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Genetic control of immunity to *Nematospiroides dubius:* a 9-day anthelmintic abbreviated immunizing regime which separates weak and strong responder strains of mice

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Summary Experiments were designed to re-examine the variables which influence the ability of single primary infections to elicit acquired immunity to *Nematospiroides dubius*, in particular the importance of the presence or absence of adult worms, as these are known to exert immunomodulatory effects. Briefly, anthelmintic abbreviated infections were considerably more effective at eliciting acquired immunity than longer infections in which adult worms were allowed to reside in the intestine. A 9-day anthelmintic abbreviated infection was extremely effective at stimulation of acquired immunity in NIH mice and very few immunising infection larvae were required. Immunity to subsequent reinfection developed rapidly after the primary infection worms had been eliminated; by day 21 post-infection, the mice were almost totally immune. Abbreviated infections were used to examine the capacity of a number of mouse strains to develop immunity to reinfection. Strains of mice were chosen to allow the effects of MHC linked and non-MHC linked (background) genes to be identified. CBA and C_3H strains (both H-2^k) were found to be weak responders to *N. dubius*. B10G (H-2^q) mice responded better than C57Bl/10 (H-2^b), although these strains have identical background genes. DBA/2 mice were stronger responders compared to BALB/c mice, both strains sharing a common MHC haplotype (H-2^d). (NIH × B10G) F_1 mice (H-2^q) were better responders than either of the parental strains. Several mouse strains all sharing the H-2^q haplotype were particularly effective at developing immunity to *N. dubius*, as were also SJL mice which were the sole representatives of the H-2^s haplotype, in the present study. The results established that the response phenotype is influenced by both background and MHC genes and demonstrated gene complementation in the capacity of mice to acquire immunity to *N. dubius*.

Keywords: nematodes, gut, immunity, *Nematospiroides dubius*, mice, genetic control

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Introduction

Like many important nematode parasites, *Nematospiroides dubius* causes a (long) chronic primary infection which can last for 8–10 months (Ehrenford 1954). There is no precipitous reduction in worms within the first month of infection in this species, as occurs in the case of *Trichinella spiralis* or *Nippostrongylus brasiliensis* infections in rats and mice. Even in those inbred mouse strains which ultimately reject primary infection *N. dubius*, the process of worm expulsion is protracted and may take several weeks with considerable variation between individual animals (Mitchell *et al.* 1982). The prolonged and variable duration of the primary infection does not lend itself to straightforward experimental manipulation and there have been few studies of genetic and immunological control.

Investigation of the genetic control of immunity to *N. dubius* has concentrated on acquired immunity but because of the variables involved, there has been little uniformity in the approaches used by workers in this field. Several of the inbred mouse strains most widely used in immunological research (C_3H, C57Bl/10, CBA and BALB/c in particular) do not acquire strong immunity following a single anthelmintic abbreviated primary infection (Liu 1966, Behnke & Wakelin 1977, Prowse *et al.* 1979, Cypess *et al.* 1977). The solution of different workers has been to use multiple immunising regimes with or without anthelmintic treatment (Behnke & Wakelin 1977, Prowse *et al.* 1979, Van Zandt, Cypess & Zidian 1973, Williams & Behnke 1983, Dobson *et al.* 1982). Following such regimes, mouse strains express varying degrees of resistance to subsequent challenge infection and can be separated into weak, intermediate or strong responder categories (Prowse *et al.* 1979, Cypess *et al.* 1977).

It is believed that acquired immunity to *N. dubius* is elicited by and acts preferentially against the L4 stages during their residence in the intestinal walls (Bartlett & Ball 1974, Hagan, Behnke & Parish 1981, Pritchard *et al.* 1983). Adult worms in the intestinal lumen do not stimulate acquired immunity nor are they affected markedly in mice otherwise totally resistant to larval challenge (Behnke, Hannah & Pritchard 1983, Jacobson, Brooks & Cypess 1982, Bartlett & Ball 1974). Furthermore there is evidence that adult parasites are immunomodulatory, suppressing the generation and expression of host protective responses (Behnke, Hannah & Pritchard 1983, Cayzer & Dobson 1983). It is clear, therefore, that where complex immunising schedules involving several superimposed infections have been adopted, the mice have been subjected to both stimulation (by larval antigen) and suppression (by adult worms). The resistance expressed against a subsequent challenge infection must therefore reflect the outcome of the interaction between these two opposing mechanisms (which may be under linked or independent genetic control), i.e., it is possible that there may be genetically determined susceptibility to suppression by the parasite as well as genetically determined ability to develop resistance.

Previous work in this laboratory has used NIH mice as a strong responder strain and C57Bl/10 mice as weak responders and complex immunising schedules aimed at ensuring consistently effective host protective acquired immunity. However, in view of the fact that adult worms have immunomodulatory activity we have re-examined the variables which may influence the success/failure of single primary infections to elicit acquired immunity to *N. dubius* (in particular the presence or absence of adult worms). In this paper we report the results of this work and we describe a 9 day anthelmintic abbreviated immunising regime which is extremely effective at discriminating between weak and strong responder strains of mice.

Materials and methods

Animals

NIH, C57Bl/10 and B10G mice and the hybrids of these strains were bred and maintained under conventional animal house conditions in the Zoology Department of Nottingham University. All other mouse strains were purchased from Olac 1976 Ltd. The animals used in this study were treated with piperazine citrate (500 mg/kg bodyweight, Sigma) at least 1 week before the start of each experiment.

