IMMUNE REACTIONS IN GUINEA PIGS DUE TO SOMATIC ANTIGENS FROM FOURTH-STAGE LARVAE OF BUNOSTOMUM TRIGONOCEPHALUM

S. KUMAR

Department of Zoology, Institute of Advanced Studies, Meerut University, Meerut

Abstract. Lyophilized somatic antigens obtained from fourth-stage larvae of *Bunostomum trigonocephalum* induced significant resistance against the development of L_{4} from L_{3} and adults from L_{4}. The higher antigen dose (1 000 LE) significantly reduced the number of adults developed from L_{4} as compared to the lower dose (800 LE) which did so only insignificantly. Inflammation of the intestinal tissue and loosening of the intestinal luminal contents were characteristics of the immunized animals.

Extensive studies available on the development of immunity by somatic antigens obtained from larval stages of nematodes, have been reviewed by Lal et al. (1983). In addition studies available on the larval somatic antigen induced resistance in the host (Soulsby 1963, Crandall and Arean 1965, Lehnert 1967, Guererro and Silverman 1969, 1971, Stromberg and Soulsby 1977, Horii et al. 1985), have produced contradictory results. A careful screening of available literature reveals that *Bunostomum trigonocephalum*, a great pathogenic hookworm and one of the most prevalent nematode parasites of Meerut region, has remained untouched as regards the immunological aspects.

The antigenic activity of somatic extracts of fourth-stage larvae of *B. trigonocephalum*, collected from intestinal mucosa and submucosa and lumen of infected animals and cultured for 5 days in vitro, is now reported.

MATERIALS AND METHODS

Infection-free, laboratory bred male and female Meerut strain 90 days old (from birth) guinea pigs weighing 500–600 g, were used throughout the experiments. The animals were housed 3–5 per cage with wire mesh floors, over sawdust at constant temperature and humidity. They received balanced commercial preparation in pellet form and water ad libitum.

For the preparation of third-stage infective larvae (L_{3}) motile *B. trigonocephalum* were obtained from the intestines of sheep and goat slaughtered freshly in a local abattoir. Female worms were separated, washed thoroughly in normal saline and teased into cow faeces-charcoal culture medium and incubated at 34 °C as recommended by Levine (1968). After about a week L_{3} were recovered from the culture with the help of Baermann method. Larvae were collected and counted.

To obtain fourth-stage larvae (L_{4}) 50 guinea pigs were each administered orally or subcutaneously with a single dose of 3 000 L_{3}. On day 10 post infection (p.i.) the guinea pigs were sacrificed and L_{4} were collected from the mucosa, submucosa and lumen of the intestine according to the pepsin digestion method of Dash (1981). The number of larvae in a sample was estimated by dilution technique.

For the preparation of antigens a known number of L_{4} was washed in distilled
water and suspended in 5 ml of double glass distilled water and extruded in a Pressure Cell Extruder at 20,000 psi. This somatic antigen was freeze-dried and stored at 4°C until needed. 4% Sodium alginate was used in doses of 0.5 ml per animal as adjuvant. Immunizing schedule included the administration of antigens in two intraperitoneal injections: the first on day 0 and the second on day 14. For challenge infection animals were orally administered on day 21 (i.e., 7 days after the second immunizing injection) with 5,000 viable L₄.

The experiment involved 4 groups subdivided into 8 sub-groups (2 sub-groups A and B per group) of 10 animals each.

Group 1: The animals received 300 larval equivalents (LE) of L₄ lyophilized somatic antigens in each injection.

Group 2: The animals received 500 LE of L₄ lyophilized somatic antigens in each injection.

Group 3: (Control): The animals received adjuvant alone in two injections.

Group 4: (Active immunization control): The animals were infected with 5,000 L₄ 21 and 7 days before the challenge infection.

For the collection of L₄ the animals of the sub-groups A were sacrificed on day 10 after the challenge infection. Adults were searched in the animals of the sub-group B sacrificed on day 24 after the challenge infection. Only those parasites were taken into account which were found viable. Intestinal tissue and the luminal contents were examined. L₄ were collected as described previously. The term larval equivalents is used to denote the number of larvae used for the preparation of somatic extract (antigen).

RESULTS

Results obtained are summarised in Table 1. Though the lyophilized somatic antigens induced significant (p < 0.01: test vs. control) resistance against the development of L₄ (around 70% of protection relative to control) and adults (around 80% of protection relative to control), the two doses of the antigen (500 and 1,000 LE) did not show a significant difference (P > 0.01) between their effects. The high dose (1,000 LE) however, reduced the number of adults developed from L₄ too, significantly (P < 0.01). The lower dose (500 LE) though reduced the number of adults developed from L₄, but insignificantly only (P > 0.01). Infection immunity control group animals offered maximum resistance (96% of protection against L₄; 97% of protection against adult).

