

**Immunological relationships during primary infection with  
*Heligmosomoides polygyrus* (*Nematospiroides dubius*): the capacity of  
adult worms to survive following transplantation to recipient mice**

J. M. BEHNKE, DIANE J. WILLIAMS, J. HANNAH  
and D. I. PRITCHARD

MRC Experimental Parasitology Research Group, Department of Zoology, University of  
Nottingham, University Park, Nottingham NG7 2RD

(Accepted 1 April 1987)

SUMMARY

Chronic primary infections with *Heligmosomoides polygyrus* (*Nematospiroides dubius*) are still relatively poorly documented, particularly in relation to the role of host resistance in limiting worm survival. In the present work the duration of infection with *H. polygyrus* was studied in CFLP mice given doses of infective larvae ranging from 50 to 500 L<sub>3</sub>. The least heavily infected (50 L<sub>3</sub>) group ceased egg production earliest (week 36) whereas eggs were still detected in the faeces of mice given 500 larvae in week 42. At autopsy (week 42) mice given 50 larvae had virtually lost their entire worm burden with 5 out of 11 mice still harbouring a single worm each. However, all the mice in the group given 500 larvae were still infected, the highest worm burden being 93. The concentration of serum IgG1 and specific antibody was highest in mice given 500 larvae, but sera taken from mice with declining worm burdens 19–38 weeks post-infection did not contain detectable host-protective antibody. During the course of infection in CFLP mice, *H. polygyrus* sustained irreversible changes in its capacity for subsequent survival. Thus, adult worms transferred to naive mice 2, 7, 14, 30 or 36 weeks post-infection did not live longer than worms of a comparable age in the respective donor group. In contrast, primary infection worms taken from jirds in which expulsion is usually completed by 6 weeks post-infection, re-established in mice and survived considerably longer than in the group of donor jirds. These results were discussed in relation to the possible interactions between parasite senility and immunomodulation, and host resistance in limiting primary infections with *H. polygyrus* in mice and jirds.

INTRODUCTION

The parasitic nematode *Heligmosomoides polygyrus* is capable of long-term survival in most strains of laboratory mice following a single administration of infective larvae (Ehrenford, 1954; Behnke & Robinson, 1985; Keymer & Hiorns, 1986). This longevity of *H. polygyrus* contrasts with the relatively brief existence of *Nippostrongylus brasiliensis* and *Trichinella spiralis*, both of which are expelled by rodents within 5 weeks of infection (Miller, 1984). *H. polygyrus* is believed to evade host-protective immune responses through immunomodulatory activity which impairs both afferent and efferent arms of the intestinal immune system (Behnke, 1987), enabling the parasites to live for 8–10 months, considerably longer than related species in rodent hosts. Although little is known about the nature of the factors involved and about their influence on the immune system at the cellular and molecular levels, it is predicted that the expression of host protective responses in infected mice must be preceded by resistance to the immunomodulatory evasive mechanisms of the parasite (Behnke & Parish, 1979).

Most strains of mice acquire some capacity for resisting *H. polygyrus* following

exposure to repeated or abbreviated infection protocols (Prowse, Mitchell, Ey & Jenkin, 1979; Behnke & Robinson, 1985) and the mechanisms involved have been studied in several laboratories in recent years (Dobson & Cayzer, 1982; Jacobson, Brooks & Cypess, 1982; Pritchard, Williams, Behnke & Lee, 1983; Pentilla, Ey, Lopez & Jenkin, 1985). However, immunological events during the chronic primary infection, particularly in the terminal stages, 6–10 months after initial exposure to larvae, have been relatively neglected. Nevertheless, this may be a particularly crucial phase of the interaction between host and parasite because there exists the possibility that the strategies used by *H. polygyrus* to evade host immunity during the initial stages of infection cease to be effective in old worms, thereby allowing the host to remove the parasites through the expression of previously suppressed mechanisms. Alternatively, the loss of 8 to 10-month-old worms may simply ensue from senility, i.e. endogenous changes within the parasites which are not influenced by the host. A third possibility is that the worms do not undergo changes within the period of infection contributing to their demise but the host, through prolonged exposure to immunomodulatory molecules, develops counter-effective factors (e.g. antibodies) which enable host-protective responses to be fully expressed.

In this paper, we report experiments which were carried out to determine whether the loss of adult *H. polygyrus* in the terminal stages of a chronic primary infection is mediated through the immunological activity of the host and is accompanied by irreversible changes to the parasite's capacity for survival. Worms from jirds (*Meriones unguiculatus*) were studied for comparison because, in contrast to mice, *M. unguiculatus* expel adult *H. polygyrus* through an intestinal immune response within 6 weeks of infection (Jenkins, 1977; Hannah & Behnke, 1982).

#### MATERIALS AND METHODS

##### *Animals*

Randomly bred CFLP and syngeneic NIH mice were used in this study. Both strains of mice and jirds were bred in the department and housed 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 carried out as reported by Behnke & Parish (1979). A detailed description of the methods used to transplant adult worms was given by Behnke, Hannah & Pritchard (1983).