N. dubius

The strain of *N. dubius* was obtained from the Wellcome Research Laboratories (Beckenham, Kent, UK) in 1975 and has been passaged since in randomly bred CFLP mice. The methods used for infection and recovery of worms have been previously described (Jenkins & Behnke 1977). Irradiated larvae were exposed to 25 krad of gamma radiation, as described by Behnke, Parish & Hagan (1980). The anthelmintic used was pyrantel embonate (Strongid—P Paste, Pfizer) and was administered orally at a dose 100 mg/kg body weight (Behnke & Wakelin 1977). Worm lengths were calculated from camera lucida drawings of female *Nematospiroides dubius* using a bit pad digitizer linked to a DEC-PDP 11/34 computer. A minimum of 10 worms were counted in each group. Faecal egg counts were carried out as described by Behnke & Parish (1979).

Analysis of results

The results are expressed as mean number of worms recovered (MWR) ± 1 s.e. Where relevant the percentage protection has also been calculated and is given for ease of comparison. The results were analysed for significance using the non-parametric Wilcoxon test (Sokal & Rohlf 1969) and values of $P < 0.05$ were considered to be significant. Worm lengths are expressed as arithmetic means ± 1 s.e. Student's *t*-test was used to assess statistical significance between means: a P value of < 0.05 was again considered significant.

Results

The course of a primary infection in weak and strong responder strains of mice

Although *N. dubius* is reputed to survive for 8 months following a primary infection in mice (Ehrenford, 1954), the exact course of infection has not been documented for different strains of mice. In the present study C57Bl/10 and NIH mice were used extensively as representing, respectively, weak and strong responders, and therefore as a preliminary to the work, the time course of a primary infection was monitored in these two strains. Groups of C57Bl/10 and NIH mice were infected with 200 larvae of *N. dubius* and faecal egg counts were recorded for the duration of infection. The results of one such experiment are shown in Figure 1.

Egg production rose rapidly 10 days after infection to approximately 30,000 e.p.g. of
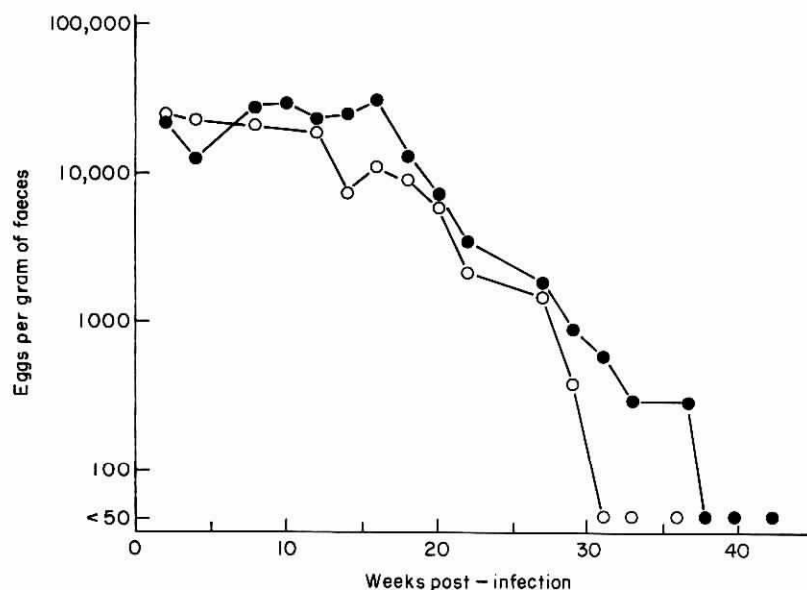
Figure 1. The time course of a primary infection with *Nematospiroides dubius* in C57Bl/10 (●) and NIH (○) female mice.

faeces and remained at this level for about 130 days. Subsequently the number of eggs in the faeces declined until 37 weeks after infection when no further eggs were detected. The duration of infection in C57Bl/10 mice was marginally longer than in NIH mice. In a replicate experiment NIH produce *N. dubius* eggs for 32 weeks following infection whereas C57Bl/10 mice had eggs in their faeces until week 38.

Acquired immunity in strong responder mice following a superimposed challenge infection

Neither of the two strains of mice studied (C57Bl/10 and NIH) expelled adult worms within 5 months of a primary infection, although the subsequent fall in egg counts was associated with a gradual loss of worms. Analysis of resistance to *N. dubius* could therefore only be directed at acquired immunity and two distinct approaches were possible. Thus the success or failure of a challenge infection could be monitored in mice still harbouring the original primary infection worms (superimposed challenge) or in mice which had received anthelmintic treatment some days before challenge (anthelmintic abbreviated infection).

Surprisingly these two approaches gave seemingly incompatible results. An example is shown in Table 1 (Expt 2). Following a brief 9-day anthelmintic abbreviated infection, resistance to a challenge infection in NIH mice was marked (94·4%, group C). In contrast resistance to a superimposed challenge infection was comparatively weak (48·7%, group D). Interestingly weak responder C57Bl/10 mice did not become immune following the anthelmintic abbreviated infection (group G) but some worm loss was identified (30·7%) when the challenge infection was superimposed.

