Journal of Helminthology (1988) **62**, 221–231

Immunity to adult *Heligmosomoides polygyrus (Nematospiroides dubius)*: survival or rejection of adult worms following transplantation to mice refractory to larval challenge

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ABSTRACT

Experiments were carried out to explore the survival of 14-day adult *H. polygyrus* following transplantation to mice of four strains, immunized by various protocols. Adult worm establishment and survival was unimpaired in CFLP mice which were totally refractory to larval challenge. Transplanted adult worms were also successful in NIH mice immunized by the 9-day abbreviated infection regime. However, NIH mice exposed to irradiated larvae or subjected to the divided primary infection, expelled transplanted adults. The 9-day abbreviated infection was further examined in SJL and (C_{57} Bl_{10} X NIH) F_1 mice which expel adult worms during a primary infection and although this regime was unsuccessful in causing NIH mice to reject adult worms, expulsion of adult worms was accelerated in SJL and F_1 mice. The survival of adult *H. polygyrus* was discussed in the context of stage-specific immunity and the delicate balance between the immunogenic stimuli from developing larvae, the immunomodulatory activities of adult stages and the host's genetically determined capacity to respond to these opposing signals.

KEY WORDS: *Heligmosomoides polygyrus*, *Nematospiroides dubius*, intestinal immunity, immunomodulation, mice, immunization

INTRODUCTION

Heligmosomoides polygyrus is a long-lived parasite in most laboratory strains of mice and is known to survive for 8–10 months following a single oral administration of infective larvae (EHRENFORD, 1954; KEYMER & HIORNS, 1986; BEHNKE *et al.* 1987). The survival of the parasite is dependent on a delicate balance between the immunogenic stimuli from developing larvae, the immunomodulatory activities of adult worms (BEHNKE, 1987) and the host's genetically determined capacity to respond to these opposing signals (BEHNKE & ROBINSON, 1985). BARTLETT & BALL (1974) concluded that *H. polygyrus* differed from most other intestinal nematodes in that the fecundity and survival of adult worms seemed to be completely unaffected by acquired resistance, the latter being considered to act only against the larvae, leaving the adult worms totally unimpaired. This remarkable ability of adult *H. polygyrus* to live in mice totally refractory to larval infections was reinforced by JACOBSON *et al.* (1982) who demonstrated that immune LAF/J mice, totally resistant to larval challenge, tolerated transplanted adult worms for 2 weeks following implantation.

Despite these studies, evidence is available that adult *H. polygyrus* are not totally resistant to immune responses in the intestine and that the host's genotype is an important factor in this equation. Some mouse strains have been shown to expel adult worms prematurely (LAF/J-CYPESS *et al.*, 1977; SJL-MITCHELL *et al.*, 1982).

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Furthermore, experiments have been reported in which effective anti-adult worm responses have been initiated in mice by appropriate laboratory manipulation. Balb/c mice immunized by intraperitoneal injections of adult worms, when challenged orally by larvae, expelled the mature parasites within 20 days of infection (DAY *et al.*, 1979). Even the weak responder strain C_{57} Bl_{10} was induced to reject adult worms by implantation of mature parasites i.p. and concurrent administration of the adjuvant, pertussigen (MITCHELL & MUNOZ, 1983; MITCHELL & CRUISE, 1984).

These studies showed that although adult worms caused chronic primary infections in many widely used laboratory strains of mice and could survive in animals totally resistant to larval infection, the parasites were still susceptible to host effector mechanisms. In the experiments reported in this paper, we began by re-examining the effectiveness if i.p. implanted adult worms in eliciting responses against adult worm challenge. Adult worms were found to survive in CFLP mice, irrespective of the immunizing protocol used even when the mice were resistant to larvae. It was then necessary to compare the efficacy of other immunizing regimes, with well established properties of inducing strong acquired resistance to *H. polygyrus* in mice and to determine the influence of host genotype on the survival of transplanted adult worms in mice immunized by these procedures.

MATERIALS AND METHODS

Animals

Four strains of female mice were used in the present study. Randomly bred CFLP, syngeneic NIH and (C_{57} Bl_{10} X NIH)F_1 were all bred in the departmental animal house. SJL mice were purchased from Harlan Olac Ltd., Bicester, Oxon. All the animals were maintained under conventional animal house conditions with access to food and water *ad libitum*.