##### *Measurement of immunoglobulins and antibodies in sera from infected mice*

Immunoglobulin levels were determined by single radial immunodiffusion (Mancini assay) as described by Williams & Behnke (1983). Goat anti-mouse subclass sera were purchased from Serotec (Bicester, U.K.) and diluted in phosphate-buffered saline (PBS) before addition to immunodiffusion plates. Immunoglobulin levels were calculated by reference to calibration curves prepared with mouse standards.

Antibodies specific for adult worm homogenate were detected using an ELISA assay. Adult homogenate in PBS was diluted in carbonate-bicarbonate buffer, pH9, to a final

concentration of 400 µg/ml and the assay conducted as described by Ruitenbergh, Steerenbergh, Brosi & Buys (1979) using a urease-conjugated second antibody (Sera Lab, Crawley).

Immune mesenteric lymph node cells (IMLNC) were prepared as described by Behnke & Parish (1981) and Williams & Behnke (1983). Recipient mice were given the cells in 0.5 ml of medium, 4 days before infection. Challenge infection larvae were exposed to 5 krad. of Cobalt 60 gamma-radiation as reported by Behnke, Parish & Hagan (1980). Infective larvae prepared in this way are particularly sensitive to protective responses by the host (Behnke & Parish, 1981) and, in comparison to normal larvae, the reduction of worm burdens in recipient mice following passive or adoptive transfer of immunity is markedly enhanced. Primary infection sera were stored at -20 °C until required and were administered to recipient mice as follows: 0.5 ml on day 0, 1.0 ml on day 1 and 0.5 ml on day 3.

#### *Statistical analysis of results*

Faecal egg counts are presented as the mean value of 4 counts on each 1 g sample of faeces (e.p.g.). Worm counts are given as the mean worm burden  $\pm$  S.E.M. When applicable the non-parametric Mann-Whitney U test was used to compare groups for significant differences (Sokal & Rohlf, 1969) and a value of  $P < 0.05$  was considered to be significant. Differences in the concentration of immunoglobulins were analysed by a Student's *t*-test (Sokal & Rohlf, 1969).

## RESULTS

### *The duration of infection in mice given varying numbers of $L_3$ larvae*

Four groups of 12 CFLP mice (3 groups of male and 1 group of female mice) were infected with different numbers of  $L_3$  and faecal egg counts were recorded until week 42 (Exp. 1). The 3 groups of male mice were given 500 (group A), 250 (group B) and 50 (group C) larvae respectively, whilst female mice (group D) received 250 larvae. Groups of 8 male (group E) and 8 female (group F) age-matched controls, which were left uninfected were also included in this experiment. The results are shown in Fig. 1 and Table 1. The least heavily infected group (C) became negative first, in week 36, but was followed within 5 weeks by groups B and D. The only group still producing parasite eggs albeit in very low numbers on the day when the animals were killed for worm counts was the most heavily infected group (A). Very few worms remained until week 42. Male mice given 50 larvae (group C) had virtually lost their entire worm burden, with 5 out of 11 mice still harbouring a single worm each. In group B only 2 mice were totally without worms, whereas all the mice in group A were still infected and the highest worm burden was 93.

### *IgG1 and parasite specific antibody in CFLP mice with a primary infection*

All the animals in the preceding experiment (Exp. 1) were bled at autopsy and individual sera were analysed for total IgG1 and for parasite-specific antibodies (Table 1). IgG1 levels in all the infected animals were significantly increased in relation to control groups and both IgG1 and specific antibody levels were highest in group A which received the most larvae.

A second experiment (Exp. 2) was carried out in which a group of 6 to 8-week-old female CFLP mice was infected with 200  $L_3$  and was bled at weekly intervals for 56 days

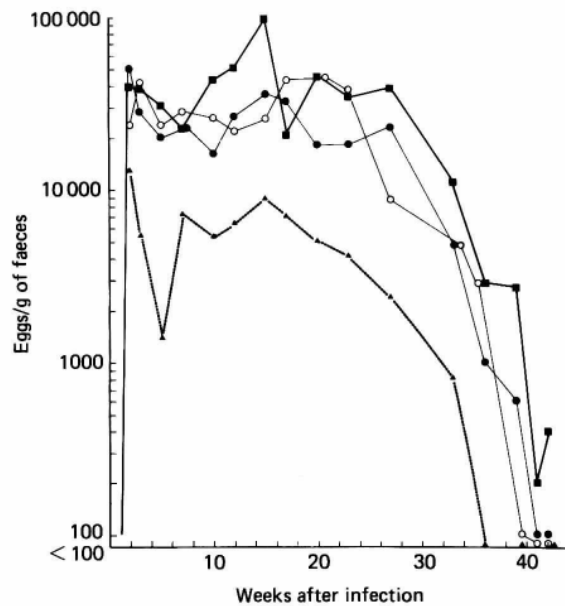


Fig. 1. Faecal egg counts during the course of a primary infection with *Heligmosomoides polygyrus* in male CFLP mice given 500 (■), 250 (●) or 50 (▲) larvae and female mice given 250 larvae (○).

