Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): H-2 linked genes determine worm survival

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SUMMARY

The course of primary infection was studied in BALB and B10 H-2 congenic mouse strains. The duration of infection, as assessed with regular faecal egg counts and worm burdens, was shorter in mice carrying the H-2^s, H-2^d or H-2^q haplotypes when compared to mice with H-2^b. Strains with H-2^k were intermediate. An experiment was carried out to test the hypothesis proposed by Wassom, Krco & David (1987) predicting that the progeny of I–E^{+ve} mouse strains crossed with I–E^{-ve} strains, would show susceptibility rather than resistance to infection. This hypothesis was not substantiated by our data and we conclude that it does not apply to primary infections with *Heligmosomoides polygyrus*. It is proposed that the gene products of at least two loci within the H-2 (associated with the H-2^b and H-2^k haplotypes) are crucial in determining the response phenotype of mice to primary infection with *H. polygyrus*. One allele, (associated with the H-2^b haplotype) may be preferentially affected by parasite-mediated immunosuppression.

Key words: *Heligmosomoides polygyrus*, nematoda, mice, H-2, MHC, immunity, evasion of immunity, immunomodulation.

INTRODUCTION

Chronic intestinal nematode infections pose a significant threat to the health of domestic animals and also to humans. The mechanisms employed to evade host immunity are still poorly understood but there is growing consensus that a number of diverse species exert an immunomodulatory influence, causing potentially host-protective effector responses to be down-regulated (reviewed by Pritchard, 1986; Behnke, 1987; Wadee, Vickery & Piessens, 1987; Klesius, 1988).

Heligmosomoides polygyrus is a long-lived intestinal nematode of mice whose longevity in the gut contrasts markedly with the relatively brief survival of *Trichinella spiralis* and *Nippostrongylus brasiliensis* (Behnke, 1987). Typically, adult *H. polygyrus* can survive for 25 weeks (Keymer & Hiorns, 1986; Robinson *et al.* 1989), only a few strains (e.g. SJL and SWR) being able to expel worms within 10 weeks of infection (Wahid, Robinson & Behnke, 1989; Cypess *et al.* 1977; Enriquez, Zidian & Cypess, 1988*a*). It has been suggested that the survival of *H. polygyrus* in otherwise immunocompetent mice reflects the capacity of adult worms to affect the expression of intestinal immunity through immunomodulatory factors (Behnke, Hannah & Pritchard, 1983; Dobson & Cayzer, 1982) and recent work has implicated low-molecular-weight components of excretory/secretory products as the principal molecules involved (Monroy, Dobson & Adams, 1989).

Studies on acquired immunity to *H. polygyrus* have demonstrated that both background and H-2 encoded genes influence the degree of protection afforded by primary exposure to the parasite (Behnke & Robinson, 1985; Enriquez *et al.* 1988*b*). At least two H-2 genes, one mapping to the left of I–E and the other towards the D end of the MHC, appear to modulate resistance (Enriquez *et al.* 1988*b*). Mice carrying the H-2^k haplotype are particularly difficult to immunize and show weak responses even after several infections (Behnke & Robinson, 1985; Enriquez *et al.* 1988*a*). Enriquez *et al.* (1988*b*) have suggested that this haplotype is preferentially suppressed by the products of adult worms in the intestine.

During primary exposure to *H. polygyrus* C57BL/10 mice (H-2^b) are also slow to develop immunity and data show that in this strain worms live for more than 30 weeks (Robinson *et al.* 1989). When C57BL/10 mice were crossed with more resistant strains, some combinations showed gene complementation and an accelerated loss of worms [e.g. (NIH × C57BL/10)F1] whereas in others the response was delayed relative to the fast parent [e.g. (SWR × C57BL/10)F1 and (SJL × C57BL/10)F1; Wahid *et al.* 1989]. Clearly, genes carried by C57BL/10 mice can interact with other genotypes in both a beneficial and deleterious manner, in deter-

