Nematospiroides dubius in the jird, *Meriones unguiculatus*: stimulation and expression of acquired immunity

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ABSTRACT

Acquired immunity to *Nematospiroides dubius* was studied in the jird. It was found that a primary infection comprising one or two worms was highly immunogenic and the animals were resistant to challenge infection. Abbreviated infections in which the adult worms were eliminated by treatment with pyrantel also elicited strong resistance to challenge indicating that larval parasites as well as adults were involved in acquired immunity. A small proportion of adult worms completed their development in immune jirds and was recovered 11 days after the challenge infection. However, these worms were rapidly eliminated and few survived to day 16. Trickle infections in which the jirds were exposed to very low numbers of worms at regular intervals were unsuccessful in prolonging the duration of a primary infection. It was concluded that the remarkable sensitivity of the jird to infection with *N. dubius* should enable small quantities of fractionated parasite derived material to be screened for immunogenicity in this host.

INTRODUCTION

Nematospiroides dubius is an intestinal parasite which is noted for its chronic survival in the mouse host (BARTLETT & BALL, 1972). Adult worm infections last for approximately 8 months indicating that the host cannot readily remove the parasite (WILLIAMS, 1982). Single short-term primary infections induce acquired immunity in only a few inbred strains of mice, e.g., Balb/C, $LAF_1/5$, NIH, SJL/01a (JACOBSON *et al.*, 1982; ROBINSON & BEHNKE, 1983). Most other strains of mice require to undergo two or more infections before they can successfully resist a challenge infection. Transplanted adult worms elicit little resistance and in some strains of mice adult worms survive in animals which are totally resistant to larval challenge (JACOBSON *et al.*, 1982; BARTLETT & BALL, 1974). It is therefore perhaps not surprising that adult worm homogenates and excretory products have proved of little value in vaccination against *N. dubius* (DAY *et al.*, 1979; HURLEY *et al.*, 1980). It has been suggested that adult worm antigens are unavailable to the host by virtue of their location in the host (JACOBSON *et al.*, 1982) but an additional and/or alternative possibility, for which there is good evidence, is that adult worms secrete immunomodulatory factors (IMF) which interfere with the stimulation and expression of anti-adult worm immunity (BEHNKE *et al.*, 1983).

In marked contrast to its ability to survive in the mouse, *N. dubius* is less successful in the jird. This host is the only other laboratory animal which, as an adult, can be successfully infected with *N. dubius* and regularly supports a patent infection (CROSS & SCOTT, 1960; JENKINS, 1977). Although jirds are susceptible to infection, the prepatent period is longer, the fecundity is lower and fewer worms mature than in control mice (CROSS & SCOTT, 1960; HANNAH & BEHNKE, 1982). The duration of the primary infection in jirds is limited by an immune response which is stimulated by the adult worms and which operates against the adult worm population, causing loss of the parasites within six weeks of infection (JENKINS, 1977). Jirds are particularly sensitive to infection with *N. dubius* and even primary infections of 25 larvae are terminated by the host (HANNAH & BEHNKE, 1982).

This remarkable sensitivity of jirds to very low numbers of primary infection worms prompted us to determine whether the jird could be used to isolate and study the functional antigens of *N. dubius*. However, little is known about acquired immunity to *N. dubius* in the jird. CROSS & SCOTT (1960) and JENKINS (1977) found that when jirds which had rejected a primary infection were challenged, eggs did not reappear in the host faeces and no worms were found at autopsy 14 days later. These experiments indicated that acquired immunity, like the primary response, was very strong, contrasting with the apparent absence of comparable immunity in the mouse. The experiments reported in this paper were carried out in order to extend these findings and in particular to determine precisely how sensitive the jird/*N. dubius* model is to the induction of acquired immunity, as a necessary preliminary to the use of this host in experiments seeking to isolate the functional antigens of *N. dubius*.

