Parasite Immunology 1979, **1**, 13–26

Expulsion of *Nematospiroides dubius* from the intestine of mice treated with immune serum

J.M.BEHNKE & HEATHER A.PARISH

Department of Zoology, University of Nottingham, University Park, Nottingham

Accepted for publication 11 October 1978

Summary This paper describes experiments which demonstrated that the survival of *Nematospiroides dubius* was severely impaired in mice treated with immune serum. CFLP donor mice were given a series of infections ranging from 25 to 200 infective larvae, at weekly intervals for 6 weeks. The mice were treated with anthelmintic on day 21 and/or day 28 to prevent the accumulation of lethal numbers of parasites in the intestine, and were bled between day 42 and day 49. Female NIH recipient mice were given a total of 2·0–2·5 ml of immune serum i.p., in several separate smaller doses at various times in relation to the day of infection. Between the administration of immune serum begun during the first 4 days of infection and the animals being killed within the next 3 weeks, the mice harboured fewer worms than control animals, the worms were stunted and their fecundity was greatly reduced. Furthermore, these worms were subsequently lost from the intestines of treated mice, during and after the fourth week of infection. These effects on *N. dubius* were not observed when mice were given normal serum nor when immune serum was administered after day 6 of infection. The delayed rejection of adult worms from mice treated with immune serum is of particular significance and suggests that immune serum contained factors which facilitated the expression of a second component in worm expulsion not normally effective in a primary infection. The possible immunological mechanisms underlying these findings are discussed and related to the immunosuppression which *N. dubius* is known to induce in the host.

Keywords: *Nematospiroides dubius*, nematode, mouse, immune expulsion, immune serum

Introduction

The generation of protective immunity to intestinal helminth parasites has been the subject of intensive research in recent years. Attempts have been made to

Correspondence: Dr J.M.Behnke, Department of Zoology, University of Nottingham, University Park, Nottingham, NG7 2RD.

0141-9838/79/0300-0013$02.00

analyse the steps involved in the immune expulsion of parasitic nematodes such as *Nippostrongylus brasiliensis, Trichinella spiralis* and *Trichuris muris*, and there is now substantial evidence that two or more components, involving antibody- and thymus-dependant lymphocytes, are required to act in a precise sequence to bring about worm loss (Ogilvie & Parrott 1977, Wakelin 1978). In contrast, there are many host–parasite relationships in which the parasite is not rejected and consequently survives for a long time following infection (Ogilvie & Wilson 1976). These situations must reflect the failure of either the afferent or the efferant arm of the immune system to function normally, but unfortunately, there is little experimental information available to explain the exact mechanisms involved (Ogilvie & Wilson 1976, Wakelin 1978).

Following infection with the third stage larvae of *Nematospiroides dubius*, the mouse develops a chronic infection of many months duration (Bartlett & Ball 1974). Furthermore, most inbred strains of mice do not readily acquire resistance to secondary infections with this parasite, which frequently, therefore, also become patent (Cypess & Zidian 1975, Behnke & Wakelin 1977). In the inbred NIH mouse, however, a divided primary infection is known to stimulate a high level of immunity, which is manifested as an arrested development of larvae in challenge infections and as the destruction of these larvae while still in the intestinal walls (Behnke 1977, Behnke & Wakelin 1977, Behnke & Parish 1979). The few larvae which complete their histotropic phase of development and return to the gut lumen are rapidly expelled (Behnke & Wakelin 1977).

Whilst several previous workers failed to passively transfer resistance to *N. dubius* with immune serum (Chaicumpa, Jenkin & Rowley 1976, Cypess 1970, Panter 1969), recent preliminary results have shown that NIH mice could be protected in this way (Behnke & Parish 1978). The purpose of the present investigation, therefore, was to determine the timing of the administration of immune serum for maximum effect against the parasite, and to clarify the time course of worm expulsion to enable mice to be autopsied immediately before and after the process of rejection.

Materials and methods

ANIMALS

Immune serum was raised in groups of male and female CFLP mice bred at random and was transferred to inbred female NIH recipients. The mice were bred under conventional animal house conditions in the Zoology Department of Nottingham University.