mining the course of primary infection. We have suggested that down-regulation of the host response, following primary exposure, may be attributed to the H-2^b haplotype, and in this respect our interpretation of the influence of host MHC on the survival of *H. polygyrus* is radically different from that proposed by Wassom *et al.* (1987) for MHC control of primary responses to *T. spiralis* and secondary responses to *H. polygyrus*. These authors have hypothesized that mouse strains in which I–E is not expressed (such as those carrying the H-2^b haplotype which do not express I–E because of a deletion in the E_α gene) should show good resistance to infection. On this basis C57BL/10 mice should rank among the fast responders rather than among the weak, and accordingly should limit primary infections earlier than is the case.

In the present investigation we examined a number of H-2 congenic mouse strains, some of which express I–E and others which do not. We report here that MHC encoded genes exert a significant influence on the survival of *H. polygyrus* following primary infection. Our observations cannot be reconciled easily with the hypothesis proposed by Wassom *et al.* (1987) and we conclude that the latter cannot be applied to primary infections with *H. polygyrus* without modification. However, our data confirm and extend our earlier work and are compatible with the known immunomodulatory effect of *H. polygyrus*.

MATERIALS AND METHODS

Animals

Congenic mouse strains were purchased from Harlan Olac Ltd (Bicester, UK) and, together with breeding stock, were maintained in the departmental animal house under conventional conditions.

Parasite

The parasite used throughout this work was *Heligmosomoides polygyrus bakeri*. The methods used to maintain the parasite, infect mice and recover worms at autopsy have all been described previously (Jenkins & Behnke, 1977). Throughout the study mice were infected with low doses of larvae not exceeding 60 L3, in order to minimize the immunodepressive influence of heavier infections (Robinson *et al.* 1989). Faecal egg counts were carried out regularly on all groups as described by Behnke & Parish (1979).

Statistical analysis of results

The results are presented as group mean values ± standard error (S.E.M.). Because of the small sample sizes, the data were analysed by non-parametric procedures. The Kruskal-Wallis (one-way analysis of variance by ranks) test was employed when more than 2 groups required comparison at a particular time point. When the Kruskal–Wallis statistic (H) indicated a significant strain effect, the Mann-Whitney U-test (Sokal & Rohlf, 1969) was used to compare each strain to the stated reference strains (H-2^b in the majority of experiments). All statistical procedures were carried out with the software package Statgraphics (A Plus Ware Product, STSC) on an IBM PC. A P value of $\leqslant 0{\cdot}05$ was considered to reflect a significant difference and the following notation was used to identify categories of significance: * $P = 0{\cdot}05$; ** $0{\cdot}05 > P \geqslant 0{\cdot}025$; *** $0{\cdot}025 > P \geqslant 0{\cdot}01$; **** $0{\cdot}01 > P \geqslant 0{\cdot}001$; ***** $P < 0{\cdot}001$.

RESULTS

Course of infection in B10 congenic mice

The course of low-intensity primary infections in B10 H-2 congenic mice, was studied in 11 different experiments each comparing 2 or more strains. The combinations of mice examined, together with their respective haplotypes are given in Table 1. Whenever possible the C57BL/10 (B10) strain, from which the others are derived, was included as a control.

The results of Exp. 1, in which B10, B10.BR, B10.D2/n and B10.S were examined, are presented in Fig. 1A. Initial establishment of worms as adjudged by worm recoveries in week 2 was uniform among all strains, but B10.D2/n and B10.S mice had lower faecal egg counts from week 5 post-infection (data not shown) and fewer worms in week 9. When the faecal egg counts of B10.S and B10.D2/n mice dropped to below 100 eggs per gram faeces (epg) in week 12 they were killed and very few worms were recovered. B10 and B10.BR mice still passed large numbers of parasite eggs at this time, although the egg counts of the latter strain had begun to fall. The remaining groups were killed in week 15 and B10.BR mice were found to harbour significantly fewer worms than B10 mice.

