Effect of H. polygyrus infection on serum IgG and IgM level in mice

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Abstract **- The science of parasitology has made remarkable progress during the last 30 years. The whole concept of 'parasite' and parasitism as understood in older days has now changed. The emphasis has been shifted to quires like why hosts fail to reject the parasite, how parasites adapt themselves to host conditions, how do they modulate host immune reactions and how specific antigens can be isolated and identified for vaccination. *Heligmosomoides polygyrus* is a natural intestinal helminth of mice. It inhibits in the duodenal region of small intestine. The present study was carried out to investigate the immunological responses and possibility of vaccine development.**

Index Terms **- Vaccine, *H. polygyrus,* IgG and IgM**

INTRODUCTION

The science of parasitology has made remarkable progress during the last 30 years. The whole concept of 'parasite' and parasitism as understood in older days has now changed. The emphasis has been shifted to quires like why hosts fail to reject the parasite, how parasites adapt themselves to host conditions, how do they modulate host immune reactions and how specific antigens can be isolated and identified for vaccination. Our knowledge about the nature, quantum and kinds of pharmacological mediators released as a result of host-parasite interactions and the effects of different types of parasitic antigens on protective mechanism of hosts in meager. Immunologists have isolated and identified hundreds of antigens, somatic and metabolic, some with protective potentialities; some for immunodiagnosis and yet others as immunosuppressive but much more remains unresolved.

Initially, much of the work on immuno-adaptation was focused on mechanism by which parasites evade or inactivate specific host effector responses (Knox and Smith, 2001). In the past decade the emphasis has shifted towards understanding how parasite might directly regulate the induction of these responses, a trend that reflects the current fascination with the interaction of parasite with the regulatory cytokine network (Sher and Coffman, 1992). The development of effective vaccines for the protection against nematodes has a high priority, because of serious limitations in the use of anthelmintics due to the emergence of drug-resistant parasites. The development of such vaccines depends on a detailed understanding of the host defense mechanism, identification of immunogens and target sequences, which induce protection.

Since the primary goal of most of the parasites is to evade host resistance, an obvious biological strategy would be to down regulate host's protective responses. Such a strategy should clearly be followed by parasite that needs to establish long-term chronic infection but that themselves are not highly virulent. Helminths and certain species of persistent protozoa are important members of this group; however, less immediately obvious is that some highly virulent parasites may actually up-regulate vertebrate defenses in order to regulate their own number, thereby ensuring the survival of the host niche. Such organisms are usually driven in to a quiescent phase by the protective immune responses they promote, re-emerging only after passing to another nonimmune definitive host or intermediate host. In contrast to the protozoans, bacteria and viruses, helminth parasites do not multiply within their host and therefore increase or decrease in worm population depends on the intake of the infective stages, rate at which mature stages disappear from the host and immune status of the host.

MATERIALS AND METHODS

Experimental Animal –

The Swiss albino mouse, *Mus musculus albinus* of either sex were selected as an experimental animal for

the present investigation. The mice were obtained from the Institute of Nutrition (NIN) Hyderabad, India and were kept in the animal house under local conditions of light, temperature ventilation and food. Food and water were provided *ad libitum*. Male and female healthy mice of 6-8 weeks old and 15-20 gms in weight were used according to the need of the experimental design. Animal experimentations were conducted according to INSA ethical guidelines for the use of animals for scientific research purpose, after permission from the ethical committee.

The Parasite: *Heligmosomoides polygyrus*-

Heligmosomoides polygyrus is a mouse intestinal nematode that establishes a chronic infection in the deodenum. It is a common nematode found in the duodenum and small intestine of woodmice and other rodents. They are 5-20 mm in length and bright red due to the pigmentation of their tissues. They are usually heavily coiled, with the female having 12-15 coils and the male 8-12. The male can be distinguished from the female by a prominent copulatory bursa and two long, thin spicules at the posterior end.