NEMATOSPIROIDES DUBIUS

The strain of *N. dubius* used in the present study was obtained in 1975 from the

Wellcome Research Laboratories, Beckenham and has since been maintained in outbred CFLP mice. The maintenance of the parasite, and the methods used for infection of animals and recovery of worms have already been described (Behnke & Wakelin 1977, Jenkins & Behnke 1977).

IMMUNIZATION OF SERUM DONORS

Approximately equal groups of male and female mice, comprising a total of 60–80 animals were given 125 larvae of *N. dubius* on days 0, 7, 14, 35 and 42. Anthelmintic was given on day 21 and day 30 to prevent lethal numbers of worms from accumulating in the intestine. The mice were killed and bled between day 42 and day 49. The serum given to recipient mice in Expts 6 and 10 was raised in donor mice according to the following schedule: 25 larvae on day 0, 50 larvae on day 7, 100 larvae on day 14, 125 larvae on day 21 and day 35, and 200 larvae on day 42. Anthelmintic was given on day 28 and the mice were killed and bled on day 45. Normal serum was obtained from CFLP mice of a similar age but never having experienced infection with *N. dubius*. The blood was allowed to clot at room temperature for approximately 1 h and then at 4°C for 4–8 h. Serum was removed after centrifugation at 3000 rpm for 10 min and was stored at −20°C in aliquots corresponding to the total volume of serum to be injected on each occasion.

ANTHELMINTIC

Pyrantel embonate (Strongid-P paste, Pfizer) was used to remove adult *N. dubius* from the infected mice. A dose of 100 mg/kg body weight was administered orally as an aqueous suspension. This dose level is known to be adequate for the removal of all adult worms from the intestinal lumen (Behnke & Wakelin 1977).

FAECAL EGG COUNTS

One gram of faeces was taken from the pooled faeces of all the mice in each group, deposited over the preceding 24 h, and was dispersed in 8 ml of 50% saturated saline. This suspension was washed through a sieve (aperture size 800 μm) with 35% zinc sulphate solution and the eggs were counted after flotation in standard McMaster counting slides, as described by Gordon & Whitlock (1939). The counts were expressed as the number of eggs per gram of whole faeces. The relative fecundity (modified from Dineen & Wagland 1966) was calculated as the mean of the egg counts recorded between day 11 and the last day of the experiment.

DETERMINATION OF THE DRY WEIGHT OF N. DUBIUS

Fifteen male and 15 female worms were separated and dried at 100°C for 24 h in a

glass vial. The worms were weighed and since female worms are much larger than males, the results were expressed as the dry weight of a pair of worms (one male and one female).

STATISTICAL ANALYSIS OF RESULTS

All the results were analysed for significance by the non-parametric Wilcoxon test (Sokal & Rohlf 1969). A value of $P < 0{\cdot}05$ was considered to be significant.

Results

THE EFFECT OF IMMUNE SERUM ON THE SURVIVAL OF N. DUBIUS IN RECIPIENT MICE

Several preliminary experiments were carried out in which groups of female NIH mice were treated with immune serum and infected, together with control groups, with 100 larvae of *N. dubius*. The experimental details and the results of three such experiments (Expts 1–3) are presented in Table 1.

Table 1. The survival of *Nematospiroides dubius* in mice treated with immune serum

		No. of worms recovered on days shown			
		Day 9		Day 37 or 42†	
Experiment	Group*	No. of mice	Mean ± s.d.	No. of mice	Mean ± s.d.
1	A Control	7	107·3 ± 9·9‡		n.d.‡
	B Immune serum	6	75·7 ± 17·3‡		n.d.
2	C Control	6	75·0 ± 12·7	7	98·3 ± 6·5§
	D immune serum	6	68·7 ± 9·1	5	25·2 ± 32·9§
3	E Control	6	99·8 ± 10·7¶**	6	103·2 ± 5·9††‡‡
	F Immune serum	6	73·8 ± 13·9¶§§	6	1·8 ± 1·9††§§
	G Immune serum	6	77·8 ± 12·8**¶¶	6	20·2 ± 33·9‡‡¶¶

* In Expt. 1, group B was given 1 ml of immune serum on days −1, 0, +1, +2, +3, +5 and +6. In Expt. 2, group D was given 0·25 ml of immune serum on day −1 and 0·5 ml on days 0, +1 and +2. In Expt. 3, group F was given 0·5 ml of immune serum on days −1, +1, +3, +5 and +7. Group G was given 1 ml of immune serum on day −1. All the groups were challenged with 100 larvae of *N. dubius* on day 0.