The results of 9 additional experiments (Exps 2–10) are summarized in Figs 1 and 2. B10 mice were clearly the slowest to expel *H. polygyrus* in 8 of the 9 experiments in which they were included. The exception was Exp. 10 (Fig. 1C) in which B10 mice lost 68% of their worms by week 20. The exact timing of worm expulsion among the other strains also varied from one experiment to another but there was consistency in the relative order in which mouse strains expelled worms. Thus B10.S were faster than B10 mice (Figs 1A, 2D and 3) and B10.BR (Fig. 2F). B10.D2/n mice were also faster than B10 in 4 experiments (Figs 1A, C, 2A, C) although Exp. 3 (Fig. 2B) did not give a significant difference in

Table 1. List of mouse strains and their respective H-2 haplotypes, in the combinations in which they were studied

Strain	H-2 haplotype	Experiment number 1	2	3	4	5	6	7	8	9	10	11	12
C57BL/10(B10)	b	+	+	+	+	+	+	+	+		+		
B10.D2/n	d	+	+	+	+					+	+		+
B10.BR	k	+	+							+	+		+
B10.S	s	+				+				+			+
B10.G	q						+	+	+		+		
(B10.D2/n × B10.BR)F1	dk												+
(B10.S × B10.BR)F1	sk												+
(B10 × B10.BR)F1	bk												+
SJL	s					+							
BALB.B	b											+	
BALB.K	k											+	
BALB/c	d											+	

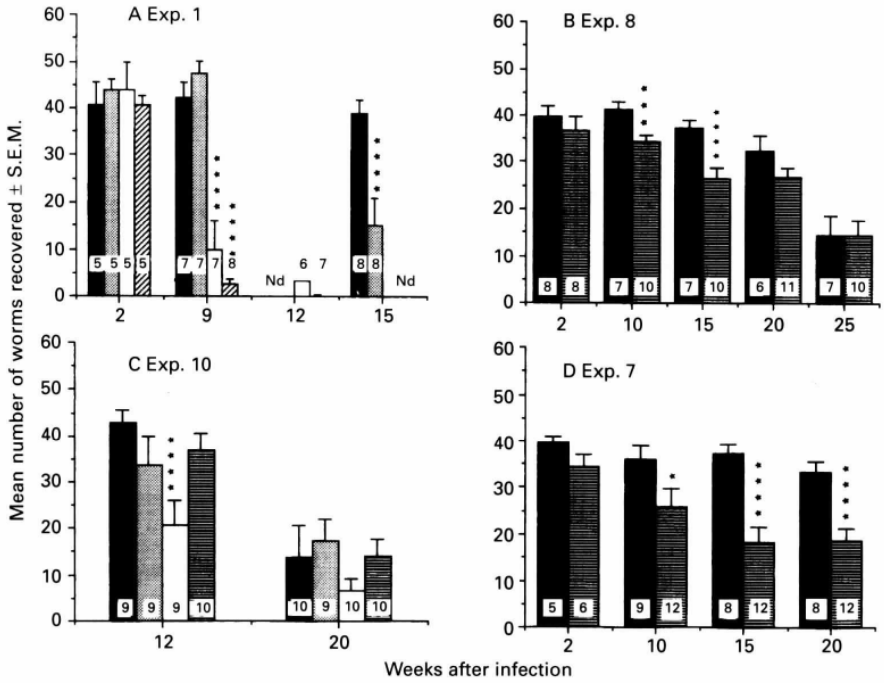

Fig. 1. Experiments 1, 7, 8 and 10. Changes in worm burden during the course of infection with *Heligmosomoides polygyrus* in B10 congenic strains of mice. B10 (■); B10.BR (▨); B10.D2n (□); B10.S (▨) and B10.G (▤). Number on column = *n*. Nd, Not done. Statistical analysis of results: (A) Experiment 1, week 2, $H = 1{\cdot}66$ (N.S.); week 9, $H = 19{\cdot}4$ ($P = 0{\cdot}000\,23$). C Experiment 10, week 12, $H = 9{\cdot}014$ ($P = 0{\cdot}029$); week 20, $H = 3{\cdot}6484$ (N.S.). Additional comparisons were made using the Mann–Whitney U-test and B10 mice as the reference strain in all cases. * $P = 0{\cdot}05$; *** $0{\cdot}025 > P \geqslant 0{\cdot}01$; **** $0{\cdot}01 > P \geqslant 0{\cdot}001$.