After two larval molts in the submucosa, the male and female adult worms emerge into the lumen of the intestine and mate, and eggs are excreted in the faeces (Wahid, *et al.,* 1994). They have a direct life cycle. After 2 days they hatch as larvae which are about 300 mm in length. The larvae moult 3 days later but retain the shed cuticle for protection. It is at this point they become infective. The larvae shed the outer protective sheath after they are eaten by a suitable host. The larvae then penetrate the submucosa of the duodenum where they under go 2 further molts. About 7 days later the male and female adult worms emerge into the lumen of the duodenum. They attach to the epithelial layer of the duodenum where they feed off the contents of the gut. The adult worms mate and eggs are shed in the feces. The complete life cycle from egg to egg takes a minimum of 15 days, and the female worms will live inside their host for 8 months.

Experimental protocol-

The mice were divided into following six groups –

1)Non Infected Non Vaccinated Control – 1

This group was utilized for collection of the blood to find out the differential counts of non-infected non-vaccinated mice (NINVC 1).

2) Infected Non –Vaccinated Control –2

300 viable infective L3 larvae were orally inoculated directly into stomach of mice by 01 ml syringe having a blunt rubber cathedral. On day 1 and day 5 post infection (pi) the mice were sacrificed for larval recovery, at the same time collection of blood and separation of serum for the assessment of other test parameters was also done. On day 13 (pi), the next batch of mice were sacrificed and adult worms were counted. Collection of blood and separation of serum for assessment of other test parameters was also done. Intestine was removed for histology. This group of mice is also called as (INVC-2).

3) Infected Vaccinated with Larval Somatic Antigens

Mice were vaccinated on day 0 with L-3 larval somatic antigen containing 20,40,60,80,100 µg protein + FCA. At the same time control mice were given culture medium + FCA. On day 5, again a booster dose of L-3 larval antigen containing the same amount of protein without FCA was given to experimental mice. The control mice were given only culture medium. On day 26 the mice were challenged with 300 L-3 larvae. On day 27 and 31, mice were sacrificed for larval recovery and assessment of the test parameters. On day 39 adult worms were recovered. Collection of blood and separation of serum for assessment of other test parameters were done. This group is also called as (IVLSAg).

4)Infected Vaccinated with Adult Somatic Antigens

First dose of homogenate, whose protein contents were preassessed containing 20,40,60,80,100 µg protein of mature worms with FCA was administered on day 0 to experimental mice (IVASAg). Culture medium + FCA was given to control mice. Again on day 5, a booster dose of vaccine in five grades without FCA was administered to experimental mice and only culture medium to control mice. On day 26 the mice were challenged with 300 L3 larvae.

On day 27 and 31 (pi), mice were sacrificed for larval recovery, collection of blood and separation of serum for assessment of other test parameters. On day 39 adult worms were recovered, collection of blood and separation of serum was done for assessment of other test parameters.

5)Infected Vaccinated with Larval ES Antigens

Different concentrations of ES antigens from L-3 larvae stage + FCA were administered on day 0. On day 5, booster dose without FCA was vaccinated. On day 26, each mice was challenged with 300 viable L-

3 larvae. Mice were sacrificed on 27 and 31 day for the recovery of L-3 larvae and collection of blood and separation of serum for assessment of other test parameters. On day 39 adult worms were recovered. Collection of blood and separation of serum was done for assessment of other test parameters. On day 39 adult worms were recovered and test parameters were assessed. This group is called as (IVLESAg).

6)Infected Vaccinated with Adult ES Antigens
Different concentration of ES antigens from adult worm stage + FCA were administrated on day 0. On day 5, booster dose without FCA was vaccinated. On 26 day each group of mice was challenged with 300 viable L-3 larvae. Mice were sacrificed on day 27 and 31, for the recovery of L-3 larvae. Collection of blood and separation of serum was done for assessment of other test parameters. This group of mice is also called as (IVAESAg).