Parasite

The methods used to maintain *H. polygyrus*, infect mice and recover worms at autopsy have all been described previously (JENKINS & BEHNKE, 1977). Faecal egg counts were monitored as reported by BEHNKE & PARISH (1979). A detailed description of the methods used to transplant adult worms was given by BEHNKE *et al.* (1983). Worms for transplantation were obtained from CFLP mice infected with 250–400 L_3 (third-stage larvae), 14 days earlier.

Immunization of mice

Several different methods were used to immunize mice to larval or adult stages of *H. polygyrus*. Most of these have been described previously but briefly the following procedures were used:

Experiments 1–3. Adult worms were isolated, washed three times in warm sterile saline and injected into the peritoneal cavity of mice in 0·5 ml of sterile saline using a sharp 18 gauge hypodermic needle. Larvae were cleaned by repeated washing in saline and were injected in 0·5 ml of sterile saline through a 21-gauge needle. Both larvae and adult worms were killed prior to injection by freezing to −40°C and thawing, twice in rapid succession.

Experiment 4. The divided primary infection protocol adopted was described by BEHNKE & WAKELIN (1977). Mice were given 100 L_3 larvae on days 0, 2, 4, 7, 9, 11

and were treated with pyrantel embonate (Strongid–P paste – Pfizer) on days 15, 22, 29, 35 and 42. The animals were challenged on day 51. This protocol allows the overlapping development of larval stages, prolonging larval exposure in the intestine and limits adult worm populations by the anthelmintic treatments administered from day 15 onwards. It is extremely effective at inducing acquired resistance to larval challenge.

Experiments 5, 8 and 9. The 9-day abbreviated infection regime used was described by BEHNKE & ROBINSON (1985). Mice were given a single dose of L_3 on day 0 and were treated with pyrantel on days 9, 10, 14 and 21. Challenge infections were administered a week later. This protocol allows the synchronized normal development of L_3 and L_4 but the anthelmintic treatment from day 9 onwards removes the adult worms from the gut lumen as they emerge from the submucosa without affecting parasites which are still developing in the tissues. This regime has been found to be very effective in discriminating between weak and strong responder mouse strains.

Experiments 6, 7 and 9. Immunization by irradiated larvae was carried out as described by BEHNKE *et al.* (1980) and HAGAN *et al.* (1981). Infective larvae were exposed to 25 krad of gamma radiation from a Cobalt 60 source. Mice were given 250 L_3 on day 0 and were challenged on day 21 (Experiment 6), day 24 (Experiment 7) or day 28 (Experiment 9). This protocol does not permit the development of adult parasites but the L_3 and L_4 stages are believed to persist as arrested forms in the intestinal tissues (ALI & BEHNKE, 1985).

Statistical analysis of results

Faecal egg counts are presented as the mean value of 4 counts on each 1 sample of faeces. Worm counts are given as the mean worm burden ±S.E.M. When applicable the non-parametric Mann-Whitney U test was used to compare groups for significant difference (SOKAL & ROHLF, 1969) and a value of $P<0{\cdot}05$ was considered to represent a significant difference.

RESULTS

Survival of H. polygyrus *in CFLP mice immunized by 3 intraperitoneal injections of adult worms or 3 doses of L_3 stages*

Initially, several exploratory experiments were carried out in CFLP mice in order to determine whether i.p. injections of adult worms would sensitize mice and enable them to reject a challenge infection with transplanted adult worms. These preliminary studies confirmed that adult *H. polygyrus* could survive in mice resistant to larval challenge. Table I summarizes the results from three such experiments in which the treatments used to induce resistance were 50 adult worms injected i.p. on days −25, −23 and −21, 500 L_3 injected on the same days or 200 L_3 given orally, again on days −25, −23 and −21. All the mice, together with a naive control group, were treated with pyrantel 14 and 7 days before challenge to eliminate worms which had established in the intestine and the animals were challenged either with 50 adult worms by laparotomy or 200 L_3 orally.

Adult worms survived without loss for 56 days in mice which showed varying levels of resistance to larval challenge (Table I). When 3 doses of 50 adult worms injected i.p. were used, up to 28·5% protection from larval challenge was observed (Experiment 3, groups BvD). Mice immunized by 3 doses of 500 L_3 given i.p. or

TABLE I. Survival of adult *H. polygyrus* in CFLP mice immunized by 3 different protocols.