MATERIALS AND METHODS

Nematospiroides dubius

The origin and maintenance of our strain of *N. dubius* and the methods used for infection and recovery of adult worms have already been described (BEHNKE & WAKELIN, 1977; BEHNKE & PARISH, 1979). Faecal egg counts were monitored as reported by HANNAH & BEHNKE (1982). Only female worms were measured. Where possible 10 female worms were selected at random from each animal in the group. 15 worms from the pooled population were drawn by camera lucida and the drawings were measured with Bitpad (Summagraphics) and a PDP 11/34 A computer (Digital Equipment Co. Ltd.).

Animals

Random bred jirds and CFLP mice were used throughout this work, except in the experiment illustrated in Fig. 1. Inbred male NIH mice were used on this occasion. The animals were bred and maintained under conventional animal house conditions in the Zoology Department of Nottingham University. The minimum age of the animals used for experiments was eight weeks for jirds and five weeks for mice.

Statistical analysis

The results are presented as group mean $\pm$ S.E. Differences in adult worm recoveries were analysed for significance by the nonparametric Wilcoxon test (SOKAL & ROHLF, 1969). A value of $p < 0.05$ was considered to be significant.

RESULTS

Stimulation of acquired immunity to N. dubius in jirds by different levels of primary infection

When jirds were given a primary infection of 25 larvae, the worms were expelled some 10 days earlier than in groups infected with 500 larvae (HANNAH & BEHNKE, 1982). We report here experiments which were carried out to extend this work and to determine precisely how many larvae would be required to stimulate acquired immunity. Several experiments were carried out and the results of one such experiment are presented in Table I. Thus groups of six female jirds were infected with 5, 10, 50, 100 or 500 larvae of *N. dubius*. The primary infection was followed by faecal egg counts and the infectivity of the inoculum was monitored in five groups of three mice which were killed 21 days after infection. In mice given only five larvae, 1.7 ± 0.3 worms were recovered but the jirds given this level of infection proved to be negative for egg counts
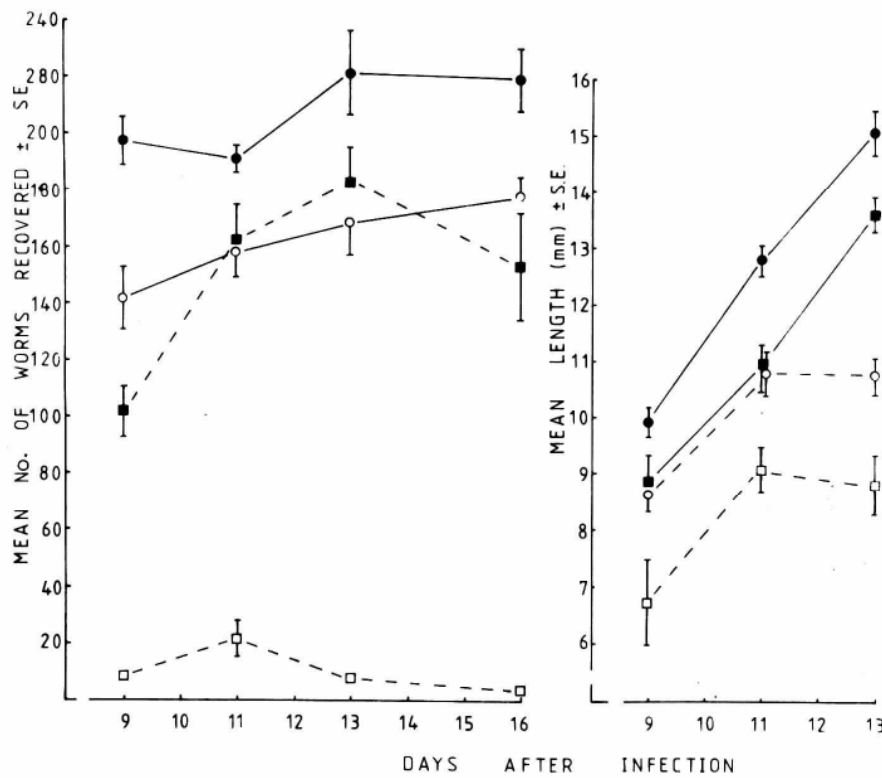
FIG. 1. The survival and growth of *N. dubius* in naive and immune jirds and mice. Control mice (●—●), control jirds (■—■), immune mice (○—○), immune jirds (□—□).