worm burdens in week 24. There was an indication that B10.S mice were slightly faster than B10D2/n in so far as mean worm burdens were lower in the former strain in Exps 1 and 9 (Figs 1A and 2F) but these differences were not significant. B10.BR mice were always slower than B10.S and B10.D2/n (Figs 1A, C, 2A, F) but were faster than B10 (Figs 1A, 2A). Finally, B10.G mice were faster than B10 (Figs 1B, D, 2E).

Course of infection in BALB congenic mice

A single experiment examined the course of infection in 3 congenic strains on the BALB background. The results are presented in Fig. 4 and show that worm expulsion occurred earlier in BALB/c mice than in BALB.K and BALB.B mice. By week 10, 41·2% of the established worms (week 2) had been rejected, and by week 15 worm burdens were reduced by

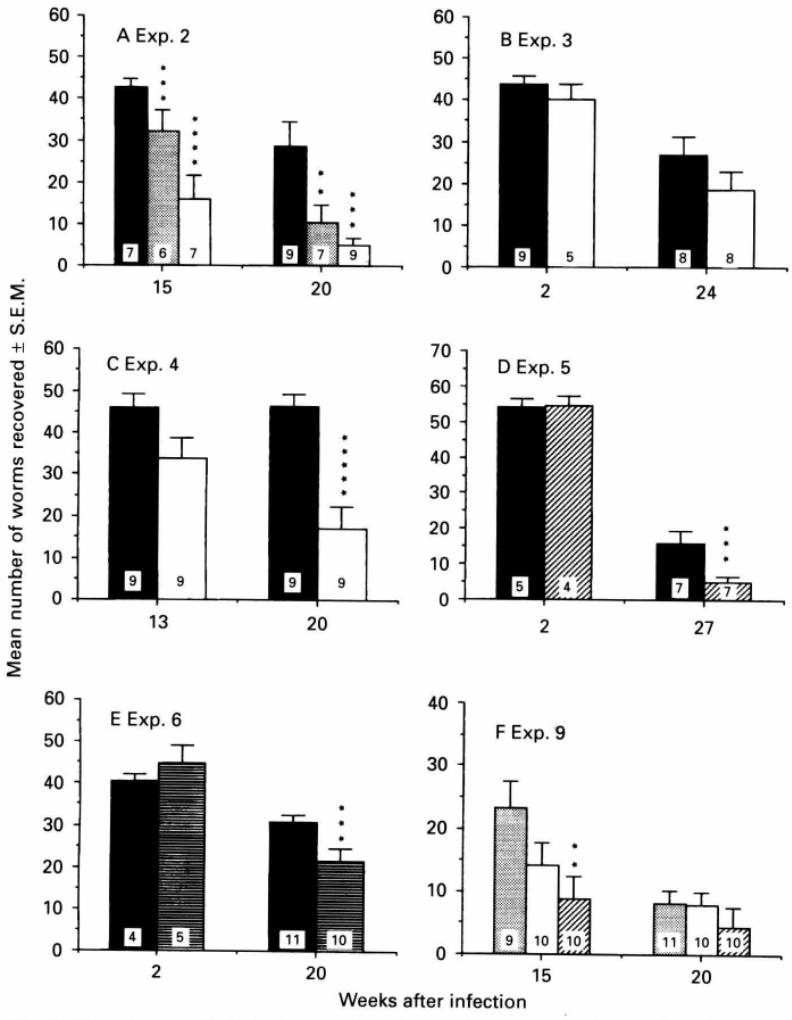

Fig. 2. Experiments 2, 3, 4, 5, 6 and 9. Changes in worm burden during the course of infection with *Heligmosomoides polygyrus* in B10 congenic strains of mice. B10 (■); B10.BR (▨); B10.D2n (□); B10.S (▨) and B10.G (▤). Number on column = *n*. Statistical analysis of results: (A) Experiment 2, week 15, $H = 10{\cdot}95$ ($P = 0{\cdot}0042$); week 20, $H = 5{\cdot}83$ ($P = 0{\cdot}05$). (F) Experiment 9, week 15, $H = 5{\cdot}8536$ ($P = 0{\cdot}053$); week 20, $H = 3{\cdot}8722$ (N.S.). Additional comparisons were made using the Mann–Whitney U-test and B10 mice as the reference strain in (A–E) and B10BR in (F). ** $0{\cdot}05 > P \geqslant 0{\cdot}025$; *** $0{\cdot}025 > P \geqslant 0{\cdot}01$; **** $0{\cdot}01 > P \geqslant 0{\cdot}001$; ***** $P < 0{\cdot}001$.