During examination of the intestine, inflammation of the intestinal wall and loosening of the intestinal contents were observed in infected animals which were more pronounced on day 24 p.i. The degree of the two characteristics correlated positively (Spearman’s rank correlation \( r_{s} = 0.72; \) SokaI and Rohlf 1969) with the per cent of protection relative to control. The L₄ present were almost normal and viable. No L₄ live or dead, could be observed on day 24 p.i.

DISCUSSION

A number of investigators have tried somatic antigens obtained from the inside host moulled stages of a variety of nematodes, recovered from the host’s tissue, to induce resistance against the respective nematode infections and have obtained contradictory results in their efforts (Lal et al. 1985). Present studies reveal that the lyophilized somatic antigens obtained from L₄ are potent enough to induce resistance into the host. The degree of resistance induced, however, is not up to the level that may

<table>
<thead>
<tr>
<th>Group</th>
<th>Description of group</th>
<th>Recovery of L₄</th>
<th>% of protection (relative to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500 LE somatic antigen + isolated infected</td>
<td>284 ± 87</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>1,000 LE somatic antigen + isolated infected</td>
<td>187 ± 39</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>Isolated alone</td>
<td>285 ± 136</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>Infection immunity control</td>
<td>38 ± 14</td>
<td>96</td>
</tr>
</tbody>
</table>

**P < 0.05 (test vs. control),** **N = 10** (adult vs. L₄), Student's t-test (SokaI and Rohlf 1969)
be expected to be required to eliminate completely the L₁ or adult stages of B. trigonocephalum from the host or that could completely inhibit the development of L₃ into L₄ or later into adult.

Though the somatic antigens could bring about the elimination of L₂ stages (P < 0.01) from the host, it seems that they failed to put a check on the development of L₃ into L₄. Had it been the case some of the stunned or retarded L₃ stages would have been expected to recover on day 10 or 24 after infection as were recovered due to B. trigonocephalum L₁ somatic antigen induced effects (Kumar, unpublished observations). It can be inferred, therefore, that the per cent of protection from development of L₃ from L₂ was achieved not by inhibition of L₂ development, but by elimination of L₁ from the immunized host. It is interesting to note that these effects of lyophilized somatic antigens of L₃ stand in marked contrast to those of lyophilized somatic antigens obtained from L₂ (Kumar, unpublished observations). It can be inferred from the results obtained that the resistance induced by the L₃ somatic antigens of B. trigonocephalum, is as a consequence of such immune responses that offer obstacle in the establishment of the parasite in the host. It can also be inferred that the L₃ somatic antigens result in relatively two fold elimination of L₁ as compared to that of the L₂, keeping in view the per cent precautions. Results of the infection immunity control group animals support a number of previous reports (Levine 1968) that the repeated infections result in development of resistance in the host against the infection.

After studies on Ascaris suum, Guerrero and Silverman (1969) obtained similar results and concluded that somatic antigens from third-stage larvae (similar to L₃ of B. trigonocephalum in the way that both moult in vitro) induce a limited protection to infection in mice. Earlier Soulsby (1965) reported a 92.3% protection in guinea pigs with lyophilized somatic antigens of the same kind used during the present studies. He, however, obtained variable results later on; he found a 63% and 82% resistance to infections with embryonated eggs of A. suum using fresh antigens. This induced resistance was previously suggested by Soulsby (1958) to be due to the release of functional antigens when the larvae were disrupted.

The inflammatory reaction of the intestinal tissue and the loss of intestinal luminal contents may well be explained by what has been previously caused by host's immune responses as has been reported earlier in case of Nippostrongylus brasiliensis (Wells 1962). A moderate degree of positive correlation between these responses and the per cent of protection achieved, provide strong evidence to this view.

It can be safely concluded from the results that lyophilized somatic antigens obtained from L₃ of B. trigonocephalum may be used to induce partial protective immunity in guinea pigs against the hookworm.

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IMMUNITARY REACTIONS IN MICE TO THE INTESTINAL ELIMINATION OF ASCARIS SUUM L. LIVIDAL Y NACH LAUTKENTERMIERUNG DES CUMAR

C. KUMAR

Résumé. Les antigènes somatiques lyophilisés, obtenus à partir de larves de l'Ascaris suum, inhibent complètement la croissance et la transformation de L₃ en L₄. Ils n'ont pas d'effet sur les larves adultes. Les antigènes lyophilisés de L₃ provoquent une protection à l'infection par l'Ascaris suum. Les antigènes lyophilisés de L₂ n'ont pas d'effet sur l'Ascaris suum. Les antigènes lyophilisés de L₃ inhibent complètement la croissance et la transformation de L₃ en L₄. Ils n'ont pas d'effet sur les larves adultes. Les antigènes lyophilisés de L₃ provoquent une protection à l'infection par l'Ascaris suum. Les antigènes lyophilisés de L₂ n'ont pas d'effet sur l'Ascaris suum.