Maintenance of the strain of *H. polygyrus* in mice-
Heligmosomoides polygyrus strain was originally obtained from the Experimental Parasitology unit, Department of Zoology, S.V. Veterinary University, Tirupati. *H. polygyrus* was maintained in the Helminthology–Immunology laboratory by infecting fresh batches of 25 young mice with 300 larvae/mice after every three months. Third stage infective filariform larvae were obtained by the petridish method of Van Zandit (1961). Faecal pallets from infected mice were collected on a damp filter paper. Two pieces of Whatmann filter paper No. 40 were placed in sterilized culture dishes with the faecal pallets placed in the centre and covered with thoroughly washed activated charcoal. The mixture was kept moist by 0.5% saline, aerated during the entire culture period daily for 30 minutes and incubated at 21-300C for about 6 days by the end of which larvae were flushed out into a jar containing distilled water. They were allowed to settle for sometime, the supernatant was discarded and the larvae re-suspended in distilled water and stored in cold.

Preparation of inoculums for infection-
A larval suspension of about 100 ml was prepared in a glass stoppered measuring cylinder of 100 ml capacity. The numbers of actively motile larvae were counted by the dilution method of Scott (1928). After vigorous shaking, 1 ml of the suspension was pipette out, transferred onto several glass slides with squares already made on their reverse with a glass marking pencil and the larvae in all squares were carefully counted under a suitable dissecting microscope. Three such counts were repeated and the average count in 1 ml was multiplied by the total volume to get the total number of the larvae. An inoculum containing the desired number of actively motile larvae was adjusted in 0.2-0.3 ml to be given to each mouse. Each mouse was orally inoculated directly into stomach with the desired number of larvae (300) by 1 ml syringe having a blunt 18 gauge-feeding needle. After inoculation, mice were kept in cages in groups of five and labeled according to the design of experiments and were fed routinely with the same standard diet.

Collection of the blood samples and separation of serum-
0.5 ml blood from experimental and control mice was collected from
orbital sinus under mild ether anesthesia with 1ml sterilized dry glass syringe
fitted with a 20 gauge needle. Blood samples were kept in 15 ml centrifuge tubes in cold overnight for clotting after which serum carefully pipetted out into clean sterilized serum collecting tubes and stored at –200 C until required.

Collection of serum-
Blood was drawn out from orbital sinus, left in cold for overnight and then serum was separated by centrifugation in cold at 2,500 rpm for 10 minutes. Sera from individual animals were pooled and stored at -200C without preservatives.

Estimation of serum IgG and IgM -
Blood was collected from orbital sinus of both control and experimental male and female albino rats after 1 days, 5 days and 13 days after pi. The IgG and IgM levels were measured in serum using Enzyme Linked Immunosorbant Assay (ELISA).

Principle-
The assay for antigen depends on being able to couple highly specific
antibody to enzyme and a solid substrate such as a plastic beads or plates (the inner walls of the wells where assay is done).

The antigen is immobilized on a solid surface. The antibody specific to first or primary antibody. This complex can be detected by enzyme substrate reaction. The intensity of this chromogenic reaction is measured.

Estimation of IgG-
Requirment-
1) Mice serum-
Blood was collected from orbital sinus of control and experimental male and female albino mice after 1, 5 and 13 days after pi. It was centrifuged to obtain the serum.
2) Goat anti-rat IgG as primary antibody-
Goat anti-rat IgG antibody was obtained from the Banglore Genei company. This goat anti-rat IgG was used as primary antibody.
3) Goat anti-rat IgG peroxidase conjugate as secondary antibody-
Goat anti-rat IgG conjugate was obtained from the Banglore Genei
Company. It was used as secondary antibody.
4) Phosphate buffer saline (PBS)-
Added 19 ml NaH2PO4 (0.2M) to 81 ml NaHPO4 and volume was made up to 200 ml with 0.9% NaCl solution
5) Substrate Solution (Ortho phenylene diamine) -
5 mg of Ortho phenylene diamine (OPD) was added in 12.5 ml of carbonate buffer and 5ml of 30 % hydrogen peroxide was added to it.
6) Microtitre plate (96 welled).