86·3%. BALB.K mice were intermediate and BALB.B mice were the slowest of the three strains. The relationship of H-2 haplotypes to response phenotype was therefore identical to that in the B10 congenic series.

Course of infection in B10 congenic hybrid strains

To test the prediction of Wassom *et al.* (1987) that the progeny of crosses between I–E positive and I–E negative strains, which should express I–E, would exhibit the poor responder phenotype of the I–E^{+ve} expressing parental strain, B10.BR (H-2^k, I–E^{+ve}) male mice were mated with B10.S (H-2^s, I–E^{-ve}) and B10 (H-2^b, I–E^{-ve}) female mice and also to B10.D2/n (H-2^d, I–E^{+ve}) mice as a control. The experiment included age-matched B10.BR, B10.D2/n and B10.S mice but unfortunately no B10 mice were available. The results of worm recoveries in weeks 2, 12 and 22 post-infection are presented in Table 2. The essential points which arise from this experiment are as follows. (1) No significant differences in worm establishment were detected between any of the parental and F1 groups. (2). Initially, all groups had comparable faecal egg counts but as expected B10.S and B10.D2/n mice showed declining egg counts from week 7 onwards, although the decline was not as marked as recorded previously (data not shown). Surprisingly, all the F1 hybrid strains also showed declining egg counts and therefore representative batches from each strain were killed in week 12 to compare worm burdens.

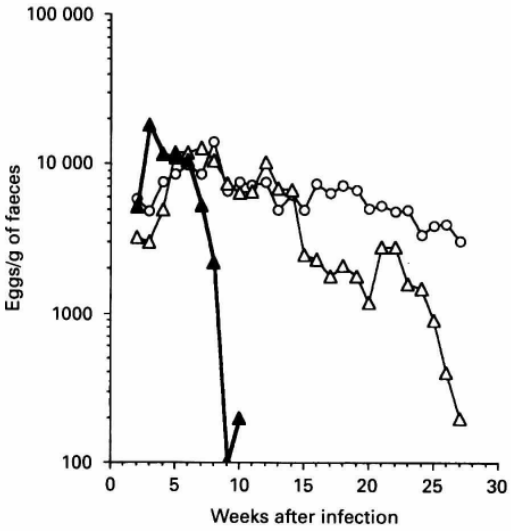

Fig. 3. Experiment 5. Faecal egg counts during the course of infection with *Heligmosomoides polygyrus* in B10, B10.S and SJL mouse strains. B10 (○); B10.S (△) and SJL (▲).