Experiment	Group (A)	Treatment (B)	No. mice	Challenge Infection					
				Route (C)	Mean No. of worms recovered ±S.E.M. Day 14	Day 56	% Reduction relative to control group		P
1	A	None	3	50 adults	33·3±4·9				
	B	50 adult worms	5	50 adults	30·6±3·1		8·1		NS
	C	500 L_3 i.p.	5	50 adults	35·4±4·7		0		NS
	D	200 L_3 orally	2	50 adults	35·5		0		NS
2	A	None	4	50 adults		32·5±8·4			
	B	50 adult worms	5	50 adults		39·8±0·6	0		NS
	C	500 L_3 i.p.	4	50 adults		31·8±3·2	2·2		NS
	D	200 L_3 orally	1	50 adults		29·0	10·8		NS
3	A	None	6	200 L_3	159·8±11·5				
	B	None	6	200 L_3		128·0±4·5		AvB	0·032
	C	50 adult worms	5	200 L_3	166·2±10·3		0	AvC	NS
	D	50 adult worms	6	200 L_3		91·5±1·8	28·5	BvD	0·001
	E	500 L_3 i.p.	6	200 L_3	120·5±9·8		24·6	AvE	NS(0·066)
	F	500 L_3 i.p.	6	200 L_3		67·0±3·9	47·7	BvF	0·001
	G	200 L_3 orally	6	200 L_3	89·3±5·7		44·1	AvG	0·002
	H	200 L_3 orally	6	200 L_3		48·0±9·4	62·5	BvH	0·001
								CvD	0·002
								EvF	0·001
								GvH	0·008

(A) All the mice were female CFLP.
(B) Each treatment was repeated on day -25, -23, -21 before challenge and then all the mice, including the control groups were given pyrantel on day -14 and -7.
(C) Adult worms were inserted into the duodenum by laparotomy; L_3 were given orally.

200 L_3 given orally showed respectively 47·7% (Experiment 3, groups BvF) and 62·5% (Experiment 3, groups BvH) reduction in parasite survival to day 56. Curiously, whilst these treatments failed to cause the rejection of transplanted adult worms, adult stages of *H. polygyrus* developing from the challenge inoculum were rejected more rapidly than in naive control mice. Thus in Experiment 3, mice immunized by 50 adult worms, 500 L_3 i.p. and 200 L_3 orally sustained respectively a further 45%, 44% and 46% reduction in worm burden between day 14 and 56. In contrast, naive control mice lost only 19·9% of their worm burden over the same period (group AvB).

Survival and rejection of H. polygyrus *in NIH and CFLP mice immunized by 3 different immunizing protocols*

The preceding experiments established that transplanted adult worms could indeed survive in CFLP mice immunized by treatments which elicited varying degrees of resistance to larval challenge. However, adult worms which survived the tissue phase of infection in immunized mice were less successful at avoiding expulsion since some were rejected within 8 weeks of infection. Thus there appeared to be an intrinsic difference in the capacity of these distinct adult worm populations to overcome the host's intestinal immune response. In an effort to explore further the resistance of transplanted adult worms to host effector mechanisms, a series of experiments was carried out using three further immunizing protocols, all known to stimulate strong acquired resistance to a challenge infection with *H. polygyrus*. Three of these experiments were carried out using NIH mice, a strain considered a strong responder to this parasite. In each experiment naive mice and mice immunized by the relevant immunizing protocol were challenged either by transplanted adult worms or by infective larvae given orally. The course of infection was then followed by faecal egg counts and parasite survival was assessed by worm recovery. The results are summarized in Table II.

As can be seen, all three procedures were highly immunogenic in NIH mice and produced respectively 98·1, 93·8, and 98·9% protection against larval challenge. The protocols differed, however, with respect to their efficacy in mediating resistance to challenge by transplanted adult worms. Two protocols, irradiated larvae (Experiment 7) and the divided primary infection (Experiment 4) caused 80·2 and 97·4% loss of adult worms respectively. Faecal egg counts (Fig. 1) showed that in Experiment 7, adult worms established in mice immunized by irradiated larvae, initially producing high egg counts, but egg production dropped promptly and stayed depressed suggesting that many of the adult worms had been expelled within 2 weeks of transplantation. In control mice (Experiment 7, group A) faecal egg counts were more steady and the parasites survived for 6 weeks post transplantation. Faecal egg count data is not shown for Experiment 4, but no eggs were detected in the faeces of group B mice (immunized by the divided primary infection) on days 13, 20 and 27, whereas the naive mice receiving adult worms by laparotomy (group A) had counts of 800, 1200 and 1100 on these days.