In this experiment it was not possible to distinguish whether it was the primary or challenge infection worms which were rejected from groups given a superimposed

Table 1. Comparison of acquired immunity following either a 9-day anthelmintic abbreviated or an uninterrupted primary infection in NIH or C57Bl/10 mice

Group	Strain*	Primary infection†	Treatment with ant- helmintic‡	Challenge infection§	MWR ± s.e.	% pro- tection¶
(A) Primary infection control	NIH	+	−	−	124·7 ± 6·8	—
(B) Challenge infection control	NIH	−	+	+	109·8 ± 5·7	—
(C) Abbreviated infection	NIH	+	+	+	6·2 ± 2.3	94·4
(D) Uninterrupted infection	NIH	+	−	+	181·0 ± 42·6	48·7
(E) Primary infection control	C57Bl/10	+	−	−	113·0 ± 10·4	—
(F) Challenge infection control	C57Bl/10	−	+	+	106·8 ± 8·9	—
(G) Abbreviated infection	C57Bl/10	+	+	+	102·7 ± 8·0	3·8
(H) Uninterrupted infection	C57Bl/10	+	−	+	181·0 ± 26·0	30·7

* Six mice in each group; † 160 infective larvae were given on day 0; ‡ Pyrantel was administered on days 9, 10, 14 and 21; § 150 infective larvae were given on day 28 and the mice were autopsied 5 weeks later.

¶ For groups given anthelmintic treatment, percentage protection was calculated as:

$$\frac{\text{MWR challenge infection control} - \text{MWR experimental group}}{\text{MWR challenge infection control}} \times 100.$$

For groups given uninterrupted primary infection percentage protection was calculated as:

$$\frac{\text{MWR challenge infection control} - (\text{MWR experimental group} - \text{MWR primary infection control})}{\text{MWR challenge infection control}} \times 100.$$

Statistical analysis of results: C *vs* B, $P = 0.001$; G *vs* F, NS.

challenge. However, it is clear from further experiments that the outcome of a superimposed challenge in NIH may be in one of three directions and that a particular results is seldom predictable. This is illustrated by the experiment described in Table 2. In this experiment (Expt 3) all the mice given a 14 day anthelmintic abbreviated infection were solidly immune to challenge. However, the worm burdens in the mice given a superimposed challenge were extremely variable ranging from 0–301. Thus two mice had lost virtually all their worms. Four mice had worm burdens of fewer than 94 worms indicating partial loss from both primary and challenge infections. Three mice had over 200 worms and one had 301 worms indicating that both primary and challenge infections had established and survived.

Acquired immunity in strong responder mice following anthelmintic abbreviated primary infections of varying duration

A further important variable which emerged as critically important in determining whether responder mice would express a weak or a strong response against a challenge infection was the duration of the primary infection prior to the administration of anthelmintic. Brief immunising infections of 9–14 days duration were considerably more

Table 2. Acquired immunity following a 14 day anthelmintic abbreviated or an uninterrupted primary infection in NIH mice

Group*	No. Mice	Primary infection†	Treatment with ant-helmintic‡	Challenge infection§	MWR ± s.e.	Range of worm burdens
(A) Primary infection control	8	+	−	−	208·5 ± 9·9	146–244
(B) Challenge infection control	7	−	+	+	94·1 ± 9·3	44–122
(C) Abbreviated infection	10	+	+	+	2·1 ± 0·7	0–7
(D) Uninterrupted infection	10	+	−	+	123·9 ± 34·6	0–301¶

* All the mice were NIH.

† 250 larvae were given on day 0.

‡ Pyrantel was administered on days 14 and 16 post-infection.

§ Groups B, C, and D were challenged with 100 larvae on day 49 and were killed 5 weeks later on day 84.

¶ The individual worm burdens in this group were 0, 2, 53, 75, 83, 89, 122, 246, 268, 301.

Statistical analysis of results: B *vs* C, $P < 0·001$.

effective in eliciting acquired immunity than regimes in which adult worms were removed after several weeks of residence in the intestine.

Because of the number of variables involved, experiments of this type could be organised logistically in a number of different ways. The results of two experiments are summarised in Table 3. Thus in experiment 4 the immunising infection was administered on the same day to all the groups. Likewise all the groups were challenged on the same day, but treatment with anthelmintic was staggered, thereby creating in addition to variable periods of primary infection, variable periods without worms for the different groups. In Expt 5 the primary infections were staggered, allowing all other treatments to be delivered simultaneously. However, this entailed some variation in the infectivity of the immunizing inoculum. Despite these variables, both experiments confirm that brief primary infections are considerably more immunogenic than infections in which adult worms are allowed to persist in the intestine for a period of several weeks. These experiments were repeated several times with similar results on each occasion.

Acquired immunity in weak and strong responder strains following a brief anthelmintic abbreviated infection or infection with irradiated larvae

In the initial exploratory experiments it was important to establish whether the brief anthelmintic abbreviated immunising regimes would be of value in discriminating between weak and strong responder mouse strains. It was also felt that a direct comparison of this immunising regime to other immunising regimes currently in use (notably immunisation by irradiated larvae) would be of value in assessing its suitability for genetic studies.