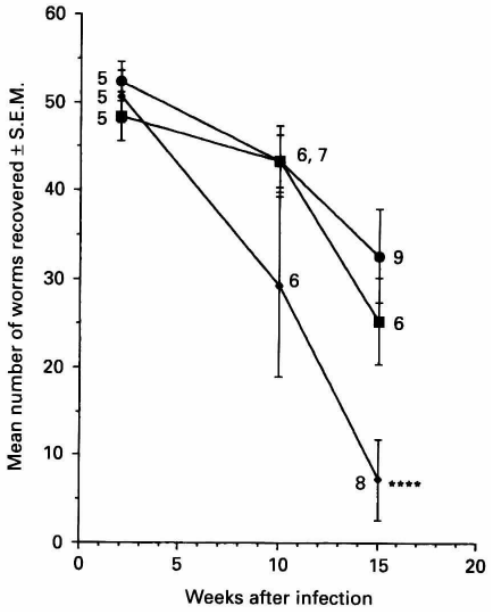

Fig. 4. Experiment 11. Changes in worm burden during the course of infection with *Heligmosomoides polygyrus* in BALB congenic strains of mice. BALB/c (◆); BALB.B (●) and BALB.K (■). Number on column = *n*. Statistical analysis of results: Week 2, $H = 1{\cdot}42$ (N.S.); week 10, $H = 0{\cdot}71$ (N.S.); week 15, $H = 9{\cdot}15$ ($P = 0{\cdot}01$). Additional comparisons were made using the Mann–Whitney U-test and BALB.B mice as the reference strain in all cases. **** $0{\cdot}01 > P \geqslant 0{\cdot}001$.

B10.BR mice had significantly more worms than all the other strains but there were no significant differences among the remaining strains. (3) By week 22 worm burdens in all the mice had declined further and although the differences between the groups were no longer significant, B10.BR mice still carried the heaviest worm burdens.

DISCUSSION

H-2 linked genes clearly exert a marked influence on the ability of mice to curtail primary infection with *H. polygyrus*. Surprisingly, there was some degree of inter-experimental variation in the exact timing of worm expulsion throughout this study. B10 mice generally did not lose worms in the first 10 weeks of infection and less than 25% by week 20 as reported previously (Robinson *et al.* 1989) but in Exp 10, 68% of worms were expelled by week 20. Differences among the congenic strains were equally evident; the loss of worms from B10.S and B10.D2/n mice occurred considerably earlier in Exp. 1 than subse-

Table 2. Expulsion of *Heligmosomoides polygyrus* from congenic mice and their F1 hybrid progeny (Exp. 12).

(Statistical analysis of results: Week 12, $H = 13{\cdot}78$ ($P = 0{\cdot}017$); Week 22, $H = 8{\cdot}55$ (P N.S.). Additional comparisons were made using the Mann–Whitney U-test and B10.BR mice as the reference strain in all cases. The key to symbols used is as follows: **** $0{\cdot}01 > P \geqslant 0{\cdot}001$; ***** $P < 0{\cdot}001$.)

	Mean ± S.E.M. number of worms recovered in weeks (*n*)		
Strain	Week 2	Week 12	Week 22
B10.BR	40·5 ± 2·0 (6)	44·3 ± 3·1 (8)	25·6 ± 3·0 (12)
B10.D2n	40·3 ± 2·6 (6)	18·9 ± 4·8 (9)*****	15·8 ± 3·3 (12)
B10.S	41·8 ± 1·5 (5)	27·7 ± 5·7 (9)****	15·3 ± 2·8 (12)
(B10.D2n × B10.BR)F1	41·6 ± 2·0(5)	27·4 ± 6·7 (9)****	18·4 ± 5·5 (10)
(B10.S × B10.BR)F1	39·6 ± 2·4 (5)	21·0 ± 5·3 (7)****	10·9 ± 3·4 (8)
(B10 × B10.BR)F1	N.D.*	25·7 ± 2·0 (6)*****	16·1 ± 4·2 (7)

* N.D., Not done.

quently but, nevertheless, overall the relative ranking of strains was not markedly affected. Mice carrying the H-2^s and H-2^d haplotypes (B10.S, B10.D2/n and BALB/c) consistently lost worms earlier than other strains. Loss occurred significantly earlier in mice with H-2^q (B10.G) than in the H-2^b carrying B10 strain and, on the basis of the 4 experiments, B10G mice showed a response phenotype marginally slower than that of H-2^s and H-2^d mice.

