

Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): dose-dependent expulsion of adult worms

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SUMMARY

The survival of *Heligmosomoides polygyrus* was monitored during primary infections in female C₅₇Bl₁₀, NIH and BALB/c mice at low and high intensities of infection. Survivorship curves were fitted for each data set and analysed. C₅₇Bl₁₀ mice, given either low or high intensities of infection, harboured parasites for 28–37 weeks, heavier infections surviving marginally but significantly longer. Essentially the survivorship curves of *H. polygyrus* in C₅₇Bl₁₀ mice could be accounted for by senility, the increased probability of worms with a longer life-span occurring at high infection intensities and, possibly, by a contribution from host-protective immune mechanisms in the terminal stages of infection. The pattern of survivorship was different in NIH and BALB/c mice. NIH mice showed weak but significant density-dependent suppression of parasite loss and infections in this strain did not exceed 27.5 weeks in duration. Primary infections in BALB/c mice were briefer still and showed marked dependence on parasite density. Thus low-level infections lasted 10–15 weeks whereas heavier infections survived for 21–34 weeks. The data suggested that both strains developed host-protective responses to adult *H. polygyrus* and that parasite survival was curtailed earlier than would be expected if senility alone was involved. The hybrid strains (C₅₇Bl₁₀ × NIH)F1 and (B10G × NIH)F1 both expelled *H. polygyrus* in a dose-dependent manner, worm loss commencing within 10 weeks of infection. In some experiments worm loss was clearly evident by weeks 4 and 6. These hybrid strains showed gene complementation in that adult worms were cleared considerably earlier than in parental strains.

INTRODUCTION

The longevity of *Heligmosomoides polygyrus* in many commonly used laboratory mouse strains is well established. Since the early work of Ehrenford (1954) experiments in various laboratories have confirmed that adult worms may survive in mice for many months following a single pulse administration of infective larvae (Liu, 1966; Day, Howard, Prowse, Chapman & Mitchell, 1979; Behnke & Robinson, 1985; Keymer & Hiorns, 1986). The inability of mice to mount rapid host-protective responses to primary infection with *H. polygyrus* contrasts markedly with the brevity of infections with *Nippostrongylus brasiliensis* and *Trichinella spiralis* in the same host. The chronic infections with *H. polygyrus* have been attributed to the immunodepressive activity of the adult worm population influencing immunoregulatory mechanisms locally in the intestinal environment (Dobson & Cayzer, 1982; Dehlawi & Wakelin, 1988; reviewed by Behnke, 1987).

Despite the general agreement that *H. polygyrus* is a long-lived parasite in the mouse, there is evidence

that the pattern of survival (survivorship curve) may be different among the mouse strains and influenced by the intensity of infection (Liu, 1966; Prowse, Mitchell, Ey & Jenkins, 1979; Dobson, Sitepu & Brindley, 1985). Detailed data for survivorship, based on worm counts are rare in the literature, most studies of longevity being based on faecal egg counts (Liu, 1966; Behnke & Robinson, 1985). Dobson *et al.* (1985) reported that high-intensity infections lasted longer than low-intensity infections in Quackenbush mice, a finding confirmed by monitoring faecal egg counts in CFLP mice (Behnke, Williams, Hannah & Pritchard, 1987). The results from both studies are consistent with the view that adult worms survive through exerting an immunodepressive influence on the host. Heavy infections with larger numbers of adult parasites would exert a stronger immunodepressive effect on the host than less intense infections. These studies, however, contrast with the report by Keymer & Hiorns (1986) who did not find a density-dependent effect on parasite survival in the randomly bred MF1 strain.

The involvement and interaction of genetic and immunological factors in determining the duration of primary infection with *H. polygyrus* is still a poorly understood aspect of the host-parasite relationship, contrasting with the wealth of information on acquired resistance to this species (Behnke, 1987).

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Nevertheless, it is during primary exposure that the adult parasite's mechanism for evading host immunity is most successful; resistance to reinfection is readily inducible even in strains which tolerate chronic primary infections (Behnke & Robinson, 1985; Prowse *et al.* 1979; Behnke & Wakelin, 1977; Mitchell & Munoz, 1983). The B₁₀ and BALB congenic series of mouse strains provide a convenient means through which the effect of H-2 linked and background genes on resistance may be investigated, but a detailed knowledge of parasite survival in the original reference strains (C₅₇Bl₁₀ and BALB/c) is first required to enable economic experimentation. In this paper we report the results of experiments in which the survivorship curves of *H. polygyrus* were determined under low and high intensities of infection in C₅₇Bl₁₀ and BALB/c mice. We also present data for NIH and (C₅₇Bl₁₀ × NIH)F1 hybrid strains.

MATERIALS AND METHODS

Animals

Syngeneic NIH, C₅₇Bl₁₀ and BALB/c mice were originally purchased from Harlan Olac Ltd and, together with the hybrid strains, were bred in the departmental animal house. The mice were 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).