TABLE I. Stimulation of acquired immunity to *N. dubius* in the jird by different levels of primary infection

Group	Species	No. of animals	No. of larvae* given in primary infection	Mean no. of worms recovered 14 d after challenge ± S.E.
A	Mouse	6	—	147·2 ± 8·9
B	Jird	6	—	124·5 ± 5·0
C	Jird	4	5	22·3 ± 16·0
D	Jird	5	10	29·3 ± 26·0
E	Jird	5	50	1·6 ± 0·6
F	Jird	6	100	2·8 ± 1·1
G	Jird	4	500	2·0 ± 0·6

*Groups of 3 mice were infected to monitor the infectivity of the primary infection. The no. of worms recovered from mice infected with 5, 10, 50, 100 and 500 larvae was 1·7 ± 0·3, 3·7 ± 1·8, 39·3 ± 5·6, 87·7 ± 3·8 and 383·7 ± 4·3 respectively.

throughout the five weeks during which they were monitored. Parasite eggs were recorded in the faeces of all the remaining infected groups in quantities which reflected the level of infection. All the groups except group G (given 500 larvae) were negative by day 28. Group G still produced eggs although in very low quantities on day 35 when all the jirds were treated with pyrantel to remove any residual worms. The challenge infection comprising 200 larvae was administered on day 42 and the animals were killed for worm counts 14 days later. The results in Table I show that all the infected groups were resistant to challenge infection. Groups C (primary infection 5 larvae) and D

(primary infection of 10 larvae) had higher worm recoveries than the other immune groups. These were caused by one animal in each group which had a far higher worm burden than the remainder (70 and 133 worms respectively).

This experiment was repeated three times covering in particular the lower levels of primary infection. The results were always similar. For example, in one replicate experiment 60.3 ± 2.3 worms were recovered from the challenge control group, but only 0.3 ± 0.3 and 0.7 ± 0.7 from jirds which had been given 5 and 15 larvae respectively during the immunizing primary infection. Additional experiments (data not shown) established that male and female jirds were equally resistant to challenge even when given very low level immunizing infections.

Stimulation of acquired immunity by primary infections of different duration

Several experiments were carried out to determine whether abbreviated primary infections would still generate resistance to challenge. In one such experiments 30 jirds were allocated to five groups of six animals each. Four groups, C, D, E and F and a group of three control mice were infected on day 0 with 250 larvae of *N. dubius*. The mice were killed on day 15 and 207.3 ± 15.8 worms were recovered from these animals. The four infected groups were treated with pyrantel on days 7, 9, 10, 13, 15, 21 and 35 but each group was started on this regime at a different time, as shown in Table I. All the jirds, together with a control group (B) and a group of three mice (A) were challenged with 200 larvae of *N. dubius* on day 42 and were killed 14 days later for worm counts.

The results, presented in Table II, show that all the jirds which had undergone the immunizing infection irrespective of the duration, were significantly resistant to challenge. However, group C, which was first treated with pyrantel on day 7, had significantly more worms than group F ($p < 0.005$). This experiment was repeated with essentially similar results.

Worm survival and growth in immune jirds

27 jirds and 28 mice were infected with 250 larvae of *N. dubius* on day 0. Four mice were killed on day 26 after this primary infection and 214.3 ± 8.8 worms were recorded. The remaining jirds and mice were treated with pyrantel on day 27 and were given a challenge infection of a further 250 larvae on day 44. 16 control jirds and 16 mice also received the challenge infection. Groups of control and immune jirds and mice were killed for worm counts on days 9, 11, 13 and 16 after the challenge infection. The results are presented in Fig. 1.

