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Original Article

Lymphocyte Migration Inhibition Response in *Trichuris muris* Infected and Vaccinated Mice

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Abstract

Background: Immunological response of host and parasite play a key role in developing vaccination and immunization. The present study deals with the immune response and effector mechanism, which was confirmed by migration inhibition factor (MIF).

Methods: The present work was conducted in Parasitological Lab of Postgraduate Department of Zoology, Government Holkar Science College, Indore (M.P.) during 2006-2007. For MIF assay, 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 Petri dish. Two wells were filled with soluble test antigen, while rest two wells were filled with medium (control wells). Petri dish was incubated overnight at 37 °C in a humidified environment at 5% CO₂ in air. Cells migrated under the agarose in a circle were fixed and stained. Diameters of the migration areas were measured with ocular micrometer.

Result: MIF reaction was maximum (44.2%) in the group IVEgESAg5 and minimum (10.8%) in the group IVASoAg1. The maximum MIF reaction was shown by eggs ES antigen and least by adult worm somatic antigen. The interesting observation was that migration inhibition increases as dose increased or we could say the reaction was dose dependent

Conclusion: Increased value of MIF response in vaccinated mice suggested the involvement of lymphocytes in cell-mediated immunity. This study also proves that excretory-secretory (ES) antigen of eggs from *Trichuris muris* was more effective in imparting immunity in mice.

Keywords: *Trichuris muris*, *Excretory-secretory Antigens*, *Somatic Antigens*, *Lymphocyte*, *Migration*

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Introduction

Helminthiasis in man through animal is burning problem of the world. Generally, no population can be considered free from immune caused by worms. The predominance of infections occurs where sanitary conditions are poor. Immunological responses of host and parasite play a key role in defense and injury mechanisms. The long lasting responses are beneficial to Immuno-pathology (host immediate and delayed hypersensitivity, antigens- antibody reactions, and activation of complement).

The development of effective vaccines for the protection of nematodes infection has a demanding priority because of serious limitations in the use of anti-helminthic drugs whose continuous use provides resistance to parasite. The development of such vaccines depends on a detailed understanding of the host defense mechanisms, identification of immunogens and target sequences, which induce production (1).

The immune mechanisms that result in the destruction and elimination of challenge infections of the *Trichuris muris* in actively immunized mice are still a subject of controversy. Immunization of the host animals against *T. muris* infection was conducted using antigenic materials from various stages of the worm (2-5).

Resistance to *T. muris*, is defined as expulsion of the parasite prior to patency. It requires the development of a T helper 2 (Th2) responses during a primary infection and High levels of serum IgG1 and cell-bound IgG1 in the colon of mice protected by the excretory-secretory (ES) vaccine, this suggest that antibody may be involved in vaccination-induced worm expulsion (6).

The role of cells in immunity to *T. muris* (7-8) and mechanisms of immune expulsion of these worms from mouse have been well-

reported (9). B-cell and antibodies are required for resistance to *T. muris* parasite (10, 11). The evasion of immunity by *T. muris* parasite caused chronic infection, which have the ability to manipulate the host immune system (12-14). The pathology of *T. muris* in primary and secondary infections is well studied in mice (2). The interleukin-9 (IL-9) enhances resistance to *T. muris* (15).

Infection of mice with the gastrointestinal nematode *T. muris* represents a valuable tool to investigate and dissect intestinal immune response. CD4 (+) T cells play a critical role in protective immunity, and that CD4 (+) T cells localize to the infected large intestinal mucosa to confer protection. Further, transfer of CD4 (+) T cells from immune mice to immunodeficient SCID mice can prevent the development of a chronic infection (16).

Typically, adult *H. polygyrus* can survive for 25 weeks (17, 18). However, in other mouse, most nematodes strains infections are curtailed in a shorter period. Longevity of parasite suggests that the adult worms are successful in avoiding host- immunity and immunosuppressant role of ES products cannot be ruled out. The vaccination of cattle with ES products reduced the fecal egg counts by 60% as compared to the counts in the control group (19).

Looking to the importance of helminth infection, the present study was undertaken to study the MIF responses in mice experimentally infected and vaccinated with *T. muris*.

Material and Methods

Animals

The Swiss albino mouse, *Mus musculus albinus* was selected as an experimental animal for the present investigations. The mice were obtained from the College of Veterinary Sci-

ence and Animal Husbandry, Mhow (M.P.) and were kept in the laboratory under local conditions of light, temperature, ventilation, and food. Food and water were provided ad libitum. Female mice of 6-8 weeks old and 15-20 gms in wt. were used according to the need of the experimental design.

Parasite

Trichuris muris strain was originally obtained from the Parasitology Laboratory, Department of Zoology, Govt. Holkar Science College, Indore, (M.P.). It was routinely maintained in the laboratory by serial passage in healthy mice, after every 31st days post infection with a dose of 100 viable embryonated eggs. The infected mice provided the various stages of parasite for experimental purposes. The method employed for maintenance, infection and recovery of various stages of *T. muris* were as described by Wakelin (9, 20).

Somatic and ES antigens of *T. muris* were prepared as described by Artis et al. (21).

Immunization of mice

The method employed for immunization of mice was described by Wakelin (9). An initial dose of 0.4 ml. of the suspension with 0.2 ml. of antigenic sample containing the required protein contents (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 of 0.2 ml, containing required amount of the protein without FCA. A challenge oral infection of a single dose of 100 embryonated eggs of *T. muris* was informally given after two week to each experimental mouse.