Statistical analysis of results

In order to allow a direct comparison between the rate of worm loss in mice given high and low intensities of infection, all experiments included groups of mice killed 14 days post-infection. The worm burdens for all the mice on day 14 and for all subsequent autopsies were then transformed into a percentage of the 14-day mean worm burden for each experimental group (mean percentage worm recovery, MPWR). Some data were presented as the mean worm recovery (MWR) ± S.E.M. using non-transformed, raw data.

Survival curves were fitted using the function

$$P = a + br^t,$$

where P = worm burden expressed as a percentage of the day 14 mean worm recovery, t = weeks after infection and a , b and r are constants describing the best fit for this function to each data set (see Keymer & Hiorns, 1986). The best fit was obtained using maximum likelihood methods (the package MLP: Ross, 1980) because it enabled us to assume Poisson-

distributed errors essential for these data. Two curves were assessed as significantly different using a likelihood ratio test (model deviance, distributed as chi-squared), by fitting the curves individually and then a single curve to all points. The maximum likelihood for the curve with Poisson-distributed errors is:-

$$-\sum_i m_i + \sum_i y_i \log m_i - \sum_i \log y_i,$$

where $m_i = a + br^t$.

Data for particular time points post-infection were also compared using the Mann-Whitney U test (Sokal & Rohlf, 1969).

RESULTS

Experimental design

Nine experiments were carried out and the composition of each, including the strain and sex of mouse and the intensity of infection administered, is presented in Table 1. The number of parasites establishing in each experimental group as reflected in worm burdens 14 days post-infection, is also given. Low intensities of infection were established by giving mice 50–100 L₃ and, as can be seen from Table 1, the MWR 14 days post-infection varied from 15.0 ± 1.7 (Exp. 7a) to 67.0 ± 8.0 (Exp. 4a), but there were no significant differences (Mann-Whitney U test) in parasite establishment between groups within any particular experiment. High-intensity infections were achieved by giving 250–300 L₃ and the range of MWR on day 14 was from 156.5 ± 6.1 (Exp. 9a) to 280.2 ± 11.2 (Exp. 6b). Again, no significant differences were detected between groups within any one experiment.

Survival of *Heligmosomoides polygyrus* in C₅₇Bl₁₀ mice at low infection intensity

The survival of *H. polygyrus* in C₅₇Bl₁₀ mice was studied in two experiments and the results are presented in Fig. 1 (Exp. 1) and Fig. 2 (Exp. 2). Survivorship curves were fitted to the data and the values for the constants are given in Table 2. Estimated maximum survival times for worms were high, being 33.9 and 28.3 weeks respectively.

Survival of *Heligmosomoides polygyrus* in NIH and BALB/c mice at low infection intensity

A direct comparison between C₅₇Bl₁₀, NIH and BALB/c mice was made in Exp. 2 and the results are shown in Fig. 2. Details of the survivorship curves calculated for these data together with the statistical analysis are given in Table 2 (Exp. 2a, b and d). It is apparent that *H. polygyrus* did not live as long in BALB/c and NIH mice, compared to C₅₇Bl₁₀ mice. A further, albeit limited, comparison between

Table 1. Summary of experiments and details of the groups including the number of worms recovered on day 14 post-infection

Exp.*	Strain of mouse	Sex†	Infection intensity‡	No. of mice	Mean no. of worms§ ± S.E.M., 2 weeks
1a	C ₅₇ Bl ₁₀	F	Low	11	36.0 ± 1.5
1b	C ₅₇ Bl ₁₀	F	High	11	206.5 ± 4.1
1c	NIH	F	Low	10	39.6 ± 1.6
1d	(C ₅₇ Bl ₁₀ × NIH)F1	M	Low	7	32.0 ± 2.0
1e	(C ₅₇ Bl ₁₀ × NIH)F1	M	High	7	205.1 ± 2.7
1f	(C ₅₇ Bl ₁₀ × NIH)F1	F	Low	7	30.7 ± 5.3
2a	C ₅₇ Bl ₁₀	F	Low	6	52.0 ± 2.8
2b	NIH	F	Low	8	49.0 ± 3.3
2c	NIH	F	High	8	245.4 ± 8.5
2d	BALB/c	F	Low	5	43.6 ± 2.8
2e	BALB/c	F	High	5	272.2 ± 5.5
3a	NIH	F	Low	5	39.2 ± 3.3
3b	(C ₅₇ Bl ₁₀ × NIH)F1	F	Low	8	41.0 ± 2.0
4a	NIH	F	Low	6	67.0 ± 8.0
4b	NIH	F	High	6	188.3 ± 3.7
4c	(C ₅₇ Bl ₁₀ × NIH)F1	F	Low	4	65.6 ± 3.9
4d	(C ₅₇ Bl ₁₀ × NIH)F1	F	High	5	196.6 ± 5.5
5a	(C ₅₇ Bl ₁₀ × NIH)F1	F	Low	8	48.6 ± 6.0
6a	(C ₅₇ Bl ₁₀ × NIH)F1	M	Low	5	34.6 ± 4.5
6b	(C ₅₇ Bl ₁₀ × NIH)F1	M	High	5	280.2 ± 11.2
7a	NIH	F	Low	6	15.0 ± 1.7
7b	NIH	F	High	6	162.5 ± 7.5
7c	(B10G × NIH)F1	F	Low	6	16.0 ± 3.2
7d	(B10G × NIH)F1	F	High	6	206.2 ± 9.3
8a	BALB/c	F	Low	8	37.1 ± 3.3
8b	BALB/c	F	High	7	179.6 ± 11.0
9a	BALB/c	F	Low	10	26.8 ± 1.7
9b	BALB/c	F	High	10	156.5 ± 6.1
9c	BALB/c	M	Low	4	34.0 ± 1.9

