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## The suppression of homologous immunity by soluble adult antigens of *Nematospiroides dubius*

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### ABSTRACT

An established adult infection of *Nematospiroides dubius* was unaffected by the administration of immune lymphocytes and immune sera whereas an incoming larval infection was expelled. Past experiments have shown that the immune inoculum at least had the ability to recognize adult stages, leading to the hypothesis that adult stages secrete or excrete an immunomodulatory shield around themselves in the gastrointestinal tract. This hypothesis was given further credence by the demonstration that soluble antigens derived from adults abolished the generation of homologous immunity to this parasite. Modulation of immunity was reflected by increased fecundity, increased worm size, and increased survival time in the gut.

### INTRODUCTION

It has been suggested that *Nematospiroides dubius* adults survive in the murine intestine in defiance of the immune system by producing immunomodulatory factors. Evidence for this is provided by the fact that infections modulate immune responses to homologous or heterologous antigenic stimulation (BEHNKE *et al.*, 1983; ALI & BEHNKE, 1983). Therefore, it would seem reasonable to postulate that established adults secrete or excrete an immunomodulatory barrier around themselves to promote their own survival, particularly as soluble adult antigens have been shown to modulate immune responsiveness *in vivo* (PRITCHARD *et al.*, 1984 a).

To test this hypothesis, two lines of approach were used. Firstly, lymphocytes and sera from immunized mice were passively transferred to naive syngeneic mice harbouring either already established adult infections or newly acquired larval infections. As it is known that these immune components at least have the capability of recognizing both larval and adult *N. dubius* antigens (PRITCHARD *et al.*, 1983), their failure to affect adults *in vivo* could be attributable to modulation of the efferent arm of the homologous immune response.

Secondly, animals subject to a very effective and reproducible immunization schedule involving truncation of a primary infection with the anthelmintic pyrantel (PRITCHARD *et al.*, 1984 b) were concurrently injected with soluble adult antigens in an attempt to identify modulation of the afferent arm of the immune response.

### MATERIALS AND METHODS

#### *Animals*

Inbred NIH mice were used throughout the study. The mice were bred and maintained under conventional conditions in the animal house of the Zoology Department, University of Nottingham.

#### *Nematospiroides dubius*

The origin and maintenance of our strain of *N. dubius* and the methods used for infection, recovery of worms and faecal egg counts have been described elsewhere (BEHNKE & PARISH, 1981). Adult *N. dubius* antigen was prepared by homogenization

(AH) in sterile 0.9% saline. The suspension was centrifuged at 10,000 rpm for one hour at 4°C and the supernatant recovered. The antigen was filter sterilized (22  $\mu$ ) before injection.

Immune lymphocytes (IC) were obtained from the mesenteric lymph nodes of female NIH mice immunized by a divided primary infection (BEHNKE & PARISH, 1981), challenged with 150 infective larvae of *N. dubius* on day 50, and killed for cell transfer eight days later. Immune sera (IS) were prepared using a similar immunization regime in outbred CFLP mice. Cells and sera were administered intraperitoneally according to the schedule illustrated in Fig. 1.

Worms were recovered from the intestines using a modified Baermann technique (BEHNKE & PARISH, 1981). Worm lengths were calculated from camera lucida drawings of *N. dubius* using a bit pad digitizer linked to a DEC-PDP 11/34 computer. A minimum of 20 worms were counted in each group.

#### Experimental design

The experiments described in this paper were designed to test the ability of *N. dubius* adults and their antigens to modulate both the generation of a homologous immune response and the expression of an already established (passive) immune response. Schematic diagrams of the experimental approaches and the numbers of worms used in infection and challenge are shown in Figs 1 and 2.

#### Statistical analysis of results

Mean worm recoveries and worm lengths were analysed for significance using Student's t-test.

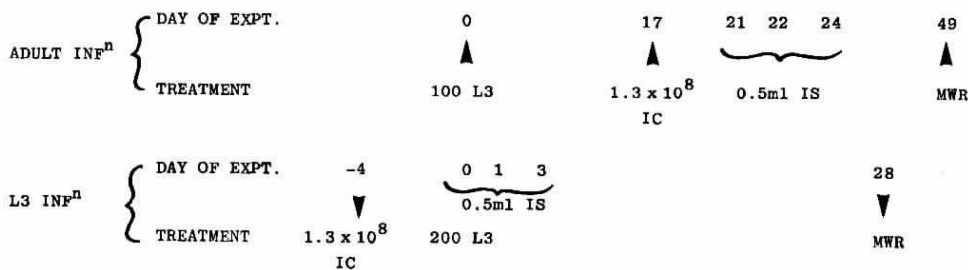


FIG. 1. The effect of passive immunity on newly acquired or established *N. dubius* infections. Experimental plan.