Accordingly Table 4 summarises the results from two separate experiments. In Expt 6, a 14 day anthelmintic abbreviated infection was given to NIH and C57Bl/10 mice. NIH mice expressed strong resistance to challenge infection as reflected in the number of worms recovered (78·7% protection) whereas all the challenge infection worms estab-

Table 3. Acquired immunity in NIH mice following anthelmintic abbreviated infections of varying duration

Duration of primary infection	Expt 4*					Expt 5†				
	Group	No. Mice	MWR ± s.e.	% protection	No. mice >95% protected	Group	No. mice	MWR ± s.e.	% protection	No. mice >95% protected
None (control groups)	A	6	94·2 ± 4·4	—	—	A	8	118·9 ± 5·4	—	—
7 days	B	6	10·2 ± 8·6	89·2	5	B	7	25·0 ± 11·7	79·0	4
14 days	C	6	55·7 ± 5·1	40·9	0	C	8	46·1 ± 10·6	61·2	2
28 days										
35 days						D	8	93·0 ± 6·2	21·8	0
42 days	D	6	67·5 ± 8·0	28·3	0					

Male NIH mice were used in both experiments.

*In Expt 4 the groups were immunised with 200 normal *N. dubius* on day 0. An infectivity control group (four mice) killed on day 17 had a MWR of 177·5 ± 11·0 worms. Group B was treated with anthelmintic on days 14 and 16. Group C received anthelmintic on days 28 and 30 and group D on days 42 and 44. All the mice together with a control group (A) were challenged with 100 larvae on day 50 and were killed 5 weeks later.

†In Expt 5, the primary infections were staggered. Thus all groups received 300 larvae 7(B), 14(C) or 35(D) days prior to anthelmintic treatment. The infectivity controls (three mice group) showed that 128·3 ± 19·0, 183·3 ± 8·2 and 301·7 ± 14·4 worms established in these groups respectively. Anthelmintic was given to all mice daily for a week with a further dose a week later. All the groups were challenged with 150 larvae of *N. dubius* after 3 weeks and were killed for worm counts 5 weeks later.

Statistical analysis of results: Expt 4—A *vs* B *P* < 0·005; B *vs* D *P* = 0·005; A *vs* D *P* = 0·025. Expt 5—A *vs* B *P* < 0·001; B *vs* D *P* = 0·001; A *vs* D *P* = 0·005.

Table 4. Acquired immunity in C57Bl/10 and NIH mice following a brief anthelmintic abbreviated infection or an infection with irradiated larvae

Expt	Group	Strain	No. mice	MWR ± s.e.	% protection	Mean length ± s.e. (mm) of female worms
6	A Control	NIH	6	69·0 ± 3·0	—	19·7 ± 0·3
	B Immune*	NIH	6	14·7 ± 6·5	78·7	11·3 ± 0·5
	C Control	C57Bl/10	6	67·3 ± 3·1	—	20·0 ± 0·4
	D Immune*	C57Bl/10	6	67·7 ± 2·8	0	16·3 ± 0·6
7	A Control	NIH	6	99·0 ± 5·9	—	
	B Immune† (anthelmintic abbreviated infection)	NIH	6	37·5 ± 16·4	62·1	
	C Immune‡ (irradiated larvae)	NIH	6	4·2 ± 4·0	95·8	
	D Control	C57Bl/10	5	105·0 ± 8·8	—	
	E Immune† (anthelmintic abbreviated infection)	C57Bl/10	5	91·6 ± 8·8	12·8	
	F Immune‡ (irradiated infection)	C57Bl/10	5	0·8 ± 0·4	99·2	

*The immune mice were given an infection of 250 larvae on day 0. Anthelmintic was given on days 14, 16 and 21 to all the groups. The challenge infection of 100 larvae was given on day 49 and the mice were killed 3 weeks later on day 70. Two groups of three mice given the immunising infection alone had 203·0 ± 6·2 (NIH) and 202·5 ± 3·5 (C57Bl/10) when killed 14 days after the primary infection.

†The immune mice were given an infection of 200 larvae on day 0. Anthelmintic was given on days 6, 7, 8, 9 and 10 and the challenge infection on day 48. The mice were killed 32 days later on day 80.

‡The immune mice were given 200 larvae irradiated at 25 krad on day 0 and the groups were subsequently treated as described for †.

Statistical analysis of results: Expt 6—Worms recovered: A *vs* B, $P < 0.005$; C *vs* D, N S. Worm length: A *vs* B, $P < 0.001$; C *vs* D, $P < 0.001$; B *vs* D, $P < 0.001$. Expt 7—A *vs* B, $P = 0.005$; D *vs* E, N S, B *vs* E, $P = 0.025$.

lished and survived in C57Bl/10 mice. The few surviving worms recovered from NIH mice were severely stunted in comparison to the control group and were significantly smaller than those recovered from immune C57Bl/10 mice ($P < 0.001$).

In Expt 7 a shorter anthelmintic abbreviated infection was used. Anthelmintic was given on days 6–10. The overall level of protection in NIH mice following this immunising regime was 62% (cf. 78·7%, Expt 6) and in C57Bl/10 mice 13·0% (cf. 0 in Expt 6). However, irradiated larvae elicited potent resistance in both strains, overriding the inability of C57Bl/10 mice to respond effectively to the anthelmintic abbreviated infection (99·2% and 95·8% protection in C57Bl/10 and NIH mice, respectively).

