Role of Excretory–Secretory Metabolites of *Fasciola gigantica* in Modulating Delayed-type Hypersensitivity in Rats

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**ABSTRACT**

The role of excretory–secretory metabolites of *Fasciola gigantica* in modulating the delayed type of hypersensitivity in the host (rats) was investigated. Eighteen rats of either sex, aged 3–4 months, were assigned to three groups of 6 animals each. Rats in group 1 served as non-inoculated controls and each rat in this group was administered only Freund’s complete adjuvant on day 7. Animals in groups 2 and 3 were administered inoculation dose(s) of somatic *F. gigantica* antigen (SFgA) and excretory–secretory *F. gigantica* antigens (ESFgA) according to the experimental schedule. The delayed-type hypersensitivity was monitored by assessing alterations in the foot pad thickness, its histopathology and lymphocyte proliferation assay. It was observed that the ESFgA caused diminution in delayed-type hypersensitivity response to a significant level (p<0.01) against SFgA in rats. This finding was further confirmed by lower stimulation indices of peripheral blood mononuclear cell in rats sensitized with ESFgA prior to inoculation of SFgA (group 1) than in nonsensitized rats receiving only SFgA (group 2).

**Keywords:** delayed-type hypersensitivity, *Fasciola gigantica*, rats

**Abbreviations:** Con-A, concanavalin-A; DTH, delayed-type hypersensitivity; ESFgA, *F. gigantica* excretory–secretory antigen; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; SFgA, *F. gigantica* somatic antigen

**INTRODUCTION**

Nonspecific immune suppression in experimental parasitic infections such as malaria (Mellouk et al., 1987), schistosomiasis (Perrin and Philips, 1988) and trypanosomosis (Beltz et al., 1988) is considered to play a significant role in making susceptible hosts incapable of mounting an immune response against these aetiological agents (Wakelin, 1984). In addition, the establishment of invading stages of the parasites as adult populations and their survival within the host mainly depends on the ability of the parasites to evade the immune response of the host (Rakha et al., 1992). Excretory–secretory metabolites of the live fluke *Fasciola hepatica* were found to be toxic to lymphocytes of rats (Goose, 1978) and are known to inhibit the respiratory burst of activated sheep neutrophils (Jefferies et al., 1997). Recently, it has been documented that metabolites of *F. hepatica* cause suppression of DTH in rats (Cervi et al., 1996). It
was therefore of interest to investigate the role of excretory and secretory metabolites of *F. gigantica* and to generate data on the immunosuppressive effect of the fluke in laboratory hosts. The present study has been a modest beginning in this direction.

**MATERIALS AND METHODS**

**Animals**

Eighteen rats of either sex, aged 12–16 weeks, were obtained from the Laboratory Animal Resource Section of the Indian Veterinary Research Institute. These were maintained in polypropylene cages in the experimental sheds of the Division of Medicine, IVRI, Izatnagar. The rats were acclimatized for a period of 15 days and were fed on a balanced diet consisting of crushed wheat 62%, maize 30%, wheat bran 7% and common salt 1%. Fresh water and green leafy vegetables were offered daily *ad libitum.*

*The somatic F. gigantica antigen (SFgA)*

Live adult *F. gigantica* flukes were recovered from the livers of sacrificed buffaloes. These were carefully washed on the spot 3 to 4 times with chilled PBS to remove material of host origin and were finally suspended in PBS and brought to the laboratory. The flukes were then homogenized in a ten Broeck tissue grinder at 4°C and were centrifuged (12 000g for 30 min). The supernatant was collected and designated as SFgA.

*The excretory–secretory F. gigantica antigens (ESFgA)*

Live, intact adult *F. gigantica* flukes were obtained from livers of sacrificed buffaloes and were washed 3–4 times with PBS at room temperature for 1 h to remove all materials of host origin (traces of blood and bile, etc.). The washed flukes (40 flukes per 100 ml) were then incubated in PBS at 37°C for 2 h. The supernatant was centrifuged at 12 000g at 4°C for 30 min to remove particulate matter. The supernatant was separated and designated as ESFgA. The final protein content was 1.8 mg/ml (Lowry *et al.*, 1951).

*Delayed hypersensitivity testing protocol*

Eighteen rats were randomly assigned to three groups of 6 animals each (3 male and 3 females) according to the experimental design given in Table I. The rats in group 1 served as untreated control. Groups 2 and 3 were inoculated subcutaneously with 1 ml of an emulsion of SFgA protein (2.5 mg/ml) and Freund’s complete adjuvant (FCA) at