† In Expt. 2 the groups were killed on day 37 whereas in Expt. 3 they were killed on day 42. Paired groups were compared and found to have the following statistical significance:

‡ $P < 0{\cdot}005$, § $P < 0{\cdot}005$, ¶ $P = 0{\cdot}005$, ** $P = 0{\cdot}01$, †† $P < 0{\cdot}005$, ‡‡ $P < 0{\cdot}005$, §§ $P < 0{\cdot}005$, ¶¶ $P < 0{\cdot}05$.

n.d. = Not done.

The data shows that in Expts 1 and 3, fewer worms were recovered from groups given immune serum (experimental groups) than from the respective control groups killed on day 9; in Expt. 2, the difference between the groups killed on day 9 was not significant. Furthermore, although the difference between the mean worm recovery from the mice in group D (Expt. 2) killed on day 9 and day 37 was not significant, four of the five mice killed on the latter day expelled the majority of the worms. Eighty-two worms were recovered from the fifth mouse in this group. Nevertheless, group D had fewer worms than the control group (C) ($P < 0{\cdot}005$), indicating that the immune serum had some effect. Expulsion of worms was clearly demonstrated in Expt. 3 where both groups treated with immune serum (F and G) lost most of the worm burden by day 42. The control group showed no such loss.

THE EFFECT OF TIMING OF ADMINISTRATION OF IMMUNE SERUM ON THE SURVIVAL OF N. DUBIUS IN RECIPIENT MICE

Since the life cycle of *N. dubius* involves a histotropic phase of development during which the larvae are deep in the intestinal submucosa and *muscularis externa*, it was of interest to determine whether this stage of the parasite would be more susceptible to the effects of immune serum than the adult worms which live in the gut lumen. Therefore, in the following three experiments (Expts 4, 5 & 6) groups of mice were infected with *N. dubius* and treated with immune serum at different times in relation to the day of infection (day 0). The experimental design and the results are shown in Table 2. The pattern of daily egg output from Expt. 4 is illustrated in Fig. 1.

It can clearly be seen from the results (Table 2, Fig. 1) that in all three experiments normal serum did not affect the survival of *N. dubius*, nor was there any evidence of a reduction in the fecundity of worms from treated animals. In marked contrast, the survival of *N. dubius* was severely impaired when treatment with immune serum overlapped with the first 4 days of infection. Furthermore, the relative fecundity of worms from such mice was greatly reduced. The lower worm recoveries from groups given immune serum at the optimum time (day -1 to day $+4$) reflect not only the loss of some worms during the tissue phase of development, but also the expulsion of worms which had reached the gut lumen. In Expt. 4, a group of 12 mice was given immune serum on day -1 and day $+1$. Six mice were then killed on day 10 when the mean worm recovery was $57{\cdot}3 \pm 14{\cdot}7$ and the remainder on day 28 when $6{\cdot}5 \pm 4{\cdot}8$ worms were recovered (Table 2), indicating that expulsion occurred between the second and fourth weeks of infection ($P < 0{\cdot}005$).

These results indicate that immune serum only evoked the expulsion of worms when the treatment of recipient mice was initiated within the first 4 days of infection. In Expt. 5, there was a significant reduction in the relative fecundity of worms in the group given immune serum on days $+4$ and $+6$, but the worm

Table 2. The effect of varying the time of administration of immune serum on the survival of *N. dubius* in recipient mice