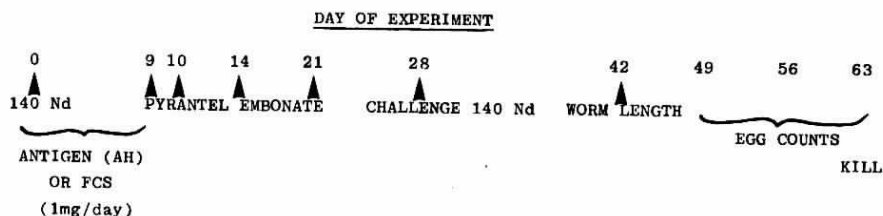


FIG. 2. Immunomodulation of homologous immunity by soluble antigens of *N. dubius*. Experimental plan.

## RESULTS

### *The effect of passive immunity on newly acquired or established N. dubius infections*

The design of this experiment is shown in Fig. 1. Immune mesenteric lymph node cells (IC) and immune sera (IS) were passively transferred to mice harbouring either established adults (ADULT INF<sup>n</sup>) or newly acquired larvae (L3 INF<sup>n</sup>). The effect of passive immunity was assessed by the mean number of adults recovered from each group (MWR). It can be seen from the results of this experiment (Fig. 3) that immune cells and sera, given either alone or in conjunction, failed to expel an established adult infection but transferred complete immunity against newly acquired infection ( $p < 0.001$ ).

### *Immunomodulation of homologous immunity by soluble antigens of adult N. dubius*

To test the ability of soluble adult antigens to modulate the induction of homologous immunity to *N. dubius*, mice were immunized using an anthelmintic-abbreviated infection schedule (PRITCHARD *et al.* 1984 b). An immunized group was compared directly to a control (challenge only) group and to an immunized group treated daily with antigen (AH) from days 0 to 9 (Fig. 2). Foetal calf serum (FCS) was used as an irrelevant antigen control.

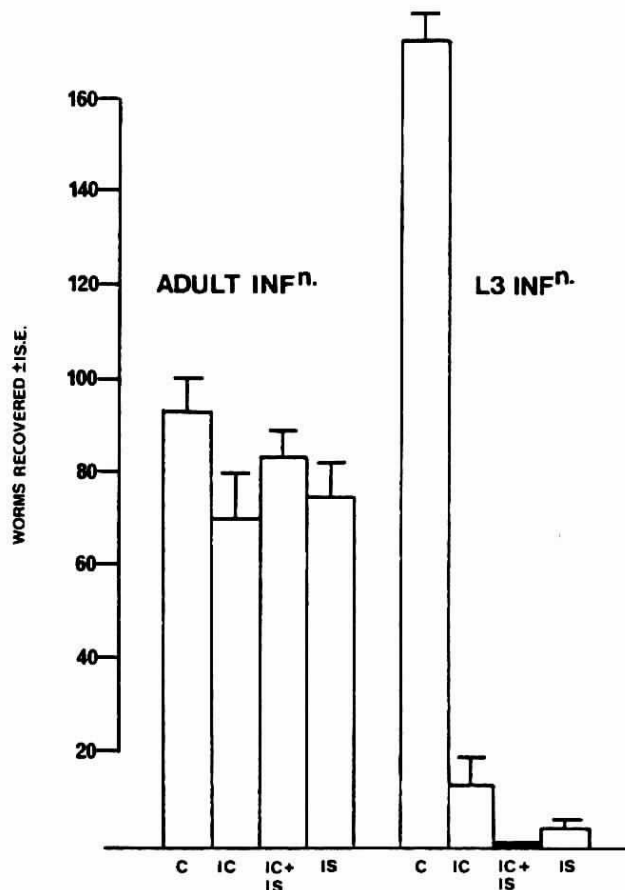


FIG. 3. The effect of passive immunity on newly acquired or established *N. dubius* infections. Immune mesenteric lymph node cells (IC) and immune sera (IS) were passively transferred to mice harbouring either established adults ( $n = 10$ ) or newly acquired L3 larvae ( $n = 6$ ). The effect of passive immunity was assessed by comparing the number of worms recovered to that of a challenge only (C) control group ( $n = 6$ ).