MIF Assay

Lymphocytes were separated employing the method of density gradient separation using Ficoll Hypaque Gradient (22). 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 Petri dish (15 x 90mm). Agarose was prepared according to the method of Noel (23). Two wells were filled with soluble test antigen, while rest two wells were filled with medium (control wells). Petri dish was incubated overnight at 37 °C in a humidified environment at 5% CO₂ in air. Cells migrated under the agarose in a circle were fixed and stained. Diameters of the migration areas were measured with ocular micrometer, while migration was calculated by following formula:-

Mean area of migration
in presence of antigen
Migration index (MI) =-----
Mean area of migration
in absence of antigen
Per cent migration = 100 x Migration index
Percentage migration Inhibition = 100 – Per cent Migration
Migration inhibition above 20% was considered significant.

Results

In the present study MIF reaction, greater than 20% was considered significant. In infected non-vaccinated control, MIF was 9.6%, whereas, in experimental group vaccinated with somatic and ES antigen it was higher reaching to maximum in IVEgESAg5 (44.2%) and minimum in IVEgSoAg1 (10.8%).

The percentage of migration inhibition in Eggs, larval and adult somatic Ag1, Ag2, Ag3, Ag4 and Ag5 groups were described in Table1, while percentage of migration inhibition in eggs, larval and adult ES Ag1, Ag2, Ag3, Ag4 and Ag5 were described in Table 2.

MIF reaction was maximum (44.2%) in the group IVEgESAg5 and minimum (10.8%) in

the group IVASoAg1. So the maximum MIF reaction was shown by eggs ES antigen and least by adult worm somatic antigen. The

interesting observation was that migration inhibition increases as dose increased or we could say the reaction was dose dependent.

Table 1: MIF responses in *T. muris* infected mice, vaccinated with different concentrations of different somatic antigens

Group No.	Groups Name	Dose of antigen	MIF reaction of somatic antigen of eggs in (%) \pm SD	MIF reaction of somatic antigen of larvae in (%) \pm SD	MIF reaction of somatic antigen of adult worm in (%) \pm SD
1.	NINVC1	-	-	-	-
2.	INVC2	-	9.6 \pm 1.34	9.6 \pm 1.34	9.6 \pm 1.34
3.	IVEgSoAg1	20 μ g	29.4 \pm 1.95	15.4 \pm 0.89	10.8 \pm 1.30
4.	IVEgSoAg2	40 μ g	31.6 \pm 2.07	20.8 \pm 0.89	12.6 \pm 0.89
5.	IVEgSoAg3	60 μ g	34.6 \pm 1.94	28.4 \pm 2.60	13.4 \pm 1.92
6.	IVEgSoAg4	80 μ g	37.2 \pm 2.68	32.8 \pm 1.92	14.4 \pm 1.14
7.	IVEgSoAg5	100 μ g	40.6 \pm 2.41	36.2 \pm 1.78	15.4 \pm 0.54

Table 2: MIF responses in *T. muris* infected mice, vaccinated with different concentrations of different ES antigens

Group No.	Groups Name	Dose of antigen	MIF reaction of ES antigen of eggs in (%) \pm SD	MIF reaction of ES antigen of larvae in (%) \pm SD	MIF reaction of ES antigen of adult worm in (%) \pm SD
1.	NINVC1	-	-	-	-
2.	INVC2	-	9.6 \pm 1.34	9.6 \pm 1.34	9.6 \pm 1.34
3.	IVEgESAg1	20 μ g	32.6 \pm 1.51	17.2 \pm 0.83	12.4 \pm 0.55
4.	IVEgESAg2	40 μ g	35.8 \pm 0.83	23.00 \pm 1.58	14.4 \pm 1.67
5.	IVEgESAg3	60 μ g	39.6 \pm 0.54	30.2 \pm 1.64	15.4 \pm 0.54
6.	IVEgESAg4	80 μ g	42.6 \pm 1.81	35.00 \pm 1.58	16.8 \pm 1.30
7.	IVEgESAg5	100 μ g	44.2 \pm 0.83	38.6 \pm 1.51	10.0 \pm 0.53

Discussion

Present experiments determine the extent of inhibition of migration of sensitization splenocytes (due to a MI factor, a lymphokine, and known to be released by sensitized T-lymphocytes) which showed remarkable inhibition with sensitized cells from oral egg infection.

T-cell migration to the large intestinal mucosa is dependent on the family of G alpha (i)-coupled receptors, during *T. muris* infection (16, 24). T-cell -mediated cytotoxic responses, readily expel *T. muris* indicating that the mechanism by which CD4-T cells mediate protective immunity (25-27).

The recognition of an antigen by lymphocytes may also occur at a site distance from the concerned lymphoid organ (e.g. spleen) where the sensitizing antigen located (within the intestinal wall, in case of *T. muris*). The reacting lymphocytes return to the lymphoid organ, undergo rapid replication, resulting in the formation of large number of sensitized cells that now recognize and react with sensitizing antigen (27-28). The role of T-lymphocyte and B- lymphocyte in the immunity was discussed against *T. muris* (29).

Significance increase in MIF values denoted that the cell- mediated immunity could be imparted by immunization through sensitized lymphocyte. Activated macrophage express increased phagocytic activity when confronted with intra cellular pathogens and passed through granulomatous transformation into multinucleated giant cell (30). Remarkable increase in migration inhibition may be at the level of T-cell, producing the activating lymphokines or at the level of the macrophages effector cells (31).

The present study confirmed that different somatic and ES antigens of nematode species generating protective immunity against GI nematodes which is T-cell dependent. Migration inhibition factor (interferon) which is released from sensitized lympho-

cytes in the tissues, responding to the presence of the sensitizing antigen.

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