Procedure-
1)Added serial dilutions of primary antibody (100ml) to each well of the mocrotitre plate.
2) Incubated for 2 hrs at 370 C and transfered to 40 C overnight.
3) Next day the plate was washed with PBS.
4) Then the wells were filled with 200 ml of blocking reagent (3% skimmed milk in PBS) to block the exposed area.
5) Then added the 100 ml of mice serum as antigen.
6) Incubated for 1 hr at 370 C.
7) The plate was again washed with PBS and then 100 ml of conjugated secondary antibody was added to each well.
8) Again the plate was incubated for 1hr at 370 C and washed with PBS.
9) The substrate solution (H2O2 + OPD) was added to each well.
10) The reaction was stopped by adding 2N Sulphuric acid after 15-20 min.
11) The O.D. of each well was checked at 492 nm.
12) The first two columns of the plate were left as blank. To this, added everything except primary antibody. This acted as "zero intensity" well while taking the O.D.

Calculation-
A graph was plotted with O. D. Vs Concentration of primary antibody on a semilog graph paper. O.D of peak was multiplied by diluting factor to get the antibody concentration.

Estimation of IgM-
Requirements-
1)Mice Serum- Blood was collected from orbital sinus of control and experimental
male and female albino mice after 1, 5 and 13 days after pi. It was centrifuged to obtain the serum.
2)Goat anti-rat IgM as primary antibody-
Goat anti-rat IgM antibody was obtained from the Banglore Genei company. This goat anti-rat IgG was used as primary antibody.
3)Goat anti-rat IgM peroxidase conjugate as secondary antibody-
Goat anti-rat IgM conjugate was obtained from the Banglore Genei company. It was used as secondary antibody.
4)Phosphate buffer saline (PBS)-
Added 19 ml NaH2PO4 (0.2M) to 81 ml NaHPO4 and volume was made up to 200 ml with 0.9% NaCl solution.
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12) The first two columns of the plate were left as blank. To this, added every thing except primary antibody. This act as "zero intensity" well while taking the O. D.

Calculation-
A graph was plotted with O.D. Vs Concentration of primary antibody on a semilog graph paper. O. D. of peak was multiplied by diluting factor to get the antibody concentration.

Statistical analysis-
Student's 't' test was used, $p < 0.05$ was regarded as moderately significant and $p < 0.01$ as significant (Fischer, 1950).

OBSERVATIONS AND RESULTS

Heligmosomoides polygyrus is a natural intestinal helminth of mice. It inhibits in the duodenal region of small intestine. In the present investigation, albino mice were colonized with 300 larvae each time by placing larvae directly in the stomach through gastric lavage. The larvae migrated to duodenum, to house in the submucosa where they matured and then emerged as adult worms migrating in the intestinal lumen by 13 days of time.
The present study was carried out to investigate the immunological responses and possibility of vaccine development.
6.0 IgG and IgM responses:
6.1 IgG and IgM responses from infected mice (with 300 larvae of *H.*
polygyrus) after 1, 5 and 13 days of administration of L-3 larval somatic antigens:
IgG and IgM were estimated in serum of infected mice on 1, 5 and 13 days of infection in vaccinated mice with larval somatic antigens (IVLSA) and in case of infected non-vaccination (INVC-2). A gradual decrease in IgM was recorded both in vaccinated and non-vaccinated mice. The decrease was 1.41 µg/ml in non-vaccinated mice after 13 days of infection and 0.85(µg/ml) in vaccinated mice (IVLSA). At all the durations the decreased and increased amount of IgM and IgG respectively were less in infected vaccinated mice (IVLSA) than that in non-vaccinated (INVC-2) mice. Increased IgG level was observed after 1, 5 and 13 days of infection in vaccinated and non-vaccinated mice. The increased amount of IgG in infected non-vaccinated mice was 3.45 µg/ml and in infected vaccinated it was 3.28 µg/ml. After one day of infection IgG level in vaccinated mice (0.62 µg/ml) was more than that in non-vaccinated (0.58 µg/ml) mice [Table-4.17, 4.17.1 - 4.17.2].