Treatment*	Volume of serum given (ml)	Days after infection when serum was given	Number of *N. dubius* recovered and the relative fecundity					
			Exp. 4		Exp. 5		Exp. 6	
			Mean worm recovery ± s.d.	Relative fecundity	Mean worm recovery ± s.d.	Relative fecundity	Mean worm recovery ± s.d.	Relative fecundity
None	—	—	100·0 ± 10·6	6513	98·5 ± 13·8	4933	67·9 ± 9·8	6674
Normal serum	1	−1, +1	107·7 ± 12·0	9113	96·0 ± 17·0	6155		
Normal serum	0·5	0, +2, +4, +6, +8					71·3 ± 9·4	6611
Immune serum	1	−1, +1	6·5 ± 4·8†	275†	29·2 ± 34·2†	39†		
Immune serum	0·5	0, +2, +4, +6, +8					2·2 ± 3·1†	7†
Immune serum	1	+2, +4			19·5 ± 38·1†	378†		
Immune serum	0·5	+2, +4, +6, +8, +10					20·5 ± 26·3†	1051†
Immune serum	1	+3, +5	10·8 ± 4·8†	338†				
Immune serum	1	+4, +6			51·5 ± 46·0	406†		
Immune serum	0·5	+4, +6, +8, +10, +12					2·7 ± 2·4†	200†
Immune serum	1	+6, +8			95·8 ± 4·6	2539		
Immune serum	0·5	+6, +8, +10, +12, +14					34·2 ± 22·5†	2756†
Immune serum	1	+7, +9	104·2 ± 12·9	4050†				
Immune serum	1	+8, +10			96·0 ± 14·0	3806		
Immune serum	1	+11, +13	99·7 ± 11·3	8700				

* Control and immune serum was given i.p. to groups of six mice on the days shown and the mice were infected with 100 larvae of *N. dubius* on day 0. All the animals in each experiment were killed on the following days: Expt. 4, day 28; Expt. 5, day 24; Expt. 6, day 36.
† Means significantly lower than the group given normal serum.

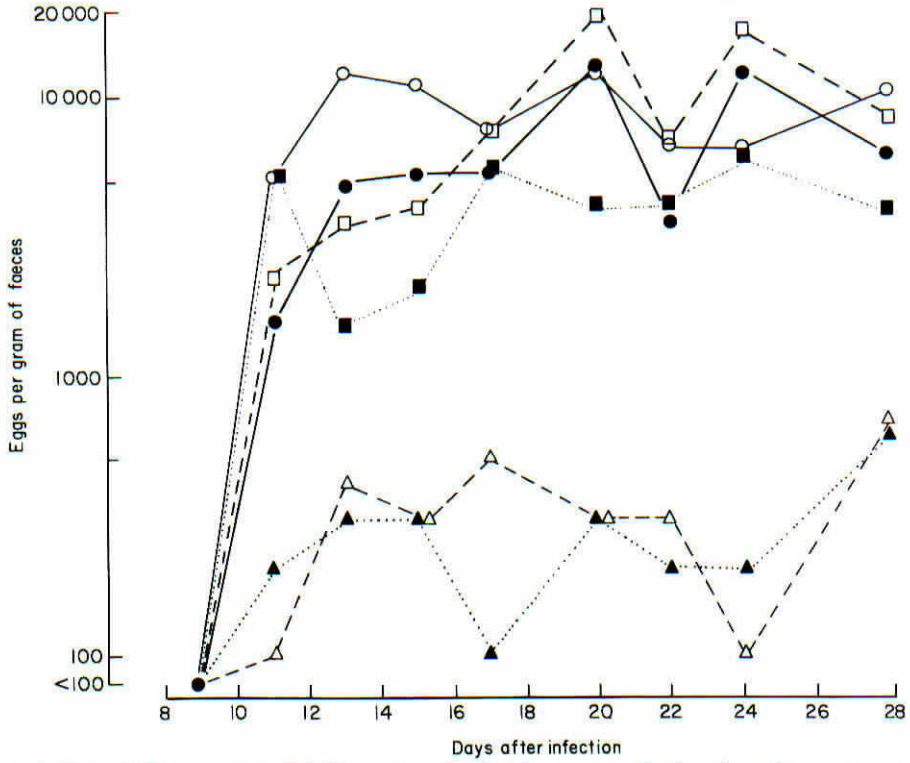

Figure 1. Exp. 4. The pattern of daily egg production in groups of mice given immune serum at different times after infection with 100 larvae of *Nematospiroides dubius*. (●) No treatment; (○) normal serum on day − 1 and day + 1; (▲) immune serum on day − 1 and day + 1; (△) immune serum on day + 3, and day + 5; (■) immune serum on day + 7 and day + 9; (□) immune serum on day + 11 and day + 13.

counts, although lower than in control mice, were not significantly different. In contrast, in Expt. 6 when immune serum was given on days + 4, 6, 8, 10 and 12, there was both a reduction in worm fecundity and in the number of worms recovered at autopsy. Furthermore, in this experiment there was also a reduction in worm counts from mice treated with immune serum on days + 6, 8, 10, 12 and 14, whereas in Expt. 5 the group given 1 ml of immune serum on days + 6 and + 8 did not expel worms. When immune serum was administered later, either from day + 7 (Expt. 4), day + 8 (Expt. 5) or day + 11 (Expt. 4) onwards, expulsion of worms did not take place and the fecundity of worms was normal, except in Expt. 4 where there was a significant reduction in the fecundity of worms in the group given immune serum on days + 7 and + 9.