As in previous experiments, fewer worms matured in control jirds than in mice, the difference between these groups being most marked on day 9, when the retarded

TABLE II. Stimulation of acquired immunity to *N. dubius* by primary infections of different duration

Group	Species	No. of animals	Primary infection	Pyrantel given* from day shown	Mean no. of worms recovered 14 d after challenge ± S.E.
A	Mouse	3	—	35	154.2 ± 13.2
B	Jird	5	—	35	108.8 ± 7.8
C	Jird	6	+	7	20.7 ± 7.8
D	Jird	4	+	10	6.0 ± 1.0
E	Jird	6	+	15	4.5 ± 2.2
F	Jird	6	+	35	1.5 ± 0.6

*Pyrantel was given on days 7, 9, 10, 13, 15, 21 and 35. The different groups were started on this regime on days 7, 10, 15 or 35.

development of some worms in jirds would have exacerbated the real difference in worm burden. However, the data also shows that the worms were smaller in jirds on days 9 to 13, although the rate of growth subsequently was comparable. In mice which had experienced the primary infection there were on average about 25% fewer worms than in their respective control groups and the worms in these mice were smaller and remained stunted after day 11. Immunized jirds were extremely resistant to reinfection, with over 90% protection on all days except day 11. The slightly higher worm recoveries on day 11 from immune jirds (day 11 v. day 16, $p < 0.025$) suggest that some worms may have completed their development in immune jirds and were probably expelled immediately on their return to the gut lumen. On the three occasions when sufficient numbers of worms were available for measurement, the worms recovered from immune jirds were very small and significantly smaller than those recovered from any of the other groups on the same day.

Resistance to chaser infections in jirds

In the experiments described thus far the primary infection was allowed to run its full course of four to five weeks before the animals were challenged for the assessment of acquired immunity. Additional experiments were carried out to investigate the fate of a superimposed chaser infection administered to jirds before the expulsion of the primary infection. The results of some of these experiments are given in Table III and in Fig. 2. Each experiment followed the same basic format in that groups of jirds were given a primary infection which was followed 4, 8, 7 or 14 days later by a chaser infection. The animals were killed either 14 days (Expts. 1 & 2) or 16 days (Expt. 3) after the chaser infection. Faecal egg counts were monitored at regular intervals throughout each experiment and a representative set of data is presented in Fig. 2.

When the chaser infection followed the primary infection by four days, the chaser infection larvae matured and superimposed on the primary infection worms. As the interval between primary and chaser infection was increased to seven days there was evidence that some but not all of the doubly infected animals had lost both worm

TABLE III. Resistance to chaser infections in jirds infected with *N. dubius*

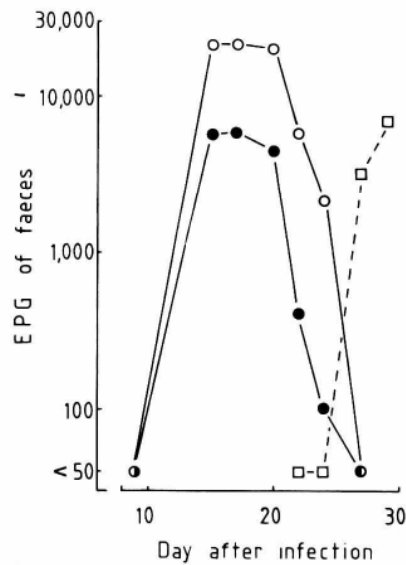|	Treatment			Mean no. of worms recovered ± S.E. (no. of jirds autopsied) on the days shown			
	Primary Infection	Chaser Infection					
	day 0	day 4	day 8	14	18	22	
Expt. 1	+	—	—	116·0 ± 7·2 (4)	142·2 ± 4·2 (5)	55·7 ± 18·6 (6)	
	+	+	—		218·0 ± 21·8 (5)		
	—	+	—		107·8 ± 17·6 (5)		
	+	—	+			123·5 ± 37·0 (6)	
	—	—	+			157·2 ± 11·5 (5)	
	day 0	day 7	day 14	14	21	28	30
Expt. 2	+	—	—	102·0 ± 6·0 (3)	45·8 ± 36·7 (4)	0·3 ± 0·3 (4)	
	+	+	—		123·0 ± 39·9 (6)		
	—	+	—		94·2 ± 9·0 (6)		
	+	—	+			29·0 ± 18·5 (6)	
	—	—	+			91·3 ± 4·7 (4)	
Expt. 3	+	—	—				1·2 ± 1·2 (6)
	+	—	+				2·2 ± 0·6 (6)
	—	—	+				35·2 ± 4·2 (6)