6.2 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of adult somatic antigens:
IgG and IgM were estimated in serum of infected mice on 1, 5 and 13 days of infection in vaccinated mice with adult somatic antigens (IVASA) and infected non-vaccinated (INVC-2) mice. Decreased level of IgM was observed in both vaccinated and non-vaccinated infected mice after 1, 5 and 13 days of infection. The decrease amount of IgM in non-vaccinated infected mice was 1.41 µg/ml and the amount was 0.85 µg/ml in vaccinated (IVASA) mice. However, IgG was found tobe increased in both vaccinated and non-vaccinated mice. Increased level of IgG in non-vaccinated infected mice was 3.45 µg/ml and 3.56 µg/ml in vaccinated mice (IVASA). When observed on 5 days of infection, IgG level in vaccinated (2.25 µg/ml) was more from that of non-vaccinated (1.26 µg/ml) mice [Table-4.18, Fig. 4.18.1 - 4.18.2].

6.3 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of L-3 larval ES antigens:
Decreased values of IgM and increased values of IgG were observed in serum of infected vaccinated (IVLESA) and infected non-vaccinated (INVC-2) mice after 1, 5 and 13 days. After 1, 5 and 13 days of infection decreased IgM and increased IgG amount

observed was in vaccinated mice as compaired to non-vaccinated mice. The decreased value of IgM in non-vaccinated infected mice was 1.41 µg/ml and that in vaccinated mice was 0.59 µg/ml. The increase in IgG level was observed in both vaccinated and non-vaccinated mice. The increased values of IgG in non-vaccinated mice was 3.45 µg/ml and in vaccinated mice 2.55 µg/ml [Table – 4.19, Fig. 4.19.1 - 4.19.2].

6.4 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of adult ES antigens:

IgG and IgM were estimated in serum after 1, 5 and 13 days after

H. polygyrus infection and vaccination (IVAESA) in the mice. At all the durations, amount of IgM was observed tobe decreased and level of IgG was increased. The decreased value of IgM was of 1.41 µg/ml in non-vaccinated infected mice and 1.02 µg/ml in vaccinated (IVAESA) mice. Increased value of IgG was 3.45 µg/ml in non-vaccinated and 2.58 µg/ml in vaccinated mice after 13 days of infection but on one day of infection IgG level was more in vaccinated mice (0.68 µg/ml) than that in non- vaccinated (0.58 µg/ml) [Table – 4.20, Fig. 4.20.1 - 4.20.2].

DISCUSSION

IgG and IgM responses:

In mice, IgG has been shown to be the principal protective factor against *H. polygyrus* in immune serum. Following primary infection (*H. polygyrus* larvae), serum IgG levels rise within 5 days and stabilize at approximately 4 times the control level (Table - 4.17, Fig. 4.17.1 - 4.17.2) after 13 days of time. Molinari, *et al.* (1978) reported double rise in IgG level after two weeks of *H. polygyrus* infection. This differences may be that of a method sensitivity. The appearance of protective antibodies and the rise in IgG levels following infections are coincidental, implying that the IgG, in immune sera at least, might be protective.

In the present investigation one day after the infection (300 larvae of *H. polygyrus*), IgM concentration was elevated to approximately double to that of control. Initial serum IgM concentration was found to be 1.82 µg/ml and one day of infection it raised to 3.26 µg/ml i.e. the rise in IgM amount is about 179%. Thereafter, it is observed that IgM concentrations declined after 5 and 13 days of infection and IgG concentration is increased from 0.85 µg/ml to 3.45 µg/ml (400%). In general the initial sudden IgM elevation indicate that IgM are first to get secreted for protection, however, IgG comes late in the picture for protection.