THE TIME-COURSE OF THE EXPULSION OF N. DUBIUS FROM MICE TREATED WITH IMMUNE SERUM

When mice infected with *N. dubius* were given immune serum during the first 4

Table 3. The time course of worm expulsion in mice treated with immune serum

Weeks after infection	No. of *N. dubius* recovered (±s.d.) on days shown*											
	Exp. 7			Exp. 8			Exp. 9			Exp. 10		
	Day killed	Control	Serum-treated	Day killed	Control	Serum-treated	Day killed	Control	Serum-treated	Day killed	Control	Serum-treated
2	10	95·4±15·5	56·6±11·4†	9	76·6±11·4	61·0±5·9†	10	88·0±9·9	74·0±10·4†	10	91·8±7·1	71·0±13·7†
	13		75·8±10·8	14		81·0±13·9§¶						
3	17		74·8±4·0‡				15		99·7±20·1**††	15	98·0±6·9	81·4±12·3†‡‡§§
	20	93·1±7·9	75·4±10·4†	21	97·0±11·9	65·3±28·2†						
4	23		72·0±7·3				22	114·5±14·2	77·3±33·7†	22	95·8±10·5	19·5±16·6†‡‡
	29	90·3±11·0	43·6±35·8†‡	28	95·5±7·7	16·3±28·9†§	27	104·5±12·6	18·2±40·6†**			
5				35	92·5±11·1	36·7±41·2†¶	34	117·1±19·2	16·3±28·3†††	35	99·3±7·1	12·0±11·8†§§

* Groups of five to seven mice were given immune serum i.p. and together with control groups of six to eight mice were infected with 100 *N. dubius* on day 0. The experimental groups were treated with immune serum as follows: Expt. 7, 1 ml of immune serum given on days −1 and +1; Expt. 8 & 9, 0·5 ml of immune serum given on days −1, +1, +3 and +5; Expt. 10, 0·5 ml of immune serum given on days −1, +1, +3, +5 and +7.

† Means significantly lower than control group killed on the same day.

Paired groups were compared and found to have the following statistical significance: ‡ $P = 0{\cdot}05$, § $P = 0{\cdot}005$, ¶ $0{\cdot}05 > P > 0{\cdot}025$, ** $P < 0{\cdot}01$, †† $P < 0{\cdot}005$, ‡‡ $P < 0{\cdot}005$, §§ $P < 0{\cdot}005$.

days of infection, fewer worms were recovered on day 9 or day 10 (Table 1). A more substantial reduction in worm numbers took place later and was essentially complete by day 28. In order to clarify the exact timing of worm expulsion, four experiments were carried out (Expts 7, 8, 9 & 10) in which groups of mice were given immune serum at the optimum time and were killed together with the respective control groups at different times after infection. The experimental details together with the results are summarized in Tables 3, 4 & 5.

Table 4. The effect of immune serum on the growth of *N. dubius* in recipient mice*

	Mean dry weight (μg per worm pair ± s.d.)			
	Expt. 9		Expt. 10	
Day after infection killed	Control	Serum-treated	Control	Serum-treated
10	68·8 ± 15·8	41·3 ± 12·8†	56·9 ± 10·0	37·0 ± 5·2†
15		45·3 ± 12·1	84·3 ± 10·3	67·8 ± 9·7†
22	80·5 ± 16·6	49·1 ± 15·6†	89·6 ± 7·2	64·8 ± 5·6†
27	97·7 ± 15·5	37·5 ± 17·6†		
35			106·0 ± 12·0	62·0 ± 25·5†

* Groups of five to seven mice were given immune serum i.p. and together with control groups of six to eight mice were infected with 100 *N. dubius* on day 0. The experimental groups were treated with immune serum as follows: Expt. 9, 0·5 ml immune serum given on days −1, +1, +3 and +5; Expt. 10, 0·5 ml immune serum given on days −1, +1, +3, +5 and +7. The worms were weighed as described in the text.
† Means significantly lower than control groups killed on the same day.