Table 1. *The worm burdens, serum IgG1 concentration and specific antibodies in CFLP mice killed 42 weeks after infection with 50, 250 or 500 larvae of Heligmosomoides polygyrus*

Group and sex of mice	No. of mice surviving to week 42	No. of L <sub>3</sub> administered	Mean worm recovery ± s.e.	Range of worm burdens	Total serum IgG1 (mg/ml)	Reciprocal ELISA titre
(A) Male	7	500	26.0 ± 11.9	4-93	5.36 ± 1.32	32
(B) Male	10	250	9.5 ± 3.0	0-25	3.92 ± 0.46	16
(C) Male	11	50	0.5 ± 0.2	0-1	2.60 ± 0.36	8
(D) Female	12	250	0.5 ± 0.4	0-5	3.13 ± 0.32	32
(E) Male	8	0	—	—	1.13 ± 0.07	2
(F) Female	8	0	—	—	1.08 ± 0.07	2

after infection. A further sample of serum was obtained on day 210. Serum immunoglobulin isotypes were measured and the results are presented in Fig. 2. With the exception of IgA on day 14, only IgG1 levels showed a significant change. These rose from a resting level of 2.1 mg/ml to a maximum of 5.0 mg/ml on day 21 and thereafter progressively declined to a concentration of 3.1 mg/ml on day 210.

The possible existence of host-protective antibodies in long-term primary infection sera was investigated by passive and adoptive transfer experiments. A group of 120 female CFLP mice was infected with 250 L<sub>3</sub> and 30 mice were bled during weeks 0, 8, 19 and 38 (Exp. 3). The course of infection was monitored by regular faecal egg counts and by the worm burdens of 6 mice killed during weeks 8, 15, 19 and 38. As in previous experiments faecal egg counts were initially (weeks 2-18) steady, ranging from 20000 to

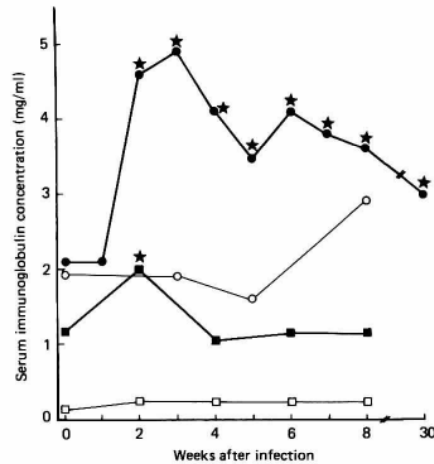


Fig. 2. The concentration of serum immunoglobulin isotypes following infection of female CFLP mice with 200  $L_3$  of *Heligmosomoides polygyrus*. IgG1 (●), IgG2 (○), IgA (■), IgM (□). \* Significantly elevated with respect to control value.

40000 e.p.g. The worm burdens in weeks 8 and 15 were  $264.5 \pm 14.5$  and  $222.4 \pm 8.5$  respectively. By week 20 faecal egg counts began to decline, culminating in a value of 200 e.p.g. during week 38. The worm burden in mice killed during weeks 19 and 38 were  $225.6 \pm 14.0$  and  $9.2 \pm 4.9$ . The pool of serum obtained during week 19 was therefore taken immediately prior to worm loss and that during week 38 when 96.5% of the worm burden had been eliminated.

Each of the 4 pools of serum was injected into 2 groups of 6 female NIH mice, alone or together with  $9 \times 10^7$  immune mesenteric lymph node cells (IMLNC). All the mice were challenged with 200 5krad. irradiated  $L_3$  and were killed 18 days later for worm counts. The mean number of worms recovered from the control group was  $181.3 \pm 4.6$ . The group receiving IMLNC alone had  $57.4 \pm 19.0$  worms, reflecting a 68.3% reduction in worm burden. All the groups of mice receiving serum alone had worm burdens indistinguishable statistically from those of the control group and the mice which were given IMLNC together with one of the serum pools had worm burdens comparable to the group given IMLNC alone (data not shown). It is clear from these results that the administration of serum from chronically infected mice had no effect on the challenge infection and did not interact synergistically with concurrently transferred IMLNC, irrespective of the time after infection when serum was taken (see Williams & Behnke, 1983; Behnke & Parish, 1981). It is therefore unlikely that the loss of primary infections from CFLP mice is accompanied by sufficient host-protective antibody in the serum to be detected in this assay. Host-protective antibody in mice which develop resistance during repeated infection with *H. polygyrus* is reliably detectable by this technique (Williams & Behnke, 1983).