Thus the present observations suggest that IgM antibodies are secreted as innate immune response. Rajan, *et al*. (2005) suggested that elimination of nematode parasites is IgM dependant for both primary and challenge infection.

Similarly, in vaccination experiments, it is observed that larval and adult somatic antigens resulted into more production of IgG, than those of larval and adult excretory-secretory antigens (Table- 4.17 to 4.20, Fig. 4.17.1 - 4.20.2).

Fig. 4.17 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of L-3 larval somatic antigens

Days after infection	I-NV-C-1		I-NV-C-2		I-V-LSA	
	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)
0	1.82 ± 0.65	0.85 ± 0.06	-	-	-	-
1	-	-	3.26 ± 0.11	0.58 ± 0.02	3.20 ± 0.26	0.62 ± 0.02
5	-	-	2.08 ± 0.09	1.26 ± 0.06	1.95 ± 0.18	1.06 ± 0.16
13	-	-	1.41 ± 0.12	3.45 ± 0.48	0.85 ± 0.05	3.28 ± 0.44

I-NV-C-2 Infected Non Vaccinated Control-2

I-V-LSA Infected and Vaccinated with L-3 larval Somatic Antigens

All values are mean ± SE of six observations.

Fig. 4.18 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of adult somatic antigens

Days after infection	I-NV-C-1		I-NV-C-2		I-V-ASA	
	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)
0	1.82 ± 0.65	0.85 ± 0.06	-	-	-	-
1	-	-	3.26 ± 0.11	0.58 ± 0.02	3.48 ± 0.56	0.56 ± 0.05

5	-	-	2.08 ± 0.09	1.26 ± 0.06	1.96 ± 0.08	2.25 ± 0.32
13	-	-	1.41 ± 0.12	3.45 ± 0.48	0.85 ± 0.02	3.56 ± 0.09

I-NV-C-2 Infected Non Vaccinated Control-2
I-V-ASA Infected and Vaccinated with Adult Somatic Antigens
All values are mean ± SE of six observations.

Fig. 4.19 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of L-3 larval ES antigens

Days after infection	I-NV-C-1		I-NV-C-2		I-V-LESA	
	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)
0	1.82 ± 0.65	0.85 ± 0.06	-	-	-	-
1	-	-	3.26 ± 0.11	0.58 ± 0.02	2.75 ± 0.22	0.70 ± 0.05
5	-	-	2.08 ± 0.09	1.26 ± 0.06	1.42 ± 0.28	1.98 ± 0.11
13	-	-	1.41 ± 0.12	3.45 ± 0.48	0.59 ± 0.05	2.55 ± 0.21

Fig. 4.20 IgG and IgM responses from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of adult ES antigens

Days after infection	I-NV-C-1		I-NV-C-2		I-V-AESA	
	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)	Ig M (µg/ml)	Ig G (µg/ml)
0	1.82 ± 0.65	0.85 ± 0.06	-	-	-	-
1	-	-	3.26 ± 0.11	0.58 ± 0.02	3.25 ± 0.15	0.68 ± 0.02
5	-	-	2.08 ± 0.09	1.26 ± 0.06	2.32 ± 0.22	1.22 ± 0.09
13	-	-	1.41 ± 0.12	3.45 ± 0.48	1.02 ± 0.09	2.58 ± 0.33

I-NV-C-2 Infected Non Vaccinated Control-2
I-V-AESA Infected and Vaccinated with Adult ES Antigens
All values are mean ± SE of six observations.