Standardization of the anthelmintic abbreviated immunizing regime

The experiments reported thus far have clearly established that brief anthelmintic abbreviated infections are extremely effective at discriminating between weak and strong responder strains of mice. However, before expanding the work to encompass a wider variety of strains it was felt that the 9 day anthelmintic abbreviated regime should be

Table 5. Dose–response; the effect of varying the number of larvae administered in a 9 day anthelmintic abbreviated primary infectin

| | | Primary infection | | Challenge infection | |
		No. of larvae administered	No. of worms recovered*	MWR following challenge infection†	% protection
Expt	Group				
	A	—	—	130·4	—
	B	500	381·0 ± 19·9	0·5	99·6
	C	250	188·3 ± 4·4	0·7	99·5
8	D	100	76·7 ± 5·2	0·2	99·8
	E	50	39·7 ± 5·2	1·7	98·7
	F	25	16·0 ± 1·0	2·8	97·9
	G	10	7·7 ± 1·7	12·3	90·6
	H	—	—	92·6 ± 5·8	—
	I	250	163·3 ± 12·1	0·9 ± 0·4	99·0
9	J	50	24·3 ± 3·5	21·0 ± 12·9	77·3
	K	10	7·0 ± 0·3	43·6 ± 14·2	52·9
	L	5	1·0 ± 0·6	48· ± 16·4	48·2

*In both experiments groups of three mice were killed 23 days (Expt 8) and 21 days (Expt 9) post-infection in order to determine the No. of worms establishing at each level of infection.

†In Expt 8 the mice were challenged on day 35 with 150 larvae and were killed 5 weeks later on day 70. Each group comprised six female NIH mice. In Expt 9 the mice were challenged on day 28 with 100 larvae and were killed 5 weeks later on day 63. Each group comprised eight female NIH mice.

optimised. Experiments were therefore, carried out to determine the importance of three variables in the system; namely, the number of larvae in the immunising infection, the length of the interval between anthelmintic treatment and challenge and the length of the interval between challenge and autopsy.

The effect of varying the number of larvae in the immunizing infection. Table 5 presents the results from two experiments (Expts 8 & 9) carried out in female NIH mice. Several infection levels were used ranging from 5 to 500 larvae. The infectivity controls,however, indicate that there was variable infectivity but nevertheless it is quite clear that the 9 day anthelmintic abbreviated infection is extremely immunogenic in NIH mice, and that even a single worm (group L, $1 \cdot 0 \pm 0 \cdot 6$) can elicit detectable immunity ($48 \cdot 2\%$ protection). An infection level of 200–250 larvae is therefore well within the range and ensures a wide safety margin in the case of poorly infective inocula.

The effect of varying the interval between anthelmintic treatment and challenge infection. The objective of this experiment (Expt 10) was to determine how soon the challenge infection could be given, after the first treatment with anthelmintic. In order to keep the regime as simple as possible only three treatments with anthelmintic were used. Some worms do not emerge from the intestinal walls until after day 9 and this is clearly illustrated by group B in Table 6, where the single dose of anthelmintic on day 9 removed $55 \cdot 8\%$ of the worm burden. It was only after the second dose of anthelmintic on day 14 that $99 \cdot 9\%$ of the worms were eliminated (group E). Inevitably, therefore, in the groups challenged on day 14 the challenge infection larvae were superimposed on the primary infection worms which had not been eliminated by the single dose of anthelmintic on day 9. The degree of acquired immunity in these mice was weak (group D). In contrast, a challenge infection on day 21 (group G) was resisted extremely effectively ($99 \cdot 0\%$ protection). These results confirmed that the 4 week interval between the immunizing infection and the challenge was well within the margin for optimum expression of acquired immunity.

Nevertheless this experiment did leave a degree of uncertainty as to how quickly immunity might have developed in the absence of complications arising from the failure to eliminate the primary infection adult worm burden. Another experiment (Expt 11) was therefore carried out using 25 krad irradiated larvae, which do not develop to maturity and therefore obviate the need for the anthelmintic treatment. Such larvae are known to elicit strong acquired immunity even in weak responder strains (C57Bl/10, Table 4), presumably because of the prolonged stimulation of the intestinal mucosa by inhibited irradiated larvae. The enhanced immunogenicity of irradiated larvae must therefore be considered in interpreting the data. The results of Table 7 show that a challenge infection with normal larvae when administered 7 days after a primary infection with irradiated larvae is resisted ($56 \cdot 6\%$ protection) and that by day 14 mice are almost totally refractory to challenge. Therefore, in the absence of adult worms immunity against *N. dubius* develops very rapidly, within 7 days and is almost absolute by day 14.

Time course of worm loss following a challenge infection. Two experiments were carried out (Expts 12 & 13) to determine the time course of worm loss following a challenge infection. The experimental details together with the results are summarised in Table 8. In expt 12 some worms survived the tissue phase of development in immunised mice, at least until day 14, but by the 3rd week of infection over 80% of the worms had been rejected. Expt 13 confirmed that by the third week of infection, immunised mice had lost the majority of challenge infection worms.