It can be seen from Fig. 4 that immune animals harboured fewer worms ( $2.9 \pm 0.6$ ) compared with control animals ( $132 \pm 4$ ) five weeks following challenge with 140 *N. dubius* L3 larvae ( $p < 0.001$ ). Foetal calf serum (FCS) was shown to have little effect on the generation of immunity ( $14.9 \pm 13.9$ ), whereas immunity was abolished in the group treated with adult homogenate (AH) during the immunization period ( $124 \pm 3.9$ ,  $p < 0.001$ ).

A similar phenomenon was observed when faecal egg counts (Table I) and worm length (Fig. 5) were taken into consideration.

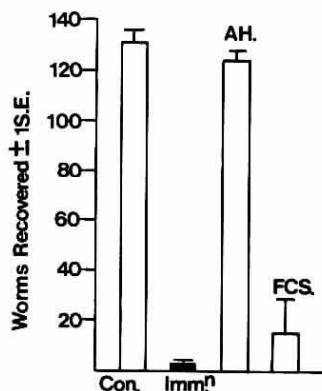


FIG. 4. Immunomodulation of homologous immunity by soluble antigens of adult *N. dubius*. Female NIH mice ( $n = 8$ ) were immunized using an anthelmintic-abbreviated infection schedule (Fig. 2). The number of worms recovered in the immunized group was compared directly to a control (challenge only) group and to an immunized group treated daily with adult antigen (AH) during the period of immunization. Foetal calf serum (FCS) was used as an irrelevant antigen control ( $n = 8$  for each experimental group).

TABLE I. Mean faecal egg counts/gm of faeces in experimental groups 49, 56 and 63 days following challenge with 140 L3 larvae of *Nematospiroides dubius*.

Group ( $n = 8$ )	Mean faecal egg count on day:		
	49	56	63
Challenge control	5100	35400	5300
Immune	200	1400	900
Immune + FCS	0	700	0
Immune + AH	2700	41200	4400

## DISCUSSION

The results presented here indicate that adult *N. dubius* can suppress or modulate the induction and expression of *homologous* immunity and are consistent with the demonstration that adults transplanted into the duodenum by laparotomy can modulate immunity generated by irradiated larvae (BEHNKE *et al.*, 1983). Furthermore, the abolition of immunity by adult homogenate is the first demonstration of homologous suppression by soluble antigens derived from adults, and supports the hypothesis that the adult produces factors capable of prolonging the fecund stage of the life-cycle.

The neutralization of an already established (passive) immunity by adult stages in the first experiment would suggest that the parasite is indeed capable of producing an immunomodulatory shield around itself, particularly when it is known that the