FIG. 2.   Faecal egg counts from jirds given 100 *N. dubius* on day 0 (○), day 14 (□), or on days 0 and 14 (●).

burdens before autopsy, i.e., prematurely in relation to the chaser infection. The faecal egg count data presented in Fig. 2 shows that when a chaser infection was administered, 14 days after the primary infection, it did not increase the group's faecal egg output. The results in Table III confirm that very few chaser infection worms survived for 14 or 16 days and presumably the larvae were either retained in the intestinal walls or more probably were lost with the primary infection worms, as soon as the former returned to the gut lumen.

Resistance to trickle infections in jirds

Finally several experiments were carried out to determine whether trickle infections with *N. dubius* would extend the period of infection in jirds. Three female jirds and six mice were given two larvae every week-day (Monday to Friday inclusive) for four weeks (i.e., a total of 40 larvae). Faecal egg counts were carried out on both groups and the results are presented in Fig. 3. The egg output for mice rose steadily to approximately 10,000 epg of faeces. When the mice were killed on day 44 the MWR was $41\cdot8\pm2\cdot9$ worms indicating that the inoculum had indeed contained approximately two larvae at each infection. However, the jirds did not produce large numbers of parasite eggs and by day 28 were totally negative. No worms were recorded from these animals on day 44.

This experiment was repeated using different numbers of larvae and different intervals between the individual infections. In none of these combinations was the duration of infection in jirds extended beyond that of jirds given a single primary infection with this parasite

DISCUSSION

Unlike the normal host of *N. dubius* (the mouse), jirds expel the adult stages of this parasite within six weeks of infection (HANNAH & BEHNKE, 1982). However, very little is known about acquired immunity to *N. dubius* in the jird. The few preliminary experiments which have been reported suggest that no adult worms develop in jirds which have previously expelled the primary immunizing infection (CROSS & SCOTT,
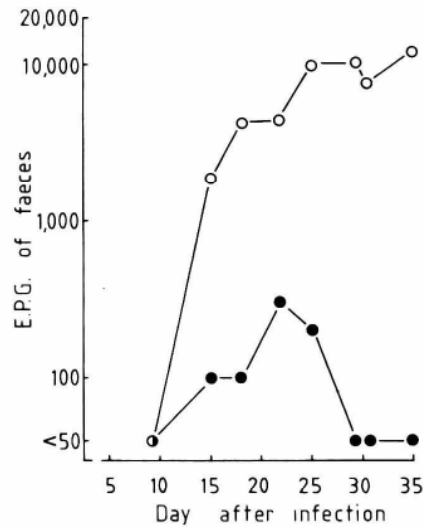
FIG. 3.   Faecal egg counts from groups of jirds (●) and mice (○) infected with two L3 every weekday for four weeks.

1960; JENKINS, 1977). The experiments reported in this paper suggest otherwise. Thus small numbers of *N. dubius* completed development and emerged into the gut lumen in immune jirds. 12·5% of the control worm burden was recovered from immune animals on day 11, but these worms were rapidly eliminated and very few remained on day 15 (Fig.1 a). These results suggest that challenge infection larvae establish in the gut, penetrate the intestinal mucosa and may be trapped in the muscularis externa as in the mouse. The worms which complete development and return to the intestinal lumen are rapidly expelled, explaining why so few worms are found at autopsy on any one occasion during the second week of a challenge infection.