In contrast to NIH mice, CFLP mice were unable to reject transplanted adult worms when immunized by irradiated larvae (Experiment 6) despite the fact that the same protocol rendered CFLP mice almost totally refractory to larval challenge (95·9% protection). Furthermore, NIH mice were unable to reject adult worms when immunized by the 9-day abbreviated infection regime (Experiment 5). Again the latter protocol was shown to be highly immunogenic and elicited 93·8% protection against larval challenge. Indeed, in order to allow for a possible delayed response to transplanted adult worms, this experiment was continued for 10 weeks,

TABLE II. Survival or rejection of adult *H. polygyrus* in NIH or CFLP mice immunized by 3 different protocols.

						Challenge Infection				
Experiment	Group	Strain of mouse	Immunizing protocol (A)	Treatment	No. mice	Parasite stage (B)	Days after challenge when killed	Mean no. worms recovered ±S.E.M.	% Reduction relative to control group	P
4	A	NIH	Divided primary infection	Naive	5	50 Adults	35	26·6±6·7		
	B	NIH		Immunized	6	50 Adults	35	0·7±0·5	97·4	0·002
	C	NIH		Naive	6	150 L_3	35	93·2±9·5		
	D	NIH		Immunized	6	150 L_3	35	1·8±1·6	98·1	0·001
5	A	NIH	9-day abbreviated primary infection	Naive	6	50 Adults	70	25·8±2·8		
	B	NIH		Immunized	5	50 Adults	70	28·4±2·3	0	NS
	C	NIH		Naive	6	50 L_3	70	45·0±10·4		
	D	NIH		Immunized	6	50 L_3	70	2·8±1·1	93·8	0·001
6	A	CFLP	Irradiated larvae	Naive	5	50 Adults	35	45·6±4·4		
	B	CFLP		Immunized	4	50 Adults	35	30·8±8·2	32·5	NS
	C	CFLP		Naive	5	250 L_3	35	204·4±16·8		
	D	CFLP		Immunized	3	250 L_3	35	8·3±3·5	95·9	0·018
7	A	NIH	Irradiated larvae	Naive	3	50 Adults	42	35·3±4·6		
	B	NIH		Immunized	5	50 Adults	42	7·0±6·3	80·2	0·036
	C	NIH		Naive	5	200 L_3	42	150·8±32·7		
	D	NIH		Immunized	6	200 L_3	42	0·8±0·5	98·9	0·002

(A) The immunizing protocol is described in Materials and Methods. All the mice were treated with pyrantel before challenge infection.
(B) Adult worms were transplanted into the duodenum by laparotomy. L_3 were given orally.

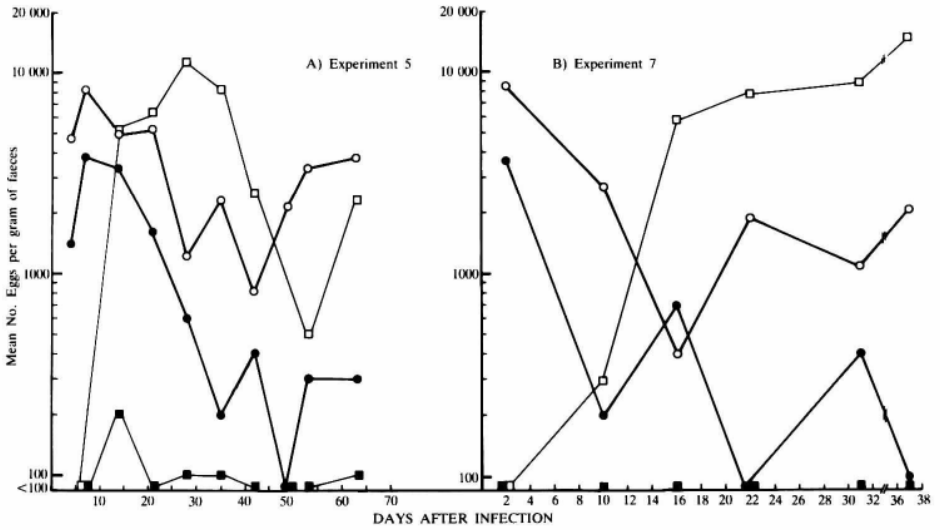

FIG. 1. Faecal egg counts in groups of naive (open symbols, ○, □) and immune (filled in symbols, ●, ■), NIH mice infected by L_3 (squares, □, ■) or receiving transplanted adult worms (circles, ○, ●). A—Experiment 5—mice immunized by the 9-day abbreviated primary infection. B—Experiment 7 —mice immunized by irradiated larvae.

but at autopsy worm counts were not significantly different in groups A and B. Nevertheless faecal egg counts in group B were depressed relative to group A throughout the period of observation and were very low in the 6–10 weeks of infection suggesting that despite the hosts failure to reject the worms, an anti-parasite response was initiated.