There was a significant reduction in the worm counts of mice treated with immune serum on all 15 occasions when control and experimental groups were killed on the same day (Table 3). When treated mice were killed during the first 3 weeks of infection, this reduction was relatively small, ranging from 16% to 41%. A far greater loss, ranging from 52% to 86%, took place only during and after the fourth week of infection. In all four experiments this loss was statistically significant.

The mean dry weight per worm pair was determined for Expts 9 & 10 and the results (Table 4) show that the worms from mice treated with immune serum were significantly lighter. The relative fecundity of the last group to be killed in these experiments was also measured (Table 5). There was a significant reduction in the egg output of the treated groups in all four experiments.

Table 5. The effect of immune serum on the relative fecundity of *N. dubius* in recipient mice

	Relative fecundity*	
Experiment	Control group	Immune serum-treated group
7	6034	2240†
8	6807	1033†
9	10524	29†
10	6065	145†

* Groups of five to seven mice were given immune serum i.p. and together with control groups of six to eight mice were infected with 100 *N. dubius* on day 0. The experimental groups were treated with immune serum as follows: Expt. 7, 1 ml immune serum given on day −1 and day +1; Expts 8 & 9, 0·5 ml immune serum given on days −1, +3 and +5; Expt. 10, 0·5 ml immune serum given on days −1, +3, +5 and +7.

† Means significantly lower than the respective control group.

Discussion

Despite the failure of several groups of workers to transfer immunity to *Nematospiroides dubius* passively with serum from immune mice (Panter 1969, Cypess 1970, Chaicumpa *et al.* 1977), the results reported in this paper clearly demonstrated that the survival of *N. dubius* was severely impaired in mice treated with immune serum. During the period between the administration of immune serum beginning in the first 4 days of infection until they were killed within the next 3 weeks, the mice harboured fewer worms than control mice, the worms were stunted and their fecundity was greatly reduced. It is possible that a proportion of the lower worm recoveries on days 9–10 represented prolonged development of the larvae in the gut mucosa as described by Bartlett & Ball (1974). Certainly, in Expts 7–10 (Table 3) there was a consistent increase in worm recoveries from treated mice during the third week of infection (days 13–20). However, even on days 15–20 (Expts 7 & 10) when worm recoveries were highest, control mice still had significantly more worms than treated mice and this suggests that not only was the rate of worm development inhibited, but that in addition there was a loss of some larvae during this early phase of the infection.

Perhaps the most interesting feature of these experiments was the observation that mice given immune serum expelled most of the worm burden during and after the fourth week of infection, some 2–3 weeks after the last injection of immune serum. The half-life of injected IgG in normal mice is about 4–8 days

(Fahey & Sell 1965), whereas in mice infected with *N. dubius* it can be as short as 2·6–3·5 days (Brown, Crandall & Crandall 1976). Therefore, in agreement with the findings from other experimental systems (Wakelin 1978), it is unlikely that in this host–parasite system, immune serum alone effected worm expulsion. However, it is possible that transferred antibody had some direct effect against *N. dubius* since the worms recovered from mice treated with immune serum were stunted throughout the experimental period (Table 4). Thus, factors in the transferred serum played a vital initial role in which worms were damaged, thereby making them susceptible to a second component in the host response which resulted in their expulsion. Such a sequence of events is well documented in the case of *Nippostrongylus brasiliensis* (Ogilvie & Love 1974) and *Trichuris muris* (Wakelin 1975a).