Table 6. The duration of primary infection required to elicit maximum immunity to challenge

Group	No. mice‡	Primary infection	Anthelmintic on day 9	14	21	Day of challenge infection†	Day of autopsy	MWR ± s.e.	% protection*
(A) Primary infection control	3	+	-	-	-	—	21	218·0 ± 13·2	
(B) Control for efficacy of anthelmintic	3	+	+	-	-	—	49	96·3 ± 38·1	
(C) Challenge control	6	-	+	-	-	14	49	87·3 ± 3·5	
(D) 2 week immunisation	8	+	+	-	-	14	49	164·9 ± 18·0	10·2
(E) Control for efficacy of anthelmintic	3	+	+	+	-	—	56	0·3 ± 0·3	
(F) Challenge control	6	-	+	+	-	21	56	126·5 ± 6·5	
(G) 3 week immunisation	8	+	+	+	-	21	56	1·3 ± 0·5	99·0
(H) Control for efficacy of anthelmintic	3	+	+	+	+	—	63	0	
(I) Challenge control	6	-	+	+	+	28	63	103·4 ± 7·9	
(J) 4 week immunisation	8	+	+	+	+	28	63	0·7 ± 0·4	99·3
(K) Challenge control	6	-	+	+	+	35	70	135·3 ± 6·2	
(L) 5 week immunisation	8	+	+	+	+	35	70	2·0 ± 0·5	98·5

*% Protection for group D was calculated as follows:

$$\frac{MWR\ B + MWR\ C - MWR\ D}{MWR\ B + MWR\ C} \times 100.$$

†The challenge infection comprised 150 larvae of *N. dubius*.

‡ The mice used were female NIH.

Table 7. The duration of primary infection with irradiated larvae (25 krads) required to elicit maximum immunity to challenge

Group*	No. mice	Primary infection with irradiated larvae†	Day of challenge infection with normal larvae‡	Day of autopsy	MWR ± s.e.	% protection
A	6	−	0	35	165·7 ± 9·3	
B§	8	+	0	35	99·9 ± 17·2	39·7
C	5	+	−	35	1·7 ± 0·9	
D	6	−	7	42	92·5 ± 9·4	
E	8	+	7	42	40·1 ± 16·6	56·6
F	6	−	14	49	112·7 ± 7·7	
G	8	+	14	49	0·5 ± 0·2	99·99
H	6	−	21	56	119·2 ± 7·8	
I	8	+	21	56	0	100
J	6	−	35	70	141·0 ± 10·2	
K	8	+	35	70	0·1 ± 0·1	100

*All groups consisted of female NIH mice.

†Primary infection comprised 250 infective larvae exposed to 25 krad of gamma radiation. An additional control group of three mice received 250 normal larvae on day 0 and 181·7 ± 24·4 worms were recovered on day 35, confirming the infectivity of the larvae prior to irradiation.

‡All groups except group C, received 150 normal larvae on the days shown.

§Note: this group received 250 irradiated + 150 normal larvae on day 0.

Mouse strain variation in acquired immunity following a 9 day anthelmintic abbreviated infection

Tables 9 & 10 summarise the results of four experiments (14, 15, 16 & 17) in which different strains of mice, including two F_1 hybrids, were subjected to the 9 day anthelmintic abbreviated infection. The tables show the degrees of acquired immunity elicited in each strain. All four experiments were controlled by the inclusion of NIH mice and it can be seen that immunity was consistently high in this strain. Three experiments included C57Bl/10 mice, but the degree of immunity ranged from 7·6 to 41·3% protection; more variable than was expected but significantly weaker than in NIH mice. Several points emerge from the results presented in Tables 9 & 10.

(1) Some strains of mice reject primary infection worms, notably SJL (Expt 14) and SWR (Expt 15). DBA/1, DBA/2 (Expt 15) and both F_1 hybrid mice (Expt 17) had fewer worms than NIH killed 5 weeks after primary infection but the reduction in worm burden was not as marked as in SJL and SWR mice.

(2) All the strains which showed evidence of an ability to reject primary infection worms acquired strong immunity to the challenge infection. Only NIH mice were

Table 8. Time course for worm loss following a challenge infection

	Expt 12†		Expt 13‡	
Days after challenge infection*	Control group	Experimental group	Control group	Experimental groups
14	134·8±1·2 (4)§	71·9±9·8 (8)	Not done	Not done
21	Not done	2·9±0·6 (8)	Not done	Not done
25	Not done	Not done	92·3±3·3 (6)	2·1±1·1 (8)
28	Not done	22·6±17·0 (8)	115·5±9·4 (6)	18·5±8·8 (8)
35	143·4±9·8 (8)	1·4±0·6 (8)	73·2±9·6 (6)	0·9±0·4 (8)

* In both experiments anthelmintic was given on days 9, 14 and 21 and the mice were challenged 28 days following the primary infection. Female NIH mice were used in both experiments.

†In Expt 12, a primary infection of 250 larvae was given on day 0 and an infectivity control group of six mice, killed 3 wks later had a MWR of 188·3±3·7.

‡In Expt 13, the primary infection also comprised 250 larvae but the infectivity control group of six mice killed on day 21 had a MWR of 223·2±11·6.

§The numbers in parenthesis denote the number of mice in each group.

classified as strong responders with respect to acquired immunity, but were incapable of rejecting primary infection worms.

(3) Some of the mouse strains most widely used for immunological research (C57Bl/10, CBA, and C_3H) were found to be weak responders to *N. dubius*.

(4) With only a few exceptions (C57Bl/10 mice, Expts 16 & 17; B10G mice, Expt 17) most strains were separated into weak responders or strong responders, there being a clear distinction between 90%+ levels of protection expressed by the latter and the considerably weaker responses of the former (generally less than 20% protection).

(5) Four strains of mice sharing the $H-2^q$ haplotype were ranked as strong responders. However, B10G mice which are $H-2^q$, but have a B10 background were generally found to be intermediate in their level of responsiveness.

