetraptera* infected mice vaccinated with different concentrations of somatic antigen of adult worm.

Group no.	Group name	MIF reaction in mm
1	$NINVC_1$	-
2	$INVC_2$	8.4±0.141
3	$IVASoAg_1$	12.2±0.413
4	$IVASoAg_2$	12.8±0.104
5	$IVASoAg_3$	13.6±0.278
6	$IVASoAg_4$	14.2±0.426
7	$IVASoAg_5$	15.8±0.228

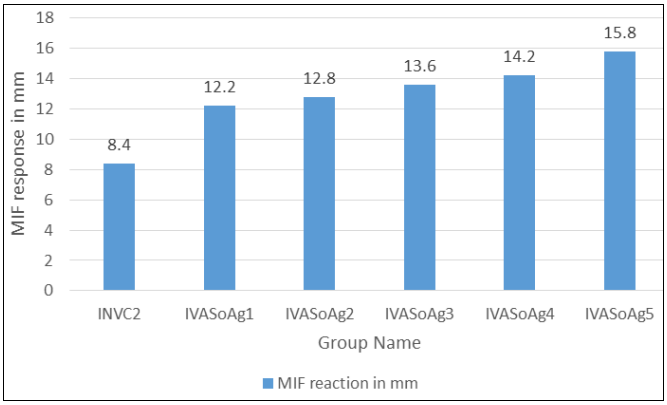

Fig 3: Showing MIF response in *Aspicularis tetraptera* infected mice vaccinated with somatic antigen of adult worm.

In the present study experiments based on MIF was conducted to determine the extent of inhibition of migration of sensitization (Splenocytes MI factor: a lymphokine which is released by sensitized T-lymphocytes) which showed a remarkable inhibition with sensitized cells from oral egg infection.

In the present study result obtained revealed that MIF reaction was directly proportional to the concentration of both egg and larval somatic antigen. Increase in MIF values suggest the cell mediated immunity may be due to immunization through sensitized lymphocyte. Bradley *et al.* (1996) and Little *et al.* (2005) [14] said that recognition of an antigen by lymphocyte occurred at lymphoid organ (spleen), while, Sher and Coffman (1992) [20] described that migration inhibition occurred at T-Cell which produces lymphokines.

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