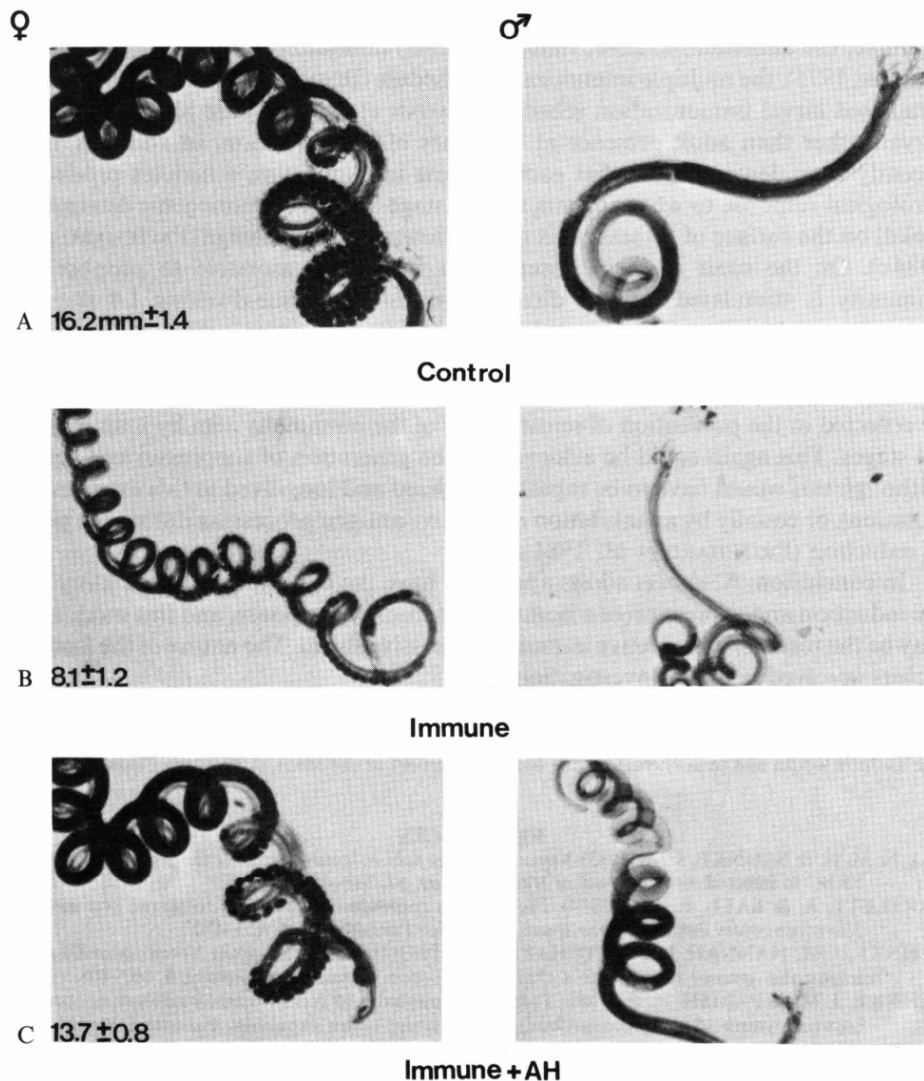


FIG. 5. The effect of adult *N. dubius* antigen on worm length. A = Normal female (left hand column) and male (right hand column) *N. dubius*. B = *N. dubius* recovered from immune animals 14 days post challenge. Note the paucity of eggs in the female nematode. C = *N. dubius* recovered from immune animals treated with AH.

components of the passive inoculum at least had the capability of recognizing adults (PRITCHARD *et al.*, 1983). In this context, it is possible that the inoculum was prevented from reaching the parasite by the interference of *N. dubius* with the increased blast cell localization seen in the intestines of parasitized mice (HAGAN & WAKELIN, 1982). In addition, it is possible that factors secreted by adults *in vivo* generated the sort of suppressor cell activity seen in heterologous systems (PRITCHARD *et al.*, 1984 a), effectively to shield the adults from immune attack. It is unlikely that the adult parasite occupies an immunologically privileged site in the intestine as adults can be rejected under certain circumstances (MITCHELL *et al.*, 1982).

The results of the second experiment indicate that soluble adult antigens are capable of preventing the induction of immunity to *N. dubius*. Immunity can be induced by a variety of means, and a common attribute of each method appears to be the

requirement for stimulation by early stages of the life-cycle in the absence of adults. For example, the anthelmintic-abbreviated (pyrantel) immunization schedule (LUEKER & HEPLER, 1975), the multiple immunization schedule (BEHNKE & PARISH, 1981) and the irradiated larval immunization schedule (HAGAN *et al.*, 1981) are all biased toward larval rather than adult presence at the times of immunization. In addition, it has recently been demonstrated that each of these immunization schedules produces a serological response to what appears to be a stage-specific immunogenic determinant (16kd) on the surface of L4 stages six days following oral challenge (PRITCHARD *et al.*, 1984c). On the basis of these observations it seems reasonable to propose that immunity is stimulated by and directed against the tissue-dwelling L4 stages, a proposition which is consistent with the generation of granulomata at the serosal surface of the gastrointestinal tract in immune animals (BARTLETT & BALL, 1974). Therefore the effect of adult *N. dubius* antigens on the induction of immunity could well be reflected in the prevention of sensitization of the immune system by immunogenic L4 stages. This again could be achieved by the generation of suppressor cell activity (although this would have to be rapidly mobilized and long-lived in this experimental situation), or equally by an inhibition of effective antigen processing during the period of induction (PRITCHARD *et al.*, 1984a).

In conclusion, *N. dubius* adults appear to have the capability of modulating both the induction and expression of a homologous immune response, and this modulation may be the result of a protective immunosuppressive shield. The nature of the factor or factors involved is under investigation.

#### ACKNOWLEDGEMENTS

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