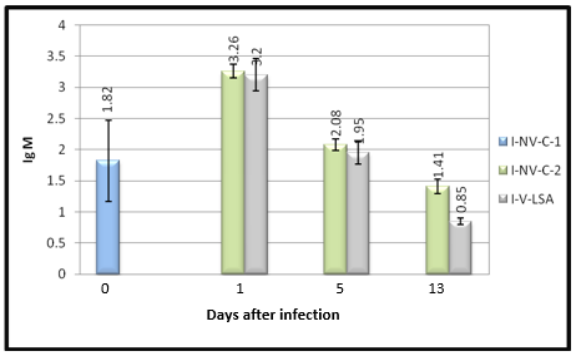

Fig. 4.17.1: IgM response from infected mice after 1, 5 and 13 days of administration of L-3 larval somatic antigens

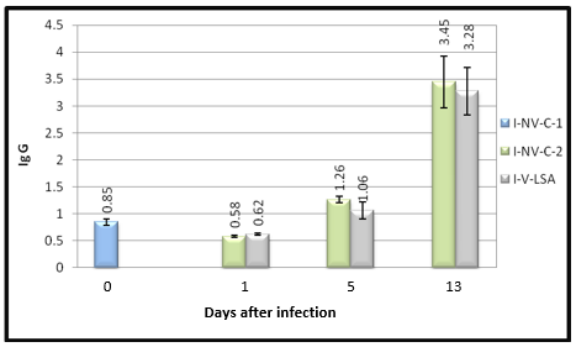

Fig. 4.17.2: IgG response from infected mice after 1, 5 and 13 days of administration of L-3 larval somatic antigens

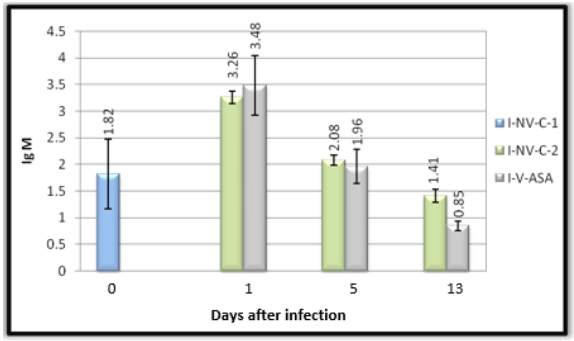

Fig. 4.18.1: IgM response from infected mice after 1, 5 and 13 days of administration of adult somatic antigens

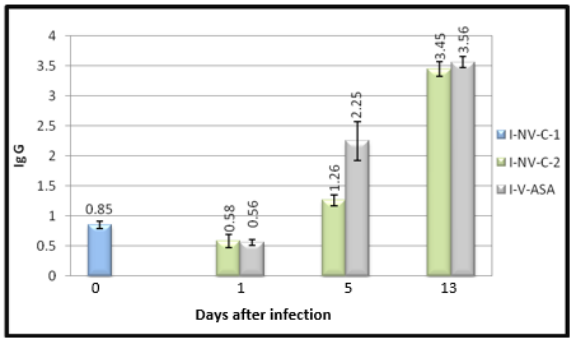

Fig. 4.18.2: IgG response from infected mice after 1, 5 and 13 days of administration of adult somatic antigens

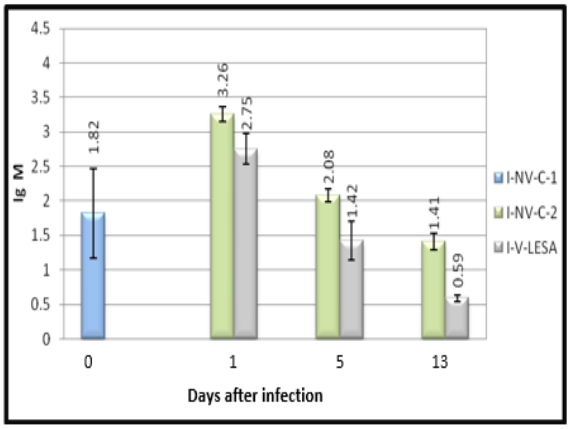

Fig. 4.19.1: IgM response from infected mice after 1, 5 and 13 days of administration of L-3 larval ES antigens