* Groups within experiments with the same number were infected concurrently.

† F, Female; M, male.

‡ Low intensity of infection = 50–100 L₃ administered; high intensity of infection = 250–300 L₃ administered.

§ The mean number of worms recovered 2 weeks after infection (14 days in each case) is given here. All subsequent calculations involving percentage survival were calculated using these data for each experimental group.

C₅₇Bl₁₀ and NIH strains was obtained in Exp. 1, (Fig. 3) in which groups of NIH mice were killed in weeks 2, 5 and 10. In week 10 the mean percentage survival in C₅₇Bl₁₀ mice was 111.7 ± 6.3 (2 versus 10 weeks, Mann-Whitney U-test, $P = 0.08$) and in NIH mice 71.7 ± 7.8 ($P = 0.03$). BALB/c mice were further studied in Exps 8 and 9 (Fig. 6) and it can be seen from Table 2 that the predicted maximum lifespan in each experiment at low intensity of infection was similar (range 10.4–15.4 weeks). Residual chi-squared values for the fitted survivorship curves for NIH and BALB/c mice, with the exception of group 9a, were higher than those calculated for C₅₇Bl₁₀ mice, suggesting that the variance within these groups was more strongly influenced by factors other than those defined in the function which was used for fitting the survivorship curves.

Survival of Heligmosomoides polygyrus in (C₅₇Bl₁₀ × NIH)F1 and (B10G × NIH)F1 hybrid mice

Five experiments were carried out in which the survival of *H. polygyrus* was studied in F1 hybrid mice. In Exp. 1, female (C₅₇Bl₁₀ × NIH)F1 and both parental strains were given a low intensity infection. The data are summarized in Fig. 3 and it is apparent that the worms were rejected by F1 mice significantly earlier than by both parental strains. In week 10, F1 mice had a MPWR of 5.6 ± 2.9 whereas in NIH and C₅₇Bl₁₀ mice the MPWR was 71.7 ± 7.8 and 111.7 ± 6.3 respectively.

This was studied further in Exps 3 and 4 and the data are presented in Fig. 4. Worm burdens in NIH mice (Exp. 3) declined marginally 2–10 weeks post-

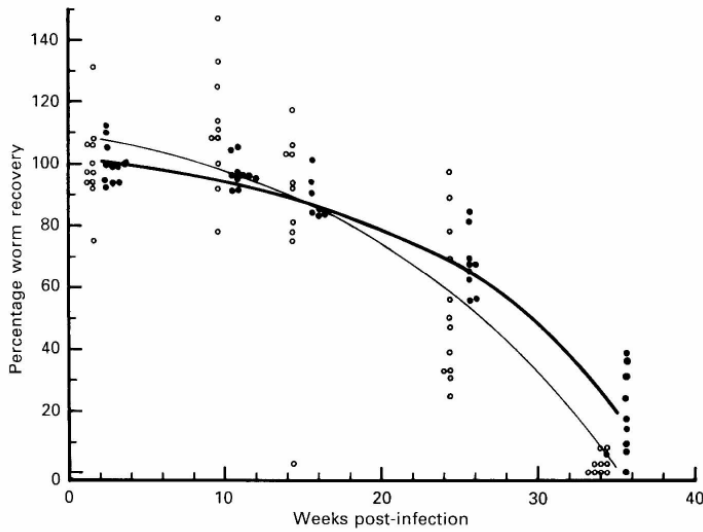


Fig. 1. (Exp. 1) Primary infections in $C_{57}Bl_{10}$ mice at low (\circ) and high (\bullet) intensities of infection. Individual worm burdens are expressed as the percentage of the mean worm recovery in week 2. The curves were fitted using procedures described in the text.