*The survival of adult worms transferred from mice harbouring a primary infection, into naive recipients*

In order to determine whether the loss of parasites in mice was attributable to irreversible damage sustained by the organisms, a series of experiments was carried out

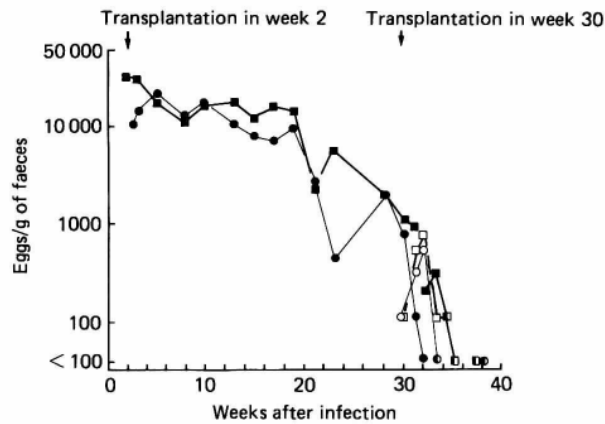


Fig. 3. Faecal egg counts in groups of mice infected with  $L_3$ , or receiving transplanted adult *Heligmosomoides polygyrus* (Exp. 4). Arrows indicate days on which worms were transplanted. (■) Group A, mice infected with 300  $L_3$  on day 0; (●) group B, mice receiving 50 worms transplanted from group A on day 14; (□) group C, mice receiving 50 worms transplanted from group A on day 210 (Week 30); (○) group D, mice receiving 50 worms transplanted from group B on day 210 (Week 30).

in which worms were transferred into naive recipients at various times in relation to infection.

In Exp. 4, a group of 12 female CFLP mice were infected with 300 larvae of *H. polygyrus*. Fourteen days later adult worms from 6 of these mice were transplanted into a group of 6 naive female CFLP mice. The faecal egg production from both groups was monitored at fortnightly intervals until week 30. Worms from half of the remaining mice in each of these 2 groups were then further transplanted into 2 groups of naive recipients, and faecal egg counts from all 4 groups were monitored until no further eggs were detected.

It is clear from Fig. 3 that transplantation of worms on day 14 did not markedly alter the survival pattern of worms. Furthermore transplantation of worms during week 30, when egg counts were falling rapidly did not improve worm survival, and consequently egg counts continued to decline in all 4 groups.

This experiment was repeated with some modifications. A group of 12 female CFLP mice were infected with 400 larvae (Exp. 5) and the faecal egg production was monitored until week 30. At this time worms were transplanted from 6 mice in this group into a group of 6 naive female recipients. Two weeks earlier, i.e. week 28, a second donor group of 12 female CFLP mice was infected with 400 larvae of *H. polygyrus* and during week 30, when these worms were only 2 weeks old, half of the donor group was killed and their parasites were transplanted into 6 recipient mice. The experiment therefore comprised 4 groups, 2 were the donor groups infected in week 0 and week 28 respectively, and 2 were recipient groups one of which received 30-week-old worms whilst the other was concurrently given 2-week-old parasites.

The faecal egg output from transplanted 30-week-old worms declined at the same rate as that of the worms in the remaining mice from the original donor group (Fig. 4). However, the faecal egg counts from the mice infected in week 28 and from those receiving 2-week-old worms during week 30 remained constant for up to 15 weeks after transplantation, but were reduced by week 57. It is clear from these results that both

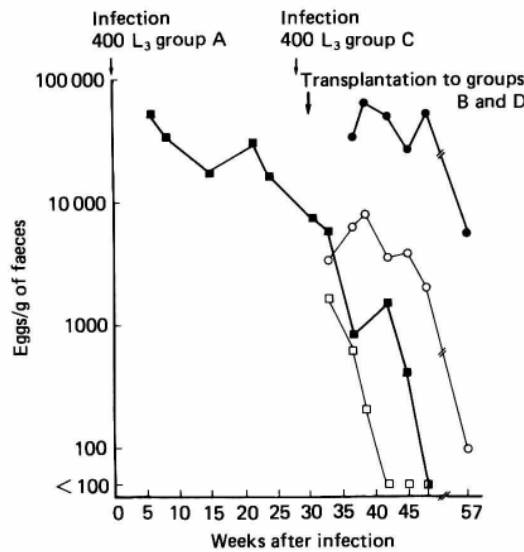


Fig. 4. Faecal egg counts in groups of mice infected with *L*<sub>3</sub>, or receiving transplanted adult *Heligmosomoides polygyrus* (Exp. 5). Arrows indicate days on which mice were infected or received worms by transplantation. (■) Group A, mice infected with 400 *L*<sub>3</sub> on day 0; (□) group B, mice receiving 50 worms transplanted from group A on day 210 (Week 30); (●) group C, mice infected with 400 *L*<sub>3</sub> on day 196 (Week 28); (○) group D, mice receiving 50 worms transplanted from group C on day 210 (Week 30).

Table 2. Plan for Experiment 6

	Weeks after infection of group A						
	0	5	7	12	14	34	36
Infect group A	—	—	Transplant to group B	—	Transplant to group E	—	Transplant to group H
—	—	Infect group C	Transplant to group D	—	—	—	—
—	—	—	—	Infect group F	Transplant to group G	—	—
—	—	—	—	—	—	Infect group I	Transplant to group J

30- and 2-week-old worms established in recipient mice, but the duration of survival of the older parasites was not prolonged beyond that in the donor group.