Discussion

The most important conclusion from the present work is that a brief anthelmintic abbreviated primary infection, which prevented adult worms from accumulating in the intestine, was extremely immunogenic in certain mouse strains. In NIH mice such an infection was more effective at stimulating acquired immunity than longer infections in which adult worms had been allowed to reside in the intestine for several weeks (Table 3). It might be expected that the more prolonged the period of immunization the greater the

Table 9. Comparison of acquired immunity elicited by a 9-day anthelmintic abbreviated infection in different strains of mice

Strain	H-2 haplotype	Expt 14			Expt 15			Expt 16			Expt 17		
		MWR±s.e. challenge control group	MWR±s.e. experimental group	% protection	MWR±s.e. challenge control group	MWR±s.e. experimental group	% protection	MWR±s.e. challenge control group	MWR±s.e. experimental group	% protection	MWR±s.e. challenge control group	MWR±s.e. experimental group	% protection
C57Bl/10	b	111·3±4·3 (6)†	102·8±9·4 (8)*†	7·6				85·8±8·4 (4)‡	50·4±12·3‡	41·3	98·0±4·7 (6)*	66·4±9·0 (10)*	32·2
CBA	k	96·7±5·8 (6)	97·2±4·7 (10)	0				74·8±3·9 (4)	77·6±5·5 (10)	0			
C3H/Hej	k	119·2±5·9 (6)†	100·2±11·2 (10)†	15·9				69·5±7·4 (4)	80·4±8·5 (8)	0			
BALB/c	d	118·3±8·4 (6)§	32·1±12·9 (10)§	72·8				75·7±4·2 (4)¶	0·7±0·2 (10)¶	99·1			
B10G	q	100·8±3·2 (6)‡	82·6±13·5 (10)‡*	18·1									
NIH	q	117·5±7·3 (6)¶	2·0±0·4 (9)¶	98·3	78·4±9·4 (5)¶	0·9±0·6¶	98·9	79·7±1·6 (6)¶	0·5±0·2 (12)¶	99·4	94·3±2·7 (3)*	32·3±8·7 (6)*	65·7
SJL	s	14·5±13·9 (6)¶	0·2±0·2 (10)¶	98·6							94·0±3·7 (6)¶	8·6±5·8 (10)¶	90·9
DBA/2	d				58·5±10·2 (5)¶	1·8±0·8 (8)¶	96·9						
DBA/1	q				50·5±10·8 (6)¶	0 (9)¶	100						
SWR	q				15·2±9·2 (6)¶	1·0±0·3 (9)*	93·4						
(NIH × C57Bl/10) F1	bq										46·2±19·2¶	3·8±3·0¶	91·8
(NIH × B10G) F1	q										39·5±16·4¶	0·9±0·7¶	97·7

In all experiments challenge control and immunised mice were killed for worm counts 5 weeks following the administration of the challenge infection.

The numbers in parenthesis denote the number of mice in each group.

Statistical analysis of results: Paired groups were compared and found to have the following statistical significance: *P < 0·05, †P > 0·1, ‡P > 0·05, §P < 0·005, ¶P < 0·001.

Table 10. Variation between mouse strains in ability to express primary and acquired immunity to *N. dubius*

Strain	H-2 haplotype	Background genes	Immunity to*		Responder status
			Primary infection	Challenge infection	
CBA	k	CBA	−	−	
C₃H	k	C₃H	−	−	Weak
C57Bl/10	b	B10	−	+	
B10G	q	B10	−	+ +	
BALB/c	d	BALB	−	+ +	Intermediate
NIH	q	NIH	−	+ + +	
DBA/1	q	DBA/1	+	+ + +	
DBA/2	d	DBA/2	+	+ + +	
(NIH × C57Bl/10) F₁	bq	NIH/B10	+	+ + +	Strong
(NIH × B10G) F₁	q	NIH/B10	+	+ + +	
SWR	q	SWR	+ +	+ + +	
SJL	s	SJL	+ +	+ + +	

*The relative ranking of stains was arranged on the basis of the experiments reported in this paper and on unpublished experiments repeating the comparison between strains. As has been pointed out recently (Wassom *et al.* 1983) the immunity expressed by a given strain will vary from experiment to experiment, but despite this interexperimental variation, the relative rankings of the strains remains constant.

subsequent response but this is evidently not so. In fact the data presented here agrees with the hypothesis that adult *N. dubius* exert a significant immunomodulatory influence on the host's ability to mount a host protective response and hence extension of the period of residence of adult worms is counterproductive to the generation of acquired immunity.

The efficacy of the 9-day anthelmintic abbreviated infection at stimulating acquired immunity in NIH mice was remarkable. Very few larvae were required to elicit acquired immunity, even a single worm inducing a detectable effect. Immunity followed rapidly after the worms were eliminated by drug treatment and by day 21, the mice were almost totally immune. The rapid onset of immunity to challenge infection, following a primary infection restricted to larval worms only, was confirmed using irradiated larvae, which do not mature. Thus significant immunity to challenge infection was demonstrated as early as 7 days post-infection with irradiated larvae and was almost complete by day 14 (Table 7). Furthermore it was established that the irradiated larvae were more effective at inducing immunity than the 9-day anthelmintic abbreviated infection because they elicited strong resistance in C57Bl/10 mice whilst the latter regime had only a weak effect (Table 4).