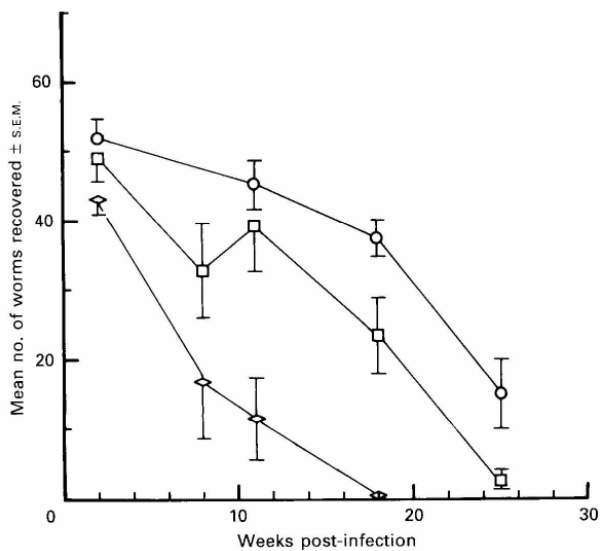


Fig. 2. (Exp. 2) Comparison of the duration of survival of adult worms in female $C_{57}Bl_{10}$ (\circ), NIH (\square) and BALB/c (\diamond) mice given low-intensity infections.

infection (13.2% reduction; 2 versus 10 weeks, Mann-Whitney U-test, $P = 0.7$) but in F1 mice a significantly greater reduction was observed (99.3%, $P = 0.0002$). Similarly, in Exp. 4, NIH mice showed little change in worm burden over the first 10 weeks of infection but F1 mice lost 58% of their worms ($P < 0.05$). In Exp. 5 the time-course of infection was studied in F1 mice alone. In week 2 the MWR

was 48.6 ± 6.0 , in week 7, 15.7 ± 4.7 (67.7% reduction; Mann-Whitney U test, $P < 0.005$) and in week 17, 2.6 ± 0.9 (94.7% reduction, $P < 0.001$). The expulsion of adult *H. polygyrus* from F1 mice occurred earlier in Exps 1, 3 and 5 compared with 4 and it may be significant that the mice used for the latter experiment were younger (6 weeks compared to 4–6 months in Exps 1, 3 and 5).

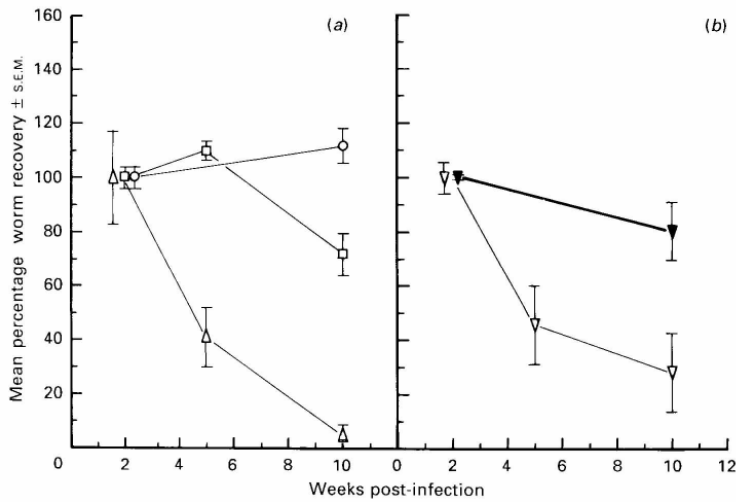


Fig. 3. (Exp. 1) Comparison of worm loss during the first 10 weeks of infection in (a) female C₅₇Bl₁₀ (○), NIH (□) and (C₅₇Bl₁₀ × NIH)F1 (△) and (b) male (C₅₇Bl₁₀ × NIH)F1 given low (▽) or high (▼) intensity infections.

Table 2. Experimental groups to which the survivorship curve given by the function $P = a + br^t$ was fitted by maximum likelihood methods

(The following curves were compared: 1 *ab*, 2 *ab*, 2 *ad*, 2 *bd*, 2 *bc*, 2 *de*, 8 *ab*, 9 *ac*. Model deviance in each case ($= \chi^2$) > 80, $P \leq 0.001$.)