A final experiment (Exp. 6) was carried out along the same lines but incorporating further modifications. A donor group (A) comprising 30 female CFLP mice was infected with 300 larvae during week 0 and worms from this group were transplanted into naive recipient mice in weeks 7 (group B), 14 (group E) and 36 (group H). Two weeks prior to each transplantation additional groups of mice were infected (groups C, F and I) so as to provide concurrently 2-week-old worms for transplantation. Thus the survival of 7, 14- and 36-week-old worms was compared to that of 2-week-old worms on each occasion. Faecal egg counts were carried out on all the donor and recipient groups until no more eggs could be detected and the experiment was terminated during week 43. The experimental design is given in Table 2 and the results are presented in Fig. 5.

Faecal egg counts remained relatively steady in the original donor group (A) until



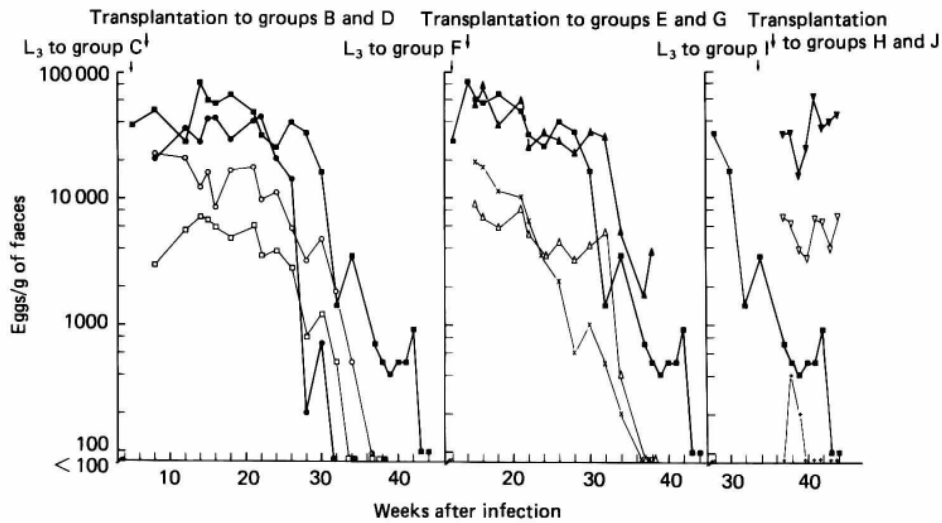


Fig. 5. Faecal egg counts in groups of mice infected with *L*<sub>3</sub>, or receiving transplanted adult *Heligmosomoides polygyrus* (Exp. 6). Arrows indicate days on which mice were infected or received worms by transplantation. (■) Group A, mice infected with 300 *L*<sub>3</sub> on day 0; (□) group B, mice receiving 50 worms transplanted from group A during week 7; (×) group E, mice receiving 50 worms transplanted from group A during week 14; (+) group H, mice receiving 50 worms transplanted from group A during week 36; (●) group C, mice infected with 300 *L*<sub>3</sub> during week 5; (○) group D, mice receiving 50 worms transplanted from group C during week 7; (▲) group F, mice infected with 300 *L*<sub>3</sub> during week 12; (△) group G, mice receiving 50 worms transplanted from group F during week 14; (▼) group I, mice infected with 300 *L*<sub>3</sub> during week 34; (▽) group J, mice receiving 50 worms transplanted from group J during week 36.

week 26, when counts began to fall steadily, but eggs were detected in the faeces until week 42. The 7-week-old worms from group A re-established in recipient mice (group B) but egg production only lasted for a further 26 weeks. Mice infected during week 5 (group C), however, showed a shorter course of egg production than expected, and faecal egg counts from group C and from group D, which received 2-week-old worms, did not extend beyond those of the original donor group A.

The transplantation of 14-week-old worms to naive recipients (group E) did not result in prolonged egg production in relation to group A. Concurrently transplanted 2-week-old worms (group G), produced fewer eggs, suggesting that establishment may have been poor, but faecal egg counts remained steady for 15 weeks in contrast to group E, before falling prematurely during week 32.

When 36-week-old worms were transplanted to naive recipients, faecal eggs were detectable for a further 2 weeks (group H). Concurrently transplanted 2-week-old worms, however, sustained high egg production until the experiment was terminated during week 43.

*The survival of adult worms transplanted from jirds harbouring a primary infection, into naive recipient mice*

Twenty-three jirds were infected with 400 larvae, and in 5 animals the course of infection was followed by regular egg counts. The remaining 18 jirds were killed in