Rejection of adult H. polygyrus *from (C_{57} Bl_{10} X NIH) F_1 hybrid and SJL mice.*

Recent studies have shown that three strains of mice expel adult *H. polygyrus* developing from orally administered L_3 within 10 weeks of primary infection (Robinson *et al.*, in press). In (C_{57} Bl_{10} X NIH)F_1 hybrids this is a dose-dependent phenomenon, low intensity infections being expelled earlier than high intensity infections. SJL mice, however, expel *H. polygyrus* in a dose-independent fashion usually within 7 weeks of larval administration. In the final section of this paper, we report the results of experiments carried out to determine whether the immunizing protocol, which failed to elicit resistance to transplanted adult worms in NIH, would accelerate worm rejection by these two strains of mice.

Experiment 8 was designed along the lines of Experiment 5. Thus 27 F_1 mice were arranged into 4 groups A(n=4), B(n=6), C(n=9) and D(n=8) which were treated identically to the mice in Experiment 5, except that groups C and D were challenged by 150 L_3 larvae and not 50. This experiment was monitored by faecal egg counts as shown in Fig. 2 and the mice were finally killed for worm counts on day 56 post challenge. Naive F_1 mice receiving transplanted adult worms (Group A), produced eggs in their faeces throughout the period of observation, but the egg counts declined towards week 5 and at autopsy 14·8±7·4 worms were recovered. Two of the mice still had high parasite numbers, the remainder had lost the majority of worms. In contrast immunized mice receiving transplanted adult worms (Group B) had rejected all the parasites by day 56 but the worms had established following transplantation as eggs were detected in the faeces from day 4 to 39. Thus

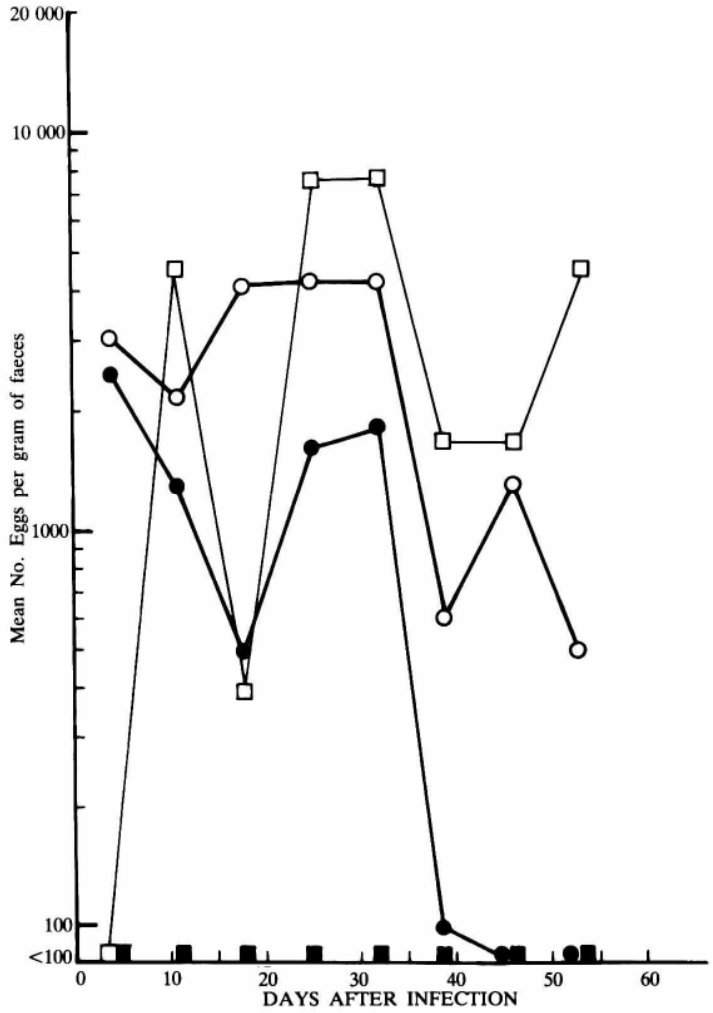

FIG. 2. Faecal egg counts in groups of naive (open symbols, ○, □) and immune (filled in symbols, ●, ■) (C_{57} Bl_{10} X NIH)F_1 hybrid mice infected by L_3 (squares, □, ■) or receiving transplanted adult worms (circles, ○, ●). The immune groups were immunized by the 9-day abbreviated primary infection.