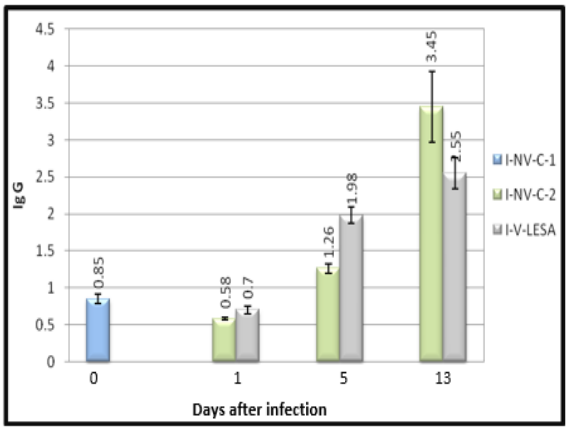

Fig. 4.19.2: IgG response from infected mice after 1, 5 and 13 days of administration of L-3 larval ES antigens

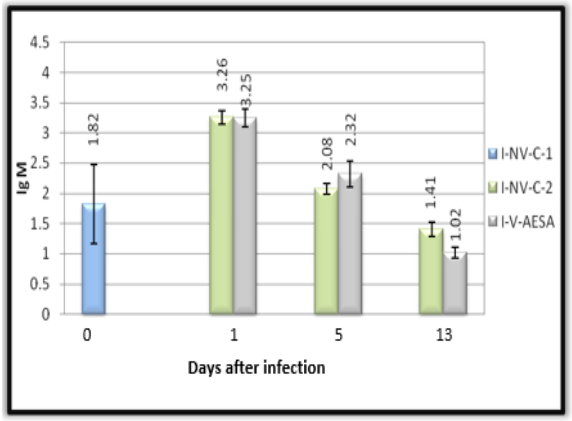

Fig. 4.20.1: IgM response from infected mice after 1, 5 and 13 days of administration of adult ES antigens

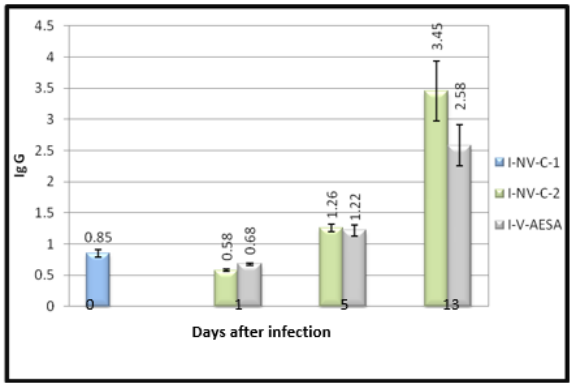

Fig. 4.20.2: IgG response from infected mice after 1, 5 and 13 days of administration of adult ES antigens

SUMMARY AND CONCLUSION

In mice, IgG has been shown to be the principal protective factor against *H. polygyrus* in immune serum. Following primary infection (*H. polygyrus* larvae), serum IgG levels rise within 5 days and stabilize at approximately 4 times the control level after 13 days of time. In mice, IgG has been shown to be the principal protective factor against *H. polygyrus* in immune serum. Following primary infection (*H. polygyrus* larvae), serum IgG levels rise within 5 days and stabilize at approximately 4 times the control level after 13 days of time. Molinari, *et al.* (1978) reported double rise in IgG level after two weeks of *H. polygyrus* infection. This difference may be that of a method sensitivity. The appearance of protective antibodies and the rise in IgG levels following infections are coincidental, implying that the IgG, in immune sera, might be protective. Similarly, in vaccination experiments, it is observed that larval and adult somatic antigens resulted into more production of IgG, than those of larval and adult excretory-secretory antigens.