Initially several experiments were carried out comparing challenge infections superimposed on uninterrupted primary infection with those administered to mice which had been immunised by abbreviated infections (Tables 1 & 2). Superimposed challenge infections were rarely effectively resisted and even when significant immunity was demonstrated (Table 2) the interpretation of the data was not straightforward. A variety of results was experienced, ranging from complete establishment and superimposition of the challenge on the primary infection (in weak responder strains and in strong responder mice with a short interval between infection and challenge) to complete expulsion of both primary and challenge infection worms (after a long primary infection in responder mice). Similar results have been reported previously (Cypess & Zidian 1975). The variation in responsiveness precludes the use of superimposed challenge infections in experiments seeking to identify, possibly subtle genetically determined differences in the immunological mechanisms governing the acquisition of immunity to *N. dubius*.

With the identification of the optimum parameters for the induction of maximum immunity in NIH mice, it was possible to extend the work by applying the 9-day anthelmintic abbreviated infection regime to other strains of mice. Some of the strains had been examined by other workers, but mostly by using complex immunising regimes involving repeated infections. In this context it is interesting that in recent work by Jacobson *et al.* (1982) a simple anthelmintic abbreviated infection was used to discriminate between the responder hybrid strain LAF_1/J and CBA/J and BALB/c mice, the latter two strains failing to acquire immunity after this regime. LAF_1/J mice, however, were good responders behaving much like NIH mice in the present work.

In general there has been close agreement between the different research groups as to which mouse strains are weak responders with only a few notable exceptions (e.g. BALB/c mice). Thus CBA and C57Bl/10 mice are universally regarded as weak responders (Cypess *et al.* 1977, Prowse *et al.* 1979, Cypess & Zidian 1975, Liu 1966). C_3H mice were designated weak responders by Behnke & Wakelin (1977) and by Liu (1966) but Prowse *et al.* (1979) found female C_3H/HeJ mice to be strong responders. There is some controversy regarding BALB/c mice. In our laboratory different mice of this strain have ranged in performance from weak ($<20\%$ protection) to strong (almost 100% protection) in response to *N. dubius*. We have therefore designated this strain as an intermediate responder, since it is not as consistent as the other strong responder strains. BALB/c mice have been found to be inferior responders relative to NIH mice (Behnke & Wakelin 1977) and LAF_1/J mice (Jacobson *et al.* 1982) but Mitchell & Prowse (1979), Prowse *et al.* (1979) and Hurley & Vadas (1983) have found BALB/c mice to be among the strong responder strains. Differences in the immunising schedules used may be responsible for these seemingly incompatible results; the latter authors all using multiple infection regimes. Jacobson *et al.* (1982) found that BALB/c mice did not show immunity after a single brief primary infection but expressed strong resistance after two such infections, and this is confirmed by our experience of this strain.

The panel of 12 mouse strains examined in Expts 14–17 (Tables 9 & 10) included mice sharing background and/or MHC genes. It is evident from the results that the response phenotype is influenced by both background and MHC genes but their relative importance is not yet understood. In general mice sharing the H-2^q haplotype were strong responders, the only exception being B10G mice which are a congenic strain on a B10 background. Nevertheless, B10G mice consistently performed better than C57Bl/10 mice suggesting that the H-2^q haplotype exerts a beneficial moderating influence.

Limited breeding experiments established that the strong responder phenotype was inherited as a dominant trait. This conclusion is consistent with reports in the literature relating to the genetic control of immunity to other helminth parasites (Wakelin 1975 1982, Wassom, De Witt & Grundmann 1974) and with the results obtained by Prowse & Mitchell (1980). The latter authors found that the F_1 progeny of C57Bl/10 ♀ (weak responders) and SJL/J ♂ (strong responders) inherited the response phenotypes of the SJL/J parents. However, our experiments have demonstrated that in the case of both (C57Bl/10 × NIH) F_1 and (B10G × NIH) F_1, the hybrids were superior responders to the NIH parental strain. F_1 hybrids of the above combinations consistently gave stronger responses than NIH mice, suggesting that whilst B10 background genes do not determine a strong responder phenotype by themselves, a combination of B10 and NIH background genes results in gene complementation enhancing the responder status of the F_1 progeny. This enhanced resistance to *N. dubius* was expressed as an ability to acquire strong immunity and by the capacity to reject primary infection worms within 5 weeks.

Although a limited range of mouse strains was examined in the present study, the strains which were chosen included mice sharing background genes but differing in MHC haplotype, sharing identical MHC haplotype but with different background genes, strains known to be weak and strong responders to *N. dubius*. It is interesting that our data has several similarities to the more extensive survey of mouse strains carried out by Wassom *et al.* (1983) in relation to susceptibility to *T. spiralis*. When mouse strains were ranked in decreasing order of susceptibility to *T. spiralis*, mice with the H-2^k haplotype were the most susceptible and those bearing H-2^q and H-2^s were most resistant, as we have found for *N. dubius*. However, Wassom *et al.* (1983) found that SJL mice ranked in mid-order and were not extremely resistant to *T. spiralis* as they are to *N. dubius* (Tables 9 & 10). Considerably more inbred, congenic and recombinant strains of mice will need to be examined before the means whereby gene products influence particular components of host protective immunity to *N. dubius* can be understood. The work described in this paper has provided baseline data on which to design such studies in the future.

Acknowledgements

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