Exp.	Strain	Sex	Intensity	Values of constants			Residual χ^2 (D.F.)	Maximum predicted longevity
				<i>a</i>	<i>b</i>	<i>r</i>		
1 <i>a</i>	C ₅₇ Bl ₁₀	F	Low	128.3 ± 7.0	-17.8 ± 4.8	1.06 ± 0.01	405 (50)	33.9
1 <i>b</i>	C ₅₇ Bl ₁₀	F	High	107.1 ± 2.1	-6.0 ± 1.9	1.08 ± 0.01	130 (44)	37.4
2 <i>a</i>	C ₅₇ Bl ₁₀	F	Low	104.1 ± 2.7	-4.2 ± 3.5	1.12 ± 0.04	186 (22)	28.3
2 <i>b</i>	NIH	F	Low	121.6 ± 3.5	-24.5 ± 3.1	1.06 ± 0.01	688 (39)	27.5
2 <i>c</i>	NIH	F	High	111.4 ± 6.3	-6.0 ± 3.6	1.12 ± 0.02	700 (37)	25.8
2 <i>d</i>	BALB/c	F	Low	9.02 ± 12.5	131.8 ± 8.3	0.83 ± 0.05	572 (15)	14.4
2 <i>e</i>	BALB/c	F	High	114.6 ± 7.2	-15.1 ± 5.5	1.10 ± 0.02	274 (22)	21.3
8 <i>a</i>	BALB/c	F	Low	-59.4 ± 0.4	181.2 ± 0.8	0.93 ± 0.01	649 (28)	15.4
8 <i>b</i>	BALB/c	F	High	-110.7 ± 15.5	221.6 ± 15.5	0.98 ± 0.01	454 (32)	34.4
9 <i>a</i>	BALB/c	F	Low	210.5 ± 4.1	-94.2 ± 2.4	1.08 ± 0.01	168 (27)	10.4
9 <i>b</i>	BALB/c	F	High	136.5 ± 13.5	-30.8 ± 12.2	1.07 ± 0.02	527 (35)	22.0
9 <i>c</i>	BALB/c	M	Low	-6.7 ± 6.5	141.4 ± 7.2	0.87 ± 0.02	524 (18)	21.9

The final experiment in this series was carried out using (B10G × NIH)F1 hybrid mice and the time-course of infection was compared to the NIH parental strain. The results in Table 3 (Exp. 7) show that there was no loss of worms from NIH mice over the 17-week period, but F1 mice had shed 56% and 90% of their worm burden by weeks 10 and 17 respectively.

The influence of the intensity of infection on survival of *Heligmosomoides polygyrus*

The influence of the intensity of infection on the

survival of *H. polygyrus* in C₅₇Bl₁₀ mice was examined in Exp. 1 (Fig. 1). Analysis of the survivorship curves which were fitted to the data (Table 2) revealed that there was a significant difference between these curves ($P \leq 0.001$). In addition, a direct comparison of the MPWR in week 35 is also significant (Mann-Whitney U-test, $P = 0.004$).

Three experiments compared the survival of *H. polygyrus* in NIH mice given low or high-intensity infections. The results of Exp. 4 (Fig. 4) and Exp. 7 (Table 3) did not reveal a significant difference between the groups given low or high intensities of infection over a period of 17 weeks. The survivor-

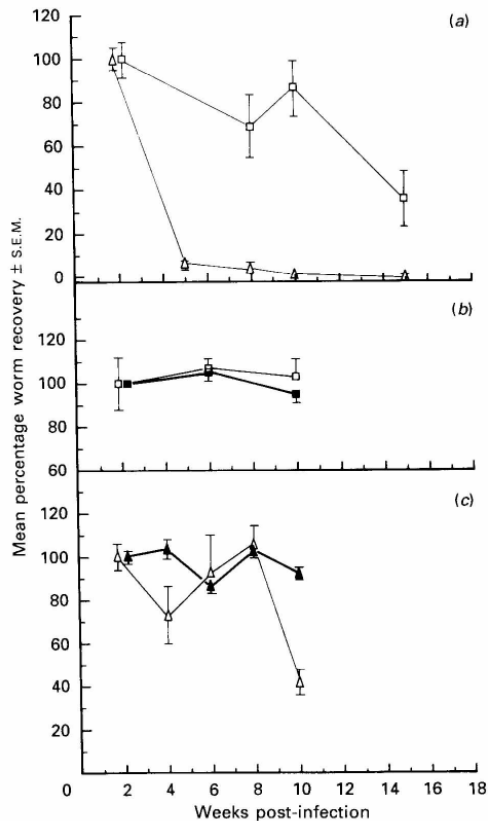


Fig. 4. Comparison of worm loss during primary infection in (a) (Exp. 3) female NIH (□) and (C₅₇Bl₁₀ × NIH)F1 (△) given low-intensity infections; (b) (Exp. 4) female NIH given low (□) or high (■) intensity infections; (c) (Exp. 4) female (C₅₇Bl₁₀ × NIH)F1 given low (△) or high (▲) intensity infections.

ship curves fitted to the data from Exp. 2 and subsequent analysis (Table 2) showed a significant difference. No significant difference was detected when the MPWR from NIH mice killed in weeks 11, 18 and 25, given low and high-intensity infections, were compared directly using the Mann-Whitney U-test.