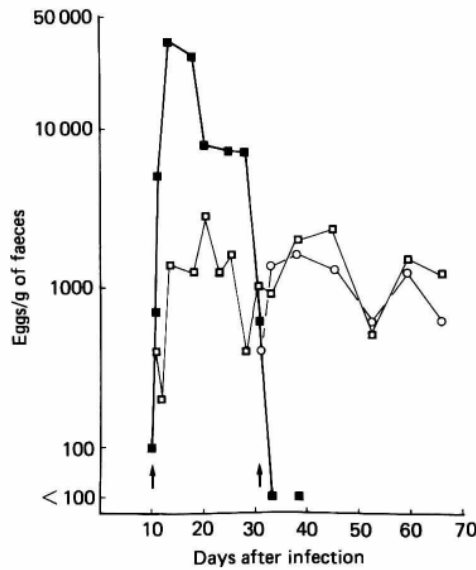


Fig. 6. Faecal egg counts from a group of donor jirds infected with 400  $L_3$  of *Heligmosomoides polygyrus* and from groups of mice receiving transplanted worms from jirds. Arrows indicate days on which worms were transplanted from jirds to mice. Data for groups of mice receiving worms from group A on days 20 and 25 is not shown. (■) Group A, jirds infected with 400  $L_3$  on day 0; (□) group B, mice receiving 20 worms by transplantation from group A on day 10; (○) group C, mice receiving 20 worms by transplantation from group A on day 31.

groups of 3–4 animals on days 10, 20, 25 and 31 and on each occasion 10 male and 10 female worms were transplanted from the donor jirds to each of 5 male CFLP mouse recipients. Faecal egg production was subsequently monitored in each group until day 70. The results are shown in Fig. 6.

Parasite eggs were first detected in jirds 10 days after infection, rose to a peak on day 14 and declined thereafter, until no more eggs could be detected on day 34. Worms established in all the recipient groups of mice and egg production continued steadily in each group until day 70. The mean number of worms recovered from groups of mice receiving worms from jirds on days 10, 20, 25 and 31 was  $7.2 \pm 2.9$ ,  $8.8 \pm 2.4$ ,  $2.9 \pm 0.9$  and  $8.3 \pm 1.5$  respectively. Despite the erratic establishment in recipient mice, some adult parasites survived for more than 4 weeks, longer than in the donor group of jirds. Five remaining jirds examined for worms on day 70 were found to have expelled all their parasites.

#### DISCUSSION

Although *H. polygyrus* is known to have the capacity to cause long-lasting infections in mice, the duration of infection is variable and influenced by the strain of mouse and the intensity of infection (Prowse *et al.* 1979; Dobson, Sitepu & Brindley, 1985). Keymer & Hiorns (1986) found that the rates of decline in parasite numbers in MFI mice were similar irrespective of the infection dose. Dobson *et al.* (1985) reported low-intensity infections to be eliminated more rapidly than heavy infections in Quackenbush mice, and our results concur with the latter study. Thus male CFLP mice, initially given 50 larvae, lost virtually their entire worm burden by week 42, whereas the more heavily

infected animals still retained significant numbers of adult worms at this time (Table 1). This is an important result because it provides a possible explanation for the data from subsequent transplantation experiments. The original donor groups were invariably given a heavy infection which lasted for at least 35 weeks. In contrast, 2-week-old worms transplanted concurrently with 7-week-old worms failed to outlive the latter or the worms in the donor groups (Fig. 5). It is possible that relatively few worms established in the recipient animals and consequently worm loss was initiated earlier. The shorter duration of low intensity infections with *H. polygyrus* is compatible with the hypothesis that worm survival is dependent on the immunomodulatory activity of adult parasites (Behnke, 1987). Support for this interpretation is found in the results of experiments analysing dose-dependent variation in the onset of the expulsion of *Trichinella spiralis* (Bell, McGregor, Woan & Adams, 1983; Bell, Adams & Ogden, 1984; Wassom, Dougherty, Krco & David, 1984; Wakelin, Donachie & Grencis, 1985). The delayed rejection of *T. spiralis* from heavily infected animals has been attributed to the slow response of a population of T lymphocytes, controlled by H-2 and non H-2 genes, which seem to be particularly sensitive to the suppressive influence of heavy parasite burdens (Wakelin *et al.* 1985). If worm loss is dependent on the sensitivity of a particular population of lymphocytes, crucial to the expression of host protective immunity, to immunomodulatory factors of both parasite species, low-intensity infections may pose less of an obstacle than heavy infections and hence expulsion would be initiated earlier.

It is clear from the results illustrated in Figs 3–5 that in no case was worm survival, as monitored by faecal egg counts, extended by transplantation of parasites into naive hosts. Therefore, it would appear that the worms undergo endogenous changes with increasing duration of infection or their evasion strategies become less effective, facilitating the expression of a protective response by the host. Although *H. polygyrus* may live for up to 46 weeks post-infection (Fig. 1) in most of our experiments survival was briefer. This variation in longevity and the fact that some inbred mouse strains (e.g. SJL) expel worms within 2 months of infection, makes it unlikely that senility is the only factor involved, and suggests that the murine host makes an active contribution to parasite loss during primary infections. Whether the host component is immunological in nature is uncertain. Our experiments did not reveal major changes in immunoglobulin or antibody levels coinciding with worm loss but we cannot exclude the possibility that intestinal IgA or cellular responses intensified during the final stages of infection, causing irreparable damage to the parasites prior to their loss from the gut.