Mice carrying H-2^b (B10 and BALB.B) were the slowest to lose worms and it is likely that in B10 mice there was little host involvement in terminating the infection, the worms dying essentially from senility (Robinson *et al.* 1989). B10 and BALB.B mice also showed the weakest antibody response to primary infection with *H. polygyrus* (Wahid and Behnke, manuscript in preparation). However, H-2^b mice are not poor at responding to other intestinal parasites and can expel *H. polygyrus* on secondary exposure. Following infection with *T. spiralis*, B10 mice were ranked as intermediate-resistant by Wakelin (1980) and by Wassom *et al.* (1983 b) and were quicker than, for example, B10.BR and only marginally slower than B10.S mice. Thus an inability to make host-protective responses against nematode infections is not an intrinsic property of this haplotype.

Mice with H-2^k (B10.BR and BALB.K) produced an intermediate response which, on occasion, was difficult to distinguish from the H-2^b response (Fig. 4) and on others from the H-2^d response (Fig. 2A). On balance it was concluded that H-2^k mice did show worm loss earlier than B10 and BALB.B mice but, nevertheless, had a relatively protracted course of infection. A number of other inbred strains sharing the H-2^k haplotype (CBA, C3H, AKR) are also known to sustain prolonged infections with *H. polygyrus* (Wahid and Behnke, unpublished observations). In *T. spiralis* this particular haplotype is associated with the weakest response phenotype (Wassom *et al.* 1983*a*, 1984) and in *Trichuris muris* infection the H-2^k haplotype rendered mice totally unable to expel worms (Else & Wakelin, 1988). In our experiments on primary rather than secondary immunity (studied by Enriquez *et al.* 1988 *b*) the H-2^k haplotype was certainly associated with a slow host-protective (worm expulsion) response but not as slow as that evident in H-2^b mice.

Finally, we tested the hypothesis put forward by Wassom *et al.* (1987) that presentation of parasite antigens to T cells in the context of I–E MHC molecules elicits a net down-regulatory effect, I–E^{+ve} mice showing susceptibility to infection. The B10.BR strain was chosen as the weakest of the I–E^{+ve} congenic strains in this study and was crossed with I–E^{-ve} B10.S and B10 mice. The F1 progeny should all be I–E^{+ve} and according to the above hypothesis should show susceptibility to infection. Our results did not confirm this prediction. All the F1 progeny of the various combinations showed a significantly earlier response to infection than the B10.BR parents. However, the original hypothesis has been modified (Wassom & Kelly, 1990) in the light of recent evidence that mice expressing I–E have a gap in their T-cell repertoire as a result of the deletion of T cells bearing particular receptors, during thymic development (Kappler, Roehm & Marrack, 1987; Marrack & Kappler, 1987). The poor response to parasites of I–E expressing mouse strains may therefore reflect the absence of T-cell clones capable of recognizing crucial parasite antigens. Our results suggest otherwise with respect to primary infections with *H. polygyrus*; I–E^{+ve} strains are not necessarily poor responders. The I–E^{+ve} F1 progeny of I–E^+ × I–E^- strains were more resistant to infection than their I–E expressing parent. Although B10 mice were unavailable for comparison, it can be safely assumed that they would have been even slower than B10.BR mice as indicated by other experiments in this paper, (an observation which is itself not consistent with the hypothesis) and hence inheritance of the faster responder phenotype or even gene complementation must have been involved. It is also pertinent that B10.S and B10 mice, neither of which express I–E, represent the extremes of response phenotype. Moreover, there is only a marginal difference between the fast responses of B10.S and B10.D2/n mice, the latter expressing I–E. Thus, however interpreted, the Wassom hypothesis is not compatible with the results of our experiments and fails to explain the variation in survival of *H. polygyrus* among congenic mouse strains exposed to primary infection.