A very obvious and marked influence of infection intensity on parasite survival was recorded for BALB/c and F1 mice. The results from Exps 2, 8 and 9, in which low and high-intensity infections were compared in BALB/c mice, are presented in Fig. 6 and Table 3. Again the curves are highly significantly different. The intensity of infection was also a major factor determining the onset of expulsion in F1 mice. The results from Exp. 1 (male F1, Fig. 3), Exp. 4 (female F1, Fig. 4), Exp. 6 (male F1, Fig. 5) and Exp. 7 [female (B10G × NIH)F1, Table 4] all concur. In each case low-intensity infections were rejected earlier than high-intensity infections.

The influence of host sex on the survival of *Heligmosomoides polygyrus*

The majority of our experiments were carried out in female mice but an opportunity was available to include male animals for comparison when Exp. 1 (F1) and Exp. 9 (BALB/c) were initiated. In Exp. 1 (Fig. 3a and b; a direct comparison is not illustrated) there was a significant difference in the MWR in week 10, female mice rejecting worms earlier than males (Mann-Whitney U-test, $P = 0.038$). Likewise, male BALB/c mice were slower than female mice at rejecting *H. polygyrus*. A significant difference was observed in week 10 ($P = 0.003$) and week 15 ($P = 0.0034$) and also through a comparison of the entire data sets for both sexes by maximum likelihood analysis (Table 2).

Table 3. Exp. 7. Comparison of the duration of survival of adult worms in female NIH and (B10G × NIH)F1 mice given low or high-intensity infections

Strain of mouse*	Infection intensity	No. of worms recovered ± S.E.M. (% reduction)		
		Week 2	Week 10	Week 17
NIH	Low	15.0 ± 1.7	16.2 ± 1.7	14.2 ± 2.3 (5.3)
NIH	High	162.5 ± 7.5	170.5 ± 12.0	166.8 ± 9.3
(B10G × NIH)F1	Low	16.0 ± 3.2	7.0 ± 3.1 (56.0)	1.6 ± 0.9 (90.0)
(B10G × NIH)F1	High	206.2 ± 9.3	199.0 ± 25.0 (3.5)	76.3 ± 47.6 (63.0)

* All groups contained 6 mice.

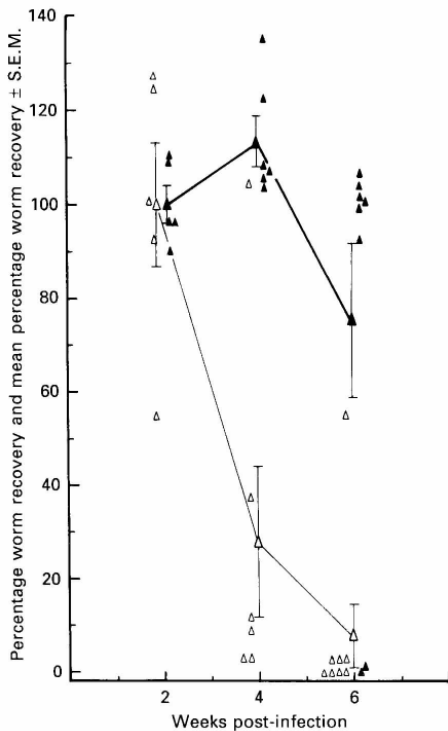


Fig. 5. (Exp. 6) Comparison of worm loss during primary infection in male ($C_{57}Bl_{10} \times NIH$)F1 mice given low (Δ) or high (\blacktriangle) intensity infections.

DISCUSSION

Although *H. polygyrus* is recognized as a long-lived parasite, little attention has been given in the past to determining accurately the pattern of survival in syngeneic strains of mice. In previous reports it has been considered that $C_{57}Bl_{10}$ mice were weak responders to *H. polygyrus* developing acquired immunity only after prolonged and repeated stimulation (Behnke & Robinson, 1985; Cypess, Lucia, Zidian & Rivera-Ortiz, 1977; Cypess & Zidian, 1975). The survival characteristics of primary infection adult worms in $C_{57}Bl_{10}$ match closely patterns observed in mouse strains where infections were recognized as chronic (Liu, 1966; Keymer & Hiorns, 1986; Behnke *et al.* 1987). Worm burdens at low and high infection intensities remained stable for 10 weeks before declining, slowly at first and more rapidly subsequently (Fig. 1). At low infection intensities virtually all worms had disappeared by week 35 but, surprisingly, more heavily infected mice still had a MPWR of 19.5 ± 4.5 at this time. This was the only occasion on which a clear significant difference was obtained in $C_{57}Bl_{10}$ mice, added to which the maximum likelihood analysis also gave a significant difference between the data sets for mice

with light and heavy infections. Whilst we cannot eliminate the possibility that the observation may reflect density-dependent suppression of worm loss and hence the involvement of host-protective immunity, the duration of both low and high-intensity infections and the relatively small difference between the groups led us to conclude that the effects of host immunity were minimal in this strain. It is more probable, that a relatively small effect towards the tail end of the survivorship curve simply reflected the greater probability of there being some, more long-lived (possibly robust) worms in the population administered in the heavier inoculum. We interpret the survivorship curves of *H. polygyrus* in $C_{57}Bl_{10}$ mice as reflecting essentially senility, with host immunity possibly intervening in the terminal stages, to accelerate worm loss in mice with residual worm burdens.