F_1 mice immunized by the 9-day abbreviated infection protocol showed enhanced resistance to adult parasites which were rejected prematurely in relation to naive controls. No eggs were detected at any time in the faeces of immunized F_1 mice challenged with L_3 (group D) and no parasites were recovered at autopsy. Group C which comprised naive F_1 mice given L_3 orally produced eggs throughout the experiment and at autopsy 28·2±15·5 worms were recovered but the worm burdens were variable and 4 of the 9 mice had expelled the parasites before day 56. Thus in this group also, expulsion of adult worms (developing from L_3 larvae) had been initiated, some mice shedding their worm burden before the termination of the experiment.

In the final experiment SJL mice were used. One group was designated the naive control group (A) and the other two groups were immunized, one by the abbreviated infection protocol (B) and the other by irradiated larvae (C). All three

groups received transplanted adult worms and the course of infection was monitored by faecal egg counts. Mice in group C ceased producing eggs on day 29, those in group B on day 44, whilst eggs were detected in group A until day 57. At autopsy, the majority of mice were totally without worms.

DISCUSSION

H. polygyrus is clearly a remarkable parasite whose adult stages survive in situations where larvae cannot form patent infections. Our results have drawn attention again to this property of adult *H. polygyrus* and we have extended previous studies by exploring the survival of adult worms in mouse strains which have the capacity to expel primary infections with this organism.

One interpretation of the results from our experiments could be that they reflect stage-specific resistance e.g. resistance induced by larvae only acting on larval and not adult parasites. Indeed, *H. polygyrus* expresses stage-specific antigens (PRITCHARD *et al.*, 1984) and in Experiment 3, mice immunized by adult worms did not express anti-larval resistance, but subsequently rejected adult worms developing from the challenge inoculum. However, the same protocol did not accelerate the rejection of transplanted adult worms and although this may seem paradoxical, there are reasons for believing that the two adult worm populations may be phenotypically distinct. Although no biochemical, enzymatic or antigenic differences have been reported, parasites developing in partially resistant hosts are stunted and less fecund than normal (BARTLETT & BALL, 1974; DOBSON, 1982; SITEPU & DOBSON, 1982) and by analogy with *Nippostrongylus brasiliensis* might be expected to show other differences (OGILVIE & JONES, 1973). Such worms may already be partially damaged as a consequence of developing in immune animals and hence may be more susceptible to host effector mechanisms.

However, stage-specific immunity is not an explanation which is entirely compatible with our data. Immunization with irradiated larvae induced strong resistance to larval challenge in both CFLP and NIH mice (Experiments 6 & 7, Table II) but allowed transplanted parasites to establish and survive in the former strain but not in the latter. Larvae subjected to 25 krad of irradiation do not develop into mature worms (BEHNKE *et al.*, 1980) and in consequence the mice would have been exposed only to prolonged stimulation by L_3 and L_4 antigens. Both strains, therefore, had comparable experience of larval antigens, but only one acquired an anti-adult worm response.

It is also pertinent that NIH mice were unable to expel transplanted adult worms when immunized by the 9-day abbreviated infection. Again, one explanation may be that the 9-day abbreviated protocol elicited anti-larval stage-specific immunity and for this reason we explored the effects of this immunizing protocol in two strains of mice which rank as stronger responders than NIH mice ($(C_{57}\ Bl_{10}$ X NIH)F_1 and SJL strains; BEHNKE & ROBINSON, 1985). In both strains, the protocol accelerated the rejection of transplanted adult worms and we conclude that stage-specific immunity was unlikely to have been involved, unless there were genetically determined qualitative differences between the strains in recognizing and responding to the inducing signals from the inoculum. Mice exposed to the 9-day abbreviated infection would have experienced only larval stages of *H. polygyrus*, but would not have been subjected to such prolonged stimulation as mice exposed to the irradiated larvae (ALI & BEHNKE, 1985). Irradiated larvae stimulate strong resistance to challenge in both NIH and $C_{57}\ B_{10}$ mice (the latter is a weak responder strain to *H. polygyrus*) but the 9-day abbreviated infection is only effective in the former (BEHNKE & ROBINSON, 1985).

Therefore, the explanation for the different efficacies of the two immunizing protocols in inducing anti-adult worm responses in NIH mice may reside in the varying intensity of stimulation which they provide.