The explanation for the differences between our conclusions and those of others may reside in the unique interrelationship between host immunity and the parasite's evasive strategies. There is good evidence that *H. polygyrus* is capable of immunomodulatory activity (reviewed by Behnke, 1987) which is likely to be most effective during primary infections but perhaps less so following secondary exposure, when the host would have had an opportunity to develop resistance. In proposing that at least two H-2 linked genes influence the level of acquired resistance in mice to *H. polygyrus*, Enriquez *et al.* (1988*b*) have suggested that the presence of adult worms in the lumen of the intestine preferentially suppresses the response of H-2^k mice. Consistent with this hypothesis H-2^k mice showed protracted primary infections in this study but our data indicate further that mice carrying the H-2^b haplotype were even less resistant, raising the possibility of a second gene, with an allele associated with the H-2^b haplotype, showing greater susceptibility to parasite immunomodulation on primary exposure than following challenge. H-2^{bk} F1 hybrids would benefit from the possession of the dominant resistant alleles in each case. Experiments involving crosses between mice with H-2^b and other haplotypes

support the conclusion that the H-2^b haplotype is associated with a net down-regulation of primary resistance to *H. polygyrus* (Robinson *et al.* 1989; Wahid *et al.* 1989). Nevertheless, some H-2^b alleles must benefit the host, because H-2^{bk} hybrids in this study showed gene complementation and the F1 progeny of NIH and C57BL/10 (which are H-2^{bq}, but also heterozygous at background loci) are known to expel worms faster than either parental strain (Robinson *et al.* 1989).

Since genes located within the MHC encode for antigen-presenting molecules, a poor responder phenotype may be explained by the possession of Class II molecules incapable of presenting key antigens to T cells in an optimal manner. However, genes for other molecules are also located in the H-2 e.g. TNFα and β, and one possibility is that the Ts-2 gene, which is important in *T. spiralis* and maps to the D end of the H-2 (Wassom *et al.* 1983*b*), may be involved. If the response phenotype to *H. polygyrus* is dependent on the Ts-2 gene, the interaction between this gene product and *H. polygyrus* following primary exposure must be quite distinct from that in *T. spiralis* infection or the secondary response to *H. polygyrus*, since H-2^b is associated with strong responsiveness in the latter cases. It is pertinent that the variation in timing of worm expulsion which we have observed contrasts vividly with that in other infections such as *T. spiralis* and *T. muris*; events in our system occurred over a time-span of months rather than days. Clearly, the overall response is on a very much slower time-frame and it is therefore likely that other factors such as variation in susceptibility to parasite-mediated immunodepression are equally if not more important in explaining the differences between our data and that of others (Behnke & Robinson, 1985; Behnke, 1987; Enriquez *et al.* 1988*b*).

The protracted nature of primary infections with *H. polygyrus* suggests that the parasite's evasive strategies are particularly successful during primary exposure. Moreover, there is evidence that adult *H. polygyrus* have a marked influence on the functional capacity of macrophages (Pritchard, Ali & Behnke, 1984; Crawford, Behnke & Pritchard, 1989). An alternative explanation for variation in response phenotype may be that parasite factors interact with Class II molecules in such a way as to impair their normal function and that some haplotypes are more susceptible than others. Binding would be dependent on receptor–ligand interactions with haplotype specific differences in Class II molecules influencing binding properties and hence determining overall response phenotype. Since H-2^b mice do not express I–E because of a deletion in the E_α gene, this interaction must involve I–A gene products as targets. There is a precedent in bacterial toxins such as staphylococcal enterotoxin which are known to bind directly to MHC Class II with resultant mitogenicity for T cells (Fleischer, 1989). It is not inconceivable therefore that parasites causing chronic infections have also evolved molecules which bind to MHC Class II and impair or even block the stimulation of T cells. Indeed such molecules would be predicted on evolutionary considerations as reflected in the arms race between hosts and pathogens (Behnke & Barnard, 1990).

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