Survival of *H. polygyrus* in NIH and BALB/c mice showed quite different characteristics. At low infection intensities the maximum life-span was calculated as 27.5 weeks in NIH mice and 10.4–15.4 weeks in BALB/c mice (Table 2). The shape of the curve which was fitted to the function defining survivorship and the constants which were calculated for NIH mice, showed broad similarity to $C_{57}Bl_{10}$ mice except that parasite longevity was severely curtailed. In contrast, BALB/c mice gave quite different constants and some of the curves which we calculated were almost linear in outline (Fig. 6 and Table 2) whilst others were clearly convex in shape (Fig. 2). Other functions were not evaluated in the present study but it is apparent that the longevity of *H. polygyrus* in NIH and BALB/c mice was markedly affected by factors which accelerated the expected rate of worm loss from senility alone. It is likely that host-protective anti-adult worm responses intervened in these strains to curtail parasite survival prematurely. An immunological analysis was undertaken as part of the present investigation and our results will be published elsewhere, but two features of the data presented here support the conclusion that immunity played a significant role in abbreviating parasite survival in BALB/c mice.

Firstly, it was apparent that in BALB/c mice heavier infections persisted considerably longer than low-intensity infections. Even at low infection intensities a correlation can be seen between initial parasite establishment (Table 1) and parasite survival (Table 2 and Fig. 6). The lightest infection was established in group 9a (Table 1) and the maximum survival was calculated as only 10.4 weeks. These observations confirm earlier reports of dose-dependent suppression of worm expulsion (Dobson *et al.* 1985; Behnke *et al.* 1987) and, as proposed by the previous authors, the phenomenon probably reflects the quantitative effect of immunomodulatory factors produced by adult worms as part of the mechanism used in evading host immunity. It fol-

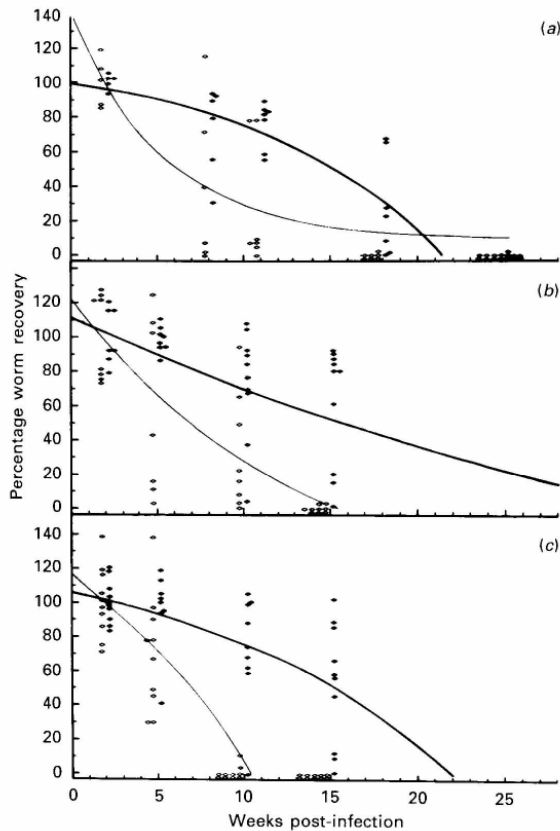


Fig. 6. Primary infection in BALB/c mice at low (\diamond) and high (\blacklozenge) intensities of infection. Individual worm burdens are expressed as the percentage of the mean worm recovery in week 2. The curves were fitted using procedures described in the text. (a) Exp. 2; (b) Exp. 8; (c) Exp. 9.

lows that BALB/c mice, whilst initially affected by immunomodulation, nevertheless had the capacity to overcome the parasite and, depending on the intensity of infection, eventually developed insensitivity to parasite immunomodulatory factors and expelled the worms. Broadly similar results have been reported for *Trichinella spiralis* in rats and mice, but on a shorter time-scale (Bell, McGregor, Woan & Adams, 1983; Bell, Adams & Ogden, 1984; Wassom, Dougherty, Krco & David, 1984; Wakelin, Donachie & Grecnis, 1985). It has been proposed that a population of T lymphocytes, controlled by genes located both within and outside the MHC, is particularly sensitive to the suppressive influence of heavy parasite burdens (Wakelin *et al.* 1985) and hence heavier infections are expelled more slowly than low-intensity infections.

Secondly, infections in male mice lasted longer than those in female mice, and the possibility arises of a direct detrimental effect of sex hormones on

parasites residing in female mice. However, the modulatory effects of sex hormones on host immunity are well recognized in the literature (Dobson, 1961, 1982; Solomon, 1969; Blazkovec, Orsini & Maginn, 1973). It is likely that the longer lasting infections in male BALB/c mice reflected slower development of immunity.