An alternate explanation for our results may lie in considering the host's response to the immunomodulatory mechanisms used by adult worms for evading immunity (reviewed BEHNKE, 1987). CFLP mice may have been more severely compromised by the immunodulatory effects of adult parasites than NIH mice and hence worms were able to survive only in immunized individuals of the former strain. There is further evidence in support of this suggestion. Thus adult worms can readily be shown to depress homologous immunity in CFLP mice immunized by irradiated larvae (BEHNKE *et al.*, 1983) and similar results have been found in C_{57} Bl_{10} mice, but we have been unable to repeat these observations in NIH mice. In one experiment, 104 adult *H. polygyrus* resulted in 12% reduction of immunity elicited by the irradiated vaccine in NIH mice, whereas in C_{57} Bl_{10} only 13 adult worms caused 84% depression (Crawford, Behnke & Pritchard, unpublished observations).

It is quite clear from our results and those of others (JACOBSON *et al.*, 1982) that under a variety of circumstances adult *H. polygyrus* can evade host protective responses, but the parasites are not always successful. An important additional factor, not investigated previously in this context, appears to be the host genotype. Mouse strains such as SJL, (C_{57} Bl_{10} X NIH)F_1 and LAF_1/J have the capacity to expel adult worms and if immunomodulation is an important component of the parasite's evasive strategies, it follows that these strains must benefit from a genetically determined insusceptibility to parasite mediated immunomodulation. The latter is an attractive, testable but as yet unproven hypothesis.

It is interesting to note here that, the expulsion of primary infection adult worms from SJL mice is not accompanied by an intestinal mast cell response, such as that mounted by mice of this strain to *Trichinella spiralis* or secondary infections with *H. polygyrus* (DEHLAWI *et al.*, 1987). SJL mice can therefore respond with a mastocytosis but fail to do so during a primary infection, presumably because the response is depressed by adult worms. In other words, *H. polygyrus* are partially successful in immunomodulating in SJL mice, in so far as the mast cell response is incapacitated, but the fact that the worms are lost implies that other component processes of the overall response in the intestine are less severely affected. These observations have not yet been extended to encompass other mouse strains which expel adult worms but such experiments are currently in progress. The key to understanding the survival strategy of *H. polygyrus* and hopefully other long-lived nematode parasites, will be to identify qualitative differences in response phenotype associated with survival or loss of adult parasites and to link these to genetic differences between the mouse strains.

ACKNOWLEDGEMENTS

We would like to thank Professor D. Wakelin and P. N. R. Usherwood for the provision of facilities for this study in the Zoology Department of Nottingham University. The work was supported by the MRC through project grant G8100159T and G8328675T to JMB. DJLW held a postgraduate studentship from the MRC and MR from the SERC, for which we are grateful. We would like to express our gratitude to K. Cosgrove who supervised the maintenance of our experimental animals.

REFERENCES

ALI, N. M. H. & BEHNKE, J. M. (1985) Observations on the gross changes in the secondary lymphoid organs of mice infected with *Nematospiroides dubius*. *Journal of Helminthology*, **59**, 167–174.

BARTLETT, A. & BALL, P. A. J. (1974) The immune response of the mouse to larvae and adults of *Nematospiroides dubius*. *International Journal for Parasitology*, **4**, 463–470.

BEHNKE, J. M. (1987) Evasion of immunity by nematode parasites causing chronic infections. *Advances in Parasitology*, **26**, 1–71.

BEHNKE, J. M., HANNAH, J. & PRITCHARD, D. I. (1983) *Nematospiroides dubius* in the mouse: evidence that adult worms depress the expression of homologous immunity. *Parasite Immunology*, **5**, 397–408.

BEHNKE, J. M. & PARISH, H. A. (1979) Expulsion of *Nematospiroides dubius* from the intestine of mice treated with immune serum. *Parasite Immunology*, **1**, 13–16.

BEHNKE, J. M., PARISH, H. A. & HAGAN, P. (1980) The effect of gamma irradiation on *Nematospiroides dubius*. Factors affecting the survival of worms in a primary infection in mice. *Journal of Helminthology*, **54**, 173–182.

BEHNKE, J. M. & ROBINSON, M. (1985) Genetic control of immunity to *Nematospiroides dubius;* a 9 day anthelmintic abbreviated immunising regime which separates weak and strong responder strains of mice. *Parasite Immunology*, **7**, 235–253.

BEHNKE, J. M. & WAKELIN, D. (1977) *Nematospiroides dubius:* stimulation of acquired immunity in inbred strains of mice. *Journal of Helminthology*, **51**, 167–176.