Recent findings which have established that *N. dubius* has the capacity to interfere with the immunological activity of the host, are also pertinent in the present context. Thus, the induction and expression of primary and secondary responses to *Trichinella spiralis* and *T. muris* was greatly impaired in mice concurrently infected with *N. dubius* (Jenkins 1977, Jenkins & Behnke 1977, Behnke, Wakelin & Wilson 1979). When mice were challenged with *N. dubius* 4–6 days after infection with either *T. spiralis* or *T. muris*, the primary response to the latter parasites was still markedly affected, suggesting that the larvae of *N. dubius* were immunosuppressive and that they rapidly interfered with the efferent component of the immune response. On the basis of these results it seems probable that in order to reject *N. dubius*, the host must have the capacity to counteract the immunosuppressive factors produced by the larvae in the first few days of infection. It is possible that transferred immune serum in the present study, besides acting directly on the larvae, neutralized parasite toxins, thus facilitating the generation of an effective immune response by the recipients which consequently expelled the damaged worms in the fourth week of infection.

The effects of immune serum on *N. dubius* were not observed when mice were given normal serum or when immune serum was administered after day 6 of infection. The failure of the immune serum given later in the infection to affect worm fecundity and to cause worm expulsion can conceivably be attributed to an already impaired or blocked host immune system which is incapable of co-operating fully with transferred serum. It is also possible that transferred serum had less access to the worms in the intestinal lumen, although Cypess, Ebersole & Molinari (1977) reported that the concentration of IgG in the gut lumen increased substantially 3–7 days after infection with *N. dubius* and they suggested that leakage of serum proteins into the lumen occurred in animals infected with this parasite. Protective activity associated with IgG in transferred immune serum has also been described in other gut dwelling helminth parasites (Di Conza 1969, Jones, Edwards & Ogilvie 1970), but it has recently been pointed out that there is no convincing evidence that the severe cytopathological changes seen in *N. brasiliensis* immediately preceding worm expulsion (Lee 1969, Ogilvie & Hockley

1968) are the direct consequence of antibody activity (Love, Ogilvie & McLaren 1975, Wakelin, 1978). Similar changes can be induced in *in vitro* culture (Love *et al.* 1975), and worm expulsion can take place normally in mice incapable of detectable antibody synthesis (Jacobson, Reed & Manning 1977).

The reasons underlying the failure of previous workers to transfer immunity to *N. dubius* are unknown, but it is possible to identify several important differences between the present work and that already reported in the literature. The donor mice used in the present study were given a series of graded infections which were controlled by treatment with anthelmintic to prevent lethal worm burdens from accumulating in the gut lumen. Bartlett & Ball (1974), who also infected donor mice with a series of overlapping infections, demonstrated a significant delay in the development of *N. dubius* in recipients, but their experiment was terminated too early to show expulsion. Another important factor is the strain of mice used in the present work. NIH mice are known to respond more rapidly to several intestinal nematode parasites (Wakelin 1975b, Wakelin & Lloyd 1976) than any other strain of inbred mice and it is possible that in the presence of transferred immune serum, the resultant more effective immune response conferred on this strain the capacity to expel worms. Clearly this point will only be resolved when the present study is expanded to involve different strains of mice. Finally, the results reported in this paper have firmly established that resistance to *N. dubius* can be transferred by immune serum and hence support the hypothesis that protection in resistant mice is immunologically mediated.

Acknowledgements

We would like to thank Professor P. N. R. Usherwood for the facilities provided for this work in the Department of Zoology, University of Nottingham. The assistance of Mr R. Gilder and Mr D. Reffin in maintaining our experimental animals is gratefully appreciated. This work was supported by MRC grant G976/935/T.

References

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

BEHNKE J.M. (1977) *Nematospiroides dubius:* Inhibition of larval development in immune mice and attempts to transfer immunity by lymphoid cells and serum. *Parasitology* **75**, xv

BEHNKE J.M. & PARISH H.A. (1978) Transfer of resistance to *Nematospiroides dubius* infection in mice with homologous antiserum. *Parasitology* (in press)

BEHNKE J.M. & PARISH H.A. (1979) *Nematospiroides dubius:* the arrested development of larvae in immune mice. *Experimental Parasitology* **47**, 116

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

BEHNKE J.M., WAKELIN D. & WILSON M.M. (1979) *Trichinella spiralis:* delayed rejection in mice concurrently infected with *Nematospiroides dubius. Experimental Parasitology* (in press)

BROWN A.R., CRANDALL R.B. & CRANDALL C.A. (1976) Increased IgG catabolism as a possible factor in the immuno-suppression produced in mice infected with *Heligmosomoides polygyrus. Journal of Parasitology* **62,** 169