As the course of infection progressed, *H. polygyrus* were observed to undergo changes which were evident at both the light and electron microscope levels (details to be reported elsewhere). The most obvious feature was a gradual darkening of adult worm haemoglobin from an initial bright red to an almost dark brown colour in the terminal stages of infection. Furthermore, electron microscopical analysis revealed depletion of the gonads and damage to the intestinal cells and to the body wall comparable to the changes encountered in *N. brasiliensis*, *T. spiralis*, *Strongyloides ratti* and *Haemonchus placei* during the loss phase of these species from their respective hosts (Kennedy & Bruce, 1981; Moqbel & McLaren, 1980; Ogilvie & Hockley, 1968; Harness, Smith & Bland, 1973). *T. spiralis* and *S. ratti* have been shown to recover from damage sustained during the immune response, upon transplantation to naive hosts (Kennedy & Bruce, 1981; Moqbel & McLaren, 1980), but in *N. brasiliensis* damage appears to be irreversible (Ogilvie & Hockley, 1968) and the survival characteristics of *H. polygyrus* in the present

work suggests that the same may be true of this species. The exact cause of the degenerative changes encountered in worms during expulsion has not been conclusively established, although antibody has been implicated (Jones & Ogilvie, 1971) and it has been proposed that worms driven into the suboptimal environment of the inflamed intestinal lumen may sustain damage through an inability to respire and feed normally under these circumstances (Lee, 1969; Love, Ogilvie & McLaren, 1975).

It is interesting that the loss of *H. polygyrus* from jirds was not accompanied by irreversible alterations in the capacity of parasites to survive after transplantation. Thus worms transferred from jirds into mice, even as late as day 31, re-established and continued egg production for at least 5 weeks, long after they would have been eliminated from the donor host. Infections in jirds are also subject to dose-dependent suppression, heavy worm burdens persisting longer than low intensity infections (Hannah & Behnke, 1982). However, the jird's immune system is not as susceptible to manipulation by the parasite as that of the mouse, since parasite survival is usually curtailed within 2 months of infection. Jirds are not a natural host for *H. polygyrus* in the wild (Hannah, 1983) and may not provide an environment which is entirely optimal in all respects; the growth of adult worms is slower initially than in mice although a comparable size is eventually attained (Behnke & Hannah, 1984; Jenkins, 1977). Under these conditions it is conceivable that expulsion can be effected without the pre-requirement of severe damage. In contrast, in the mouse intestine, where presumably the environment is more favourable, the worms immunomodulate successfully and probably compensate for and repair initial damage. Mice therefore may be unable to remove the parasites until changes within the organisms are so severe as to markedly enhance their susceptibility to other effector mechanisms. The crucial question, however, is whether these changes are entirely endogenous or whether they are brought about by a first-step process, mediated through antibodies as has been suggested for *N. brasiliensis* (Jones & Ogilvie, 1971). It will now be interesting to determine whether *H. polygyrus* from SJL mice just prior to rejection, exhibit irreversible changes which increase susceptibility to a second-step expulsive mechanism and to establish whether the capacity of these mice to terminate adult worm infections so rapidly in relation to other strains is linked to a genetically determined insusceptibility to parasite-mediated immunodulation.

We would like to thank Professors P. N. R. Usherwood and D. Wakelin for the provision of facilities for this study in the Zoology Department at Nottingham University. The work was supported by the MRC through project grants G967/935/T and G810015T to J. M. B. D. J. L. W. and J. H. held post-graduate studentships from the MRC and the University of Nottingham respectively, for which we are grateful. We would like to express our gratitude to K. Cosgrove who supervised the maintenance of our experimental animals.

#### REFERENCES

- BEHNKE, J. M. (1987). Evasion of immunity by nematode parasites causing chronic infections. *Advances in Parasitology*, (in the Press).
- BEHNKE, J. M. & HANNAH, J. (1984). *Nematospiroides dubius* in the jird, *Meriones unguiculatus*: stimulation and expression of acquired immunity. *Journal of Helminthology* **58**, 221-9.
- BEHNKE, J. M., HANNAH, J. & PRITCHARD, D. I. (1983). Evidence that adult *Nematospiroides dubius* impair the immune response to a challenge infection. *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**, 13-26.
- BEHNKE, J. M. & PARISH, H. A. (1981). Transfer of immunity to *Nematospiroides dubius*: co-operation between lymphoid cells and antibodies in mediating worm expulsion. *Parasite Immunology* **3**, 249-59.