BEHNKE, J. M., WILLIAMS, D. J., HANNAH, J. & PRITCHARD, D. I. (1987) Immunological relationships during primary infection with *Heligmosomoides polygyrus (Nematospiroides dubius)*: the capacity of adult worms to survive following transplantation to recipient mice. *Parasitology*, **95**, 569–581.

CYPESS, R. H., LUCIA, H. L., ZIDIAN, J. L. & RIVERA-ORTEZ, C. I. (1977) *Heligmosomoides polygyrus*: temporal, spatial and morphological population characteristics in LAF_1/J mice. *Experimental Parasitology*, **42**, 34–43.

DAY, K. P., HOWARD, R. J., PROWSE, S. J., CHAPMAN, C. B. & MITCHELL, G. F. (1979) Studies on chronic versus transient intestinal nematode infections in mice. 1. A comparison of responses to excretory/secretory (ES) products of *Nippostronglyus brasiliensis* and *Nematospiroides dubius* worms. *Parasite Immunology*, **1**, 217–239.

DEHLAWI, M. S., WAKELIN, D. & BEHNKE, J. M. (1987) Suppression of mucosal mastocytosis by infection with the intestinal nematode *Nematospiroides dubius*. *Parasite Immunology*, **9**, 187–199.

DOBSON, C. (1982) Passive transfer of immunity with serum in mice infected with *Nematospiroides dubius*: influence of quality and quantity of immune serum. *International Journal for Parasitology*, **12**, 207–213.

EHRENFORD, F. A. (1954) The life cycle of *Nematospiroides dubius* Baylis (Nematoda; Heligmosomidae) *Journal of Parasitology*, **40**, 480–481.

HAGAN, P., BEHNKE, J. M. & PARISH, H. A. (1981) Stimulation of immunity to *Nematospiroides dubius* in mice using larvae attenuated by cobalt 60 irradiation. *Parasite Immunology*, **3**, 149–156.

JACOBSON, R. H., BROOKS, B. O. & CYPRESS, R. H. (1982) Immunity to *Nemotospiroides dubius*: parasite stages responsible for and subject to resistance in high responder (LAF_1/JAF) mice. *Journal of Parasitology*, **68**, 1053–1058.

JENKINS, S. N. & BEHNKE, J. M. (1977) Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*. *Parasitology*, **75**, 71–78.

KEYMER, A. E. & HIORNS, R. W. (1986) *Heligmosomoides polygyrus* (Nematoda): the dynamics of primary and repeated infection in outbred mice. *Proceedings of the Royal Society of London*, **B29**, 47–67.

MITCHELL, G. F., ANDERS, R. F., BROWN, G. V., HANDMAN, E., ROBERTS-THOMSON, I. C., CHAPMAN, C. B., FORSYTH, K. P., KAHL, L. P. & CRUISE, K. M. (1982) Analysis of infection characteristics and antiparasite immune responses in resistant compared with susceptible hosts. *Immunological Reviews*, **61**, 137–188.

MITCHELL, G. F. & CRUISE, K. M. (1984) Immunization with *Nematospiroides dubius* adult worms plus pertussigen has different consequences in mice of various genotypes. *Australian Journal of Experimental Biology and Medical Science*, **62**, 523–530.

MITCHELL, G. F. & MUNOZ, J. J. (1983) Vaccination of genetically susceptible mice against chronic infection with *Nematospiroides dubius* using pertussigen as adjuvant. *Australian Journal of Experimental Biology and Medical Science*, **61**, 425–434.

OGILVIE, B. M. & JONES, V. E. (1973) Immunity in the parasitic relationship between helminths and hosts. *Progress in Allergy*, **17**, 93–144.

PRITCHARD, D. I., MAIZELS, R. M., BEHNKE, J. M. & APPLEBY, P. (1984) Stage-specific antigens of *Nematospiroides dubius*. *Immunology*, **53**, 325–335.

ROBINSON, M., WAHID, F. N., BEHNKE, J. M. & GILBERT, F. S. Immunological relationships during primary infection with *Heligmosomoides polygyrus (Nematospiroides dubius)* = dose-dependent expulsion of adult worms. *Parasitology*, in press.

SITEPU, P. & DOBSON, C. (1982) Genetic control of resistance to infection with *Nematospiroides dubius* in mice: selection of high and low immune response populations of mice. *Parasitology*, **85**, 73–84.

SOKAL, R. R. & ROHLF, F. J. (1969) *Biometry*. San Francisco: Freeman.

Accepted 12th April, 1988.