CHAICUMPA V., JENKIN C.R. & ROWLEY D. (1976) Passive transfer of immunity to infection with *Nematospiroides dubius* from immunised mice to their offspring. *The Australian Journal of Experimental Biology and Medical Science* **54,** 245

CYPESS R. (1970) Demonstration of immunity to *Nematospiroides dubius* in recipient mice given spleen cells. *Journal of Parasitology* **56,** 199

CYPESS R.M., EBERSOLE J.L. & MOLINARI J.A. (1977) Specific antibody levels in the intestinal perfusates of *Heligmosmoides polygyrus*-infected mice. *International Archives of Allergy and Applied Immunology* **55,** 496

CYPESS R.H. & ZIDIAN J.L. (1975) *Heligmosomoides polygyrus (=Nematospiroides dubius):* the development of self-cure and/or protection in several strains of mice. *Journal of Parasitology* **61,** 819

DI CONZA J.J. (1969) Protective action of passively transferred immune serum and immunoglobulin fractions against tissue invasive stages of the dwarf tapeworm *Hymenolepis nana. Experimental Parasitology* **25,** 368

DINEEN J.K. & WAGLAND B.M. (1966) The cellular transfer of immunity to *Trichostrongylus colubriformis* in an isogenic strain of guinea-pig. II. The relative susceptibility of the larval and adult stages of the parasite to immunological attack. *Immunology* **11,** 47

FAHEY J.L. & SELL S. (1965) The immunoglobulins of mice. V. The metabolic (catabolic) properties of five immunoglobulin classes. *Journal of Experimental Medicine* **122,** 41

GORDON H.M. & WHITLOCK H.V. (1939) A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research, Australia* **12,** 50

JACOBSON R.H., REED N.D. & MANNING D.D. (1977) Expulsion of *Nippostrongylus brasiliensis* from mice lacking antibody production potential. *Immunology* **32,** 867

JENKINS S. (1977) Studies on the immune response of the mouse to the nematode *Trichuris muris.* PhD Thesis, University of Glasgow

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

JONES V.E., EDWARDS A.J. & OGILVIE B.M. (1970) The circulating immunoglobulins involved in protective immunity to the intestinal stages of *Nippostrongylus brasiliensis* in the rat. *Immunology* **18,** 621

LEE D.L. (1969) Changes in adult *Nippostrongylus brasiliensis* during the development of immunity to this nematode in rats. I. Changes in ultrastructure. *Parasitology* **59,** 29

LOVE R.J., OGILVIE B.M. & MCLAREN D.J. (1975) *Nippostrongylus brasiliensis:* further properties of antibody damaged worms and induction of comparable damage by maintaining worms *in vitro. Parasitology* **71,** 275

OGILVIE B.M. & HOCKLEY D. (1968) Effects of immunity on *Nippostrongylus brasiliensis* adult worms: reversible and irreversible changes in infectivity, reproduction and morphology. *Journal of Parasitology* **54,** 1073

OGILVIE B.M. & LOVE R.J. (1974) Co-operation between antibodies and cells in immunity to a nematode parasite. *Transplantation Reviews* **19,** 147

OGILVIE B.M. & PARROTT D.M.V. (1977) The immunological consequences of nematode infection. In *Immunology of the Gut,* Ciba Foundation Symposium, **46.** North Holland, Elsevier

OGILVIE B.M. & WILSON R.J.M. (1976) Evasion of the immune response by parasites. *British Medical Bulletin* **32,** 177

PANTER H.C. (1969) The mechanism of immunity to *Nematospiroides dubius. Journal of Parasitology* **55,** 38

SOKAL R.R. & ROHLF (1969) In *Biometry*, p. 240. W. H. Freeman & Co., San Francisco

WAKELIN D. (1975A) Immune expulsion of *Trichuris muris* from mice during a primary infection: analysis of the components involved. *Parasitology* **70,** 397

WAKELIN D. (1975b) Genetic control of immune responses to parasites: immunity to *Trichuris muris* in inbred and random-bred strains of mice. *Parasitology* **71,** 51

WAKELIN D. (1978) Immunity to intestinal parasites. *Nature (London)* **273,** 617

WAKELIN D. & LLOYD M. (1976) Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a reexamination of conventional parameters. *Parasitology* **72,** 173