- 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**, 173–82.
- BEHNKE, J. M. & ROBINSON, M. (1985). Genetic control of immunity to *Nematospiroides dubius*: a 9 day anthelmintic abbreviated immunizing regime which separates weak and strong responder strains of mice. *Parasite Immunology* **7**, 235–53.
- BELL, R. G., ADAMS, L. S. & OGDEN, R. W. (1984). *Trichinella spiralis*: genetics of worm expulsion in inbred and F<sub>1</sub> mice infected with different worm doses. *Experimental Parasitology* **58**, 345–55.
- BELL, R. G., MCGREGOR, D. D., WOAN, M. C. & ADAMS, L. S. (1983). *Trichinella spiralis*: selective intestinal immune deviation in the rat. *Experimental Parasitology* **56**, 129–42.
- DOBSON, C. & CAYZER, C. J. R. (1982). Passive transfer of immunity with serum in mice infected with *Nematospiroides dubius*: *in vitro* effect of immune serum on larval infectivity. *International Journal for Parasitology* **12**, 413–21.
- DOBSON, C., SITEPU, P. & BRINDLEY, P. J. (1985). Influence of primary infection on the population dynamics of *Nematospiroides dubius* after challenge infections in mice. *International Journal for Parasitology* **15**, 353–9.
- EHRENFORD, F. A. (1954). The life cycle of *Nematospiroides dubius* Baylis (Nematoda: Heligmosomidae). *Journal of Parasitology* **40**, 480–1.
- HANNAH, J. (1983). Studies on the host–parasite relationships of *Nematospiroides dubius* in normal and abnormal hosts. Ph.D. thesis, University of Nottingham.
- HANNAH, J. & BEHNKE, J. M. (1982). *Nematospiroides dubius* in the jird, *Meriones unguiculatus*: factors affecting the course of a primary infection. *Journal of Helminthology* **56**, 329–38.
- HARNESSE, E., SMITH, K. & BLAND, P. (1973). Structural changes in the bovine nematode *Haemonchus placei*, that may be associated with host immune response. *Parasitology* **66**, 199–205.
- JACOBSON, R. H., BROOKS, B. O. & CYPRESS, R. H. (1982). Immunity to *Nematospiroides dubius*: parasite stages responsible for and subject to resistance in high responder (LAF<sub>1</sub>/J) mice. *Journal of Parasitology* **68**, 1053–8.
- JENKINS, D. C. (1977). *Nematospiroides dubius*: the course of primary and challenge infections in the Jird, *Meriones unguiculatus*. *Experimental Parasitology* **41**, 335–40.
- JENKINS, S. N. & BEHNKE, J. M. (1977). Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*. *Parasitology* **75**, 71–8.
- JONES, V. E. & OGILVIE, B. M. (1971). Protective immunity to *Nippostrongylus brasiliensis*: the sequence of events which expels worms from the rat intestine. *Immunology* **20**, 549–61.
- KENNEDY, M. W. & BRUCE, R. G. (1981). Reversibility of the effects of the host immune response on the intestinal phase of *Trichinella spiralis* in the mouse, following transplantation to a new host. *Parasitology* **82**, 39–48.
- 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, B* **229**, 47–67.
- 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**, 29–30.
- 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**, 275–83.
- MILLER, H. R. P. (1984). The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. *Veterinary Immunology and Immunopathology* **6**, 167–259.
- MOQBEL, R. & MCLAREN, D. J. (1980). *Strongyloides ratti*: structural and functional characteristics of normal and immune-damaged worms. *Experimental Parasitology* **49**, 139–52.
- OGILVIE, B. M. & HOCKLEY, D. J. (1968). Effect of immunity on *Nippostrongylus brasiliensis* adult worms: reversible and irreversible changes in infectivity, reproduction and morphology. *Journal of Parasitology* **54**, 1073–84.
- PENTILLA, I. A., EY, P. L., LOPEZ, A. F. & JENKIN, C. R. (1985). Suppression of early immunity to *Nematospiroides dubius* in mice by selective depletion of neutrophils with monoclonal antibody. *Australian Journal of Experimental Biology and Medical Science* **63**, 531–43.
- PRITCHARD, D. I., WILLIAMS, D. J. L., BEHNKE, J. M. & LEE, T. D. G. (1983). The role of IgG<sub>1</sub> hypergammaglobulinaemia in immunity to the gastrointestinal nematode *Nematospiroides dubius*. The immunochemical purification, antigen-specificity and *in vivo* anti-parasite effect of IgG<sub>1</sub> from immune serum. *Immunology* **49**, 353–65.

- PROWSE, S. J., MITCHELL, G. F., EY, P. L. & JENKIN, C. R. (1979). The development of resistance in different inbred strains of mice to infection with *Nematospiroides dubius*. *Parasite Immunology* **1**, 277-88.
- RUITENBERG, E. J., STEERENBERG, P. A., BROSI, B. J. M. & BUYS, J. (1976). Reliability of the enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of *Trichinella spiralis* infections in conventionally raised pigs. *Journal of Immunological Methods* **10**, 67-83.
- SOKAL, R. R. & ROHLF, F. J. (1969). *Biometry*. San Francisco: Freeman.
- WAKELIN, D., DONACHIE, A. M. & GRENCIS, R. K. (1985). Genetic control of immunity to *Trichinella spiralis* in mice: capacity of cells from slow responder mice to transfer immunity in syngeneic and F<sub>1</sub> hybrid recipients. *Immunology* **56**, 203-11.
- WASSOM, D. L., DOUGHERTY, A., KRICO, C. J. & DAVID, C. S. (1984). H-2 controlled, dose dependent suppression of the response that expels adult *Trichinella spiralis* from the small intestine of mice. *Immunology* **53**, 811-18.
- WILLIAMS, D. J. & BEHNKE, J. M. (1983). Host protective antibodies and serum immunoglobulin isotypes in mice chronically infected or repeatedly immunized with the nematode parasite *Nematospiroides dubius*. *Immunology* **48**, 37-47.