Two hybrid strains were investigated during this study. When $C_{57}Bl_{10}$ (H-2^b) and NIH (H-2^a) mice were crossed, their F₁ progeny (H-2^{bq}) showed a considerable advantage over both parental strains. Worm expulsion commenced within 10 weeks of infection in F₁ mice and in some experiments significantly accelerated rejection was identified by weeks 4-6 (Fig. 5). Clearly, gene complementation was involved, the unique combination of genes resulting from the cross, endowing F₁ mice with the capacity to overcome *H. polygyrus* in a comparatively short period of time. In a single experiment we examined (B10G × NIH)F₁ mice, a hybrid combination which would possess the H-2^a haplotype of both parental strains. Although the 2 hybrid strains were not compared directly, (B10G × NIH)F₁ mice behaved much like ($C_{57}Bl_{10}$ × NIH)F₁, no obvious advantage of H-2^a over H-2^{bq} being apparent. Both hybrid strains were affected by the intensity of infection and, as in BALB/c mice, worm expulsion began later in heavily infected animals. Male ($C_{57}Bl_{10}$ × NIH)F₁ mice expelled worms more slowly than female mice (Fig. 2), a result which is also consistent with an immunological explanation for the short duration of primary infections in these animals.

The demonstration that the F₁ hybrid mice exhibited considerably enhanced resistance to infection compared to either parental strain deserves further comment. NIH mice are recognized as being capable of generating rapid inflammatory responses in the intestine whereas $C_{57}Bl_{10}$ mice are slower in this respect (Alizadeh & Wakelin, 1982; Wakelin, 1980). However, $C_{57}Bl_{10}$ mice develop better host-protective antibody responses to *H. polygyrus* after repeated larval challenge (Williams & Behnke, 1983). These mechanisms may interact synergistically in F₁ mice to bring about better overall protection but, as yet, this has not been confirmed experimentally. Hybrid vigour is recognized in a variety of different contexts as endowing increased fitness in competition and survival and gene complementation is a well-established phenomenon among micro and macro-parasites (reviewed by Wakelin & Blackwell (1988)). In our system, NIH and $C_{57}Bl_{10}$ mice arose independently through inbreeding programmes for which the selection criteria are not clear, and the two strains probably carry many contrasting alleles. If we consider the two strains as analogues of different genotypes available in wild populations, then our results may have broader implications. The reasons for the existence of susceptible individuals in wild host populations are a subject for contemporary debate

(Schad & Anderson, 1985; Anderson, 1986*b*; Bundy, Cooper, Thompson, Didier, Anderson & Simmons, 1987) but one possibility may be that other advantages balance or exceed the net disadvantage of susceptibility to a particular disease (e.g. reproductive potential or possibly, as in our case, an antibody response). Furthermore, heterozygous animals may be at an overall advantage in carrying both the resistant genes and the genes for high reproductive potential or other advantageous traits. Thus, the maintenance of genetic polymorphism at loci governing responsiveness, including the traits for susceptibility, is assured and the recurrence of susceptibles among future progeny, guaranteed. The mathematical basis for this hypothesis has been reviewed by Anderson (1986*a*).

The involvement of immune responses in limiting primary infections with *H. polygyrus* is still a controversial subject, there being little evidence for the participation of acute inflammatory reactions such as those initiated by *T. spiralis* and considered to play a crucial role in terminating parasitic infections in the intestine (Miller, 1984; Wakelin, 1985). Indeed, *H. polygyrus* is known to suppress mastocytosis, a key component of the intestinal inflammatory response, even in mouse strains such as SJL in which primary infections seldom last beyond week 10 (Dehlawi, Wakelin & Behnke, 1987; Dehlawi & Wakelin, 1988). However, our experiments provide support for the role of immunity. The variations in the survival characteristics of *H. polygyrus* which we have observed between mouse strains, in relation to the intensity of infection and sex of the host, cannot be explained easily, other than through the complex interaction between host protective responses against adult worms, parasite immunomodulation and host genes controlling the ability of mice to generate the former and/or resist the latter. The next step will be to define the component processes involved, but this will not be an easy task as attempts to correlate worm loss with cellular and antibody activity during primary infection have been largely unsuccessful (Behnke, 1987; Behnke *et al.* 1987; Williams & Behnke, 1983). Nevertheless, the information which we have provided on survivorship curves of *H. polygyrus* in C₅₇Bl₁₀ (B10) and BALB/c mice should enable the B10 and BALB congenic mouse series to be exploited in identifying the host genes governing susceptibility and resistance to primary infection. The uniformity of background genes within each of these mouse series should allow MHC effects on parasite survival to be isolated and the accompanying MHC-linked immune responses to be recognized.

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