It was found that *N. dubius* grew more slowly in jirds than in mice (Fig. 1 b). JENKINS (1977) showed that 21-day-old mouse and jird worms were identical in size so although initially *N. dubius* grows more slowly in jirds than in mice, the worms eventually attain a size which is comparable to that of parasites developing in the normal host. The worms recovered from immune jirds were very small, even in comparison to worms from mice which had undergone a similar immunizing infection. Many of these worms were in fact 3rd and 4th-stage larvae, although adult worms were the more numerous.

Our experiments demonstrated that when jirds were exposed to a larval infection in which the adult worms were removed by treatment with pyrantel during the period of their re-emergence, the animals were still very resistant to a challenge infection. There was some indication that immunity was weaker in those animals in comparison to other jirds which had undergone a longer primary infection, but nevertheless this result established that larvae in addition to adults stimulate strong acquired immunity. This contrasts with the situation in mice where larvae but not adults are believed to be immunogenic (JACOBSON *et al.*, 1982).

Perhaps the most remarkable finding in the work reported in this paper was that jirds given only five larvae, a level of infection which gave $1·7 \pm 0·3$ worms in mice, were strongly resistant to challenge infection (Table I). On the basis of this result it can be concluded that one or two worms are all that is required to elicit acquired immunity to *N. dubius* in the jird. Whilst it has been shown that female *Nippostrongylus brasiliensis* are equally immunogenic in rats (OGILVIE, 1965), only one to ten worms inducing strong protective immunity (OGILVIE & JONES, 1971), the present results are particular-

ly interesting because they contrast with the apparent failure of patent infections of
N. dubius to elicit immunity in mice. It is perhaps not surprising, therefore, that the
administration of multiple small daily infections of *N. dubius* to jirds (Fig. 3) did not
extend the duration of infection, as has been described for *N. brasiliensis* in four to six-
week-old rats (JENKINS & PHILLIPSON, 1971, 1972; JENKINS, 1974). The use of mature
animals, combined with the strong immunogenicity of *N. dubius* in jirds prevented all
but a transient survival of trickle infections. A similar explanation has been proposed
for the failure of repeated low-level infection of *Trichuris muris* to build up patent
populations of worms in the mouse (WAKELIN, 1973; BEHNKE & WAKELIN, 1973).

The present demonstration that *N. dubius* is very immunogenic in the jird raises
interesting points regarding the failure of this parasite to show comparable immuno-
genicity in the mouse. Clearly the parasite does have antigens both in its larval and
adult stages which can elicit a primary and secondary host protective response in the
rodent intestine. It is, however, possible that these antigens resemble murine antigens
thus reducing their immunogenicity in the mouse, or that they are unavailable to the
host because of the position of the adult worm in the intestinal lumen (JACOBSON *et al.*,
1982). This, however, is thought to be unlikely by the present authors. Recent work has
demonstrated that mice given a single primary infection which is abbreviated by
anthelmintic 9 to 14 days after infection are as immune to reinfection as mice subjected
to multiple immunizing infections or to irradiated larvae (JACOBSON *et al.*, 1982;
ROBINSON & BEHNKE, 1983). Therefore, in the absence of adult worms the normal larval
stages of *N. dubius* are highly immunogenic in responder mouse strains. Furthermore, it
is now known that the adult worms interfere with homologous anti-larval immunity in
the mouse. It is therefore possible that the production of IMF by the adult worms,
masks or interferes with the immunogenicity of this stage in the normal host (BEHNKE
et al., 1983). This mechanism fails to operate successfully in the jird, or indeed in any
other laboratory rodent host, leaving the parasite exposed to the efferent arm of the
immune response (CROSS, 1960; CROSS & DUFFY, 1963, HANNAH, 1983). These findings
are extremely encouraging and bode well for the use of the jird as an experimental
animal in which to isolate and characterize the functional antigens of *N. dubius*. Indeed,
preliminary experiments have shown that jirds can be vaccinated by adult worm
homogenates (HANNAH, 1983). The remarkable sensitivity of the jird to infection with
N. dubius should enable small quantities of parasite-derived material to be screened for
immunogenicity in this host.

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