Immunological relationships during primary infection with *Heligmosomoides polygyrus*. Regulation of fast response phenotype by H-2 and non-H-2 genes

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SUMMARY

The inheritance of response phenotype to *Heligmosomoides polygyrus* was investigated in F1 hybrid progeny of fast and slow responder mouse strains. The fast responses of SJL(H-2^s) and SWR(H-2^q) mice were mediated by dominant genes complementing each other in F1 hybrids which lost worms earlier and produced faster parasite-specific IgG1 antibody responses than either parent. However, the response of F1 hybrids from crosses of C57BL/10 (B10, H-2^b) mice with either SJL or SWR differed from that of the parental strains and from each other: (B10 × SWR)F1 lost worms earlier than SWR whilst (B10 × SJL)F1 lost worms later than SJL mice. The F1 progeny of SJL mice with congenic strains B10.G (H-2^q) and B10.S (H-2^s) lost worms as quickly as SJL. Therefore, the response phenotype mediated by the genome of SJL mice was unaffected by H-2 heterozygosity (with H-2^q) or homozygosity (H-2^s) despite heterozygosity with B10 background genes, but was slowed significantly by heterozygosity with H-2^b. All hybrids involving heterozygosity with B10, irrespective of MHC haplotype or background, failed to clear worms completely, in each case a proportion of mice harbouring residual worm burdens after loss of worms from parental strains.

Key words: *Heligmosomoides polygyrus*, hybrid mice, H-2 congenic strains, response phenotype.

INTRODUCTION

The majority of mouse strains support chronic infections with *H. polygyrus*, lasting in some cases more than 10 months (Keymer & Hiorns, 1986; Robinson *et al.* 1989). Two strains differ radically in this context: SJL and SWR mice both lose their worm burdens by week 8 post-infection (p.i.) (Mitchell *et al.* 1982; Wahid, Robinson & Behnke, 1989). These strains bear the H-2^s and H-2^q MHC haplotypes respectively, both of which have been associated with early worm loss in mice sharing common non-MHC background genes (B10 MHC congenic mouse strains; Behnke & Wahid, 1991). However, MHC-linked genes are not solely responsible for accelerated worm loss because NIH and DBA1 mice, both of which are H-2^q, support primary infections which last for more than 10 weeks (Robinson *et al.* 1989). It has also been demonstrated that (NIH × B10)F1 hybrids, which carry H-2^{bq}, show rapid loss of worms despite the inability of either parental strain to curtail infections, indicating that gene complementation can take place at the MHC and/or background gene levels (Robinson *et al.* 1989).

The unusually rapid loss of adult worms from SJL and SWR mice is of interest; it suggests that common genes may confer an advantage on SJL and SWR mice over other strains, enabling them either to mount rapid host-protective immune responses and/or to resist the immunomodulatory mechanism employed by *H. polygyrus* to facilitate its own survival (Behnke, 1987). In contrast, it has been suggested that C57BL/10 (B10) mice are particularly sensitive to parasite-mediated immunodepression (Robinson *et al.* 1989; Behnke & Wahid, 1991) and it was pertinent, therefore, to explore further the relationship between SJL, SWR and B10 background genes and their respective MHC haplotypes in facilitating resistance/susceptibility to infection with *H. polygyrus*. In this paper we report the results of 6 experiments in which we compared the course of primary infection in SJL and/or SWR mice with that in their F1 hybrid progeny and in hybrids arising from crosses of each strain with mice carrying B10 background genes and one of the 3 MHC haplotypes (H-2^s, H-2^q and H-2^b) linked respectively to resistance and susceptibility.

MATERIALS AND METHODS

Animals

Syngeneic SWR, SJL, C57BL/10 (B10), MHC congenic B10.S, B10.G and outbred CFLP mice were either purchased from Harlan Olac Ltd or, together with hybrids, were bred in the Department

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of Life Science animal house under conventional conditions. Female mice were used in all experiments and were provided with food and water *ad libitum*.

Parasite

The parasite used in this work was *Heligmosomoides polygyrus bakeri* (Behnke, Keymer & Lewis, 1991). The methods employed for maintenance, infection of mice and recovery of worms at autopsy have all been described previously (Jenkins & Behnke, 1977).

Preparation of antigens

Outbred CFLP mice were infected with 400 L_3 of *H. polygyrus* and were killed 14 days later. Adult worms were isolated by opening the small intestine and incubating sections harbouring worms in Hanks's saline at 37 °C in gauze bags held over small glass beakers. When sufficient numbers of worms had collected, the parasites were washed 10 times in ice-cold sterile phosphate-buffered saline (PBS) and were homogenized in a minimal volume of PBS using a glass tissue homogenizer held in an ice bath. The resulting suspension was centrifuged at 10000 ***g*** for 1 h at 4 °C to remove coarse particulate matter. The supernatant fraction was filtered (0·22 μm filter, Millipore), analysed for protein concentration using a method modified from Lowry *et al.* (1951), aliquoted and stored at −40 °C.

Measurement of antibody responses

Specific anti-worm antibodies were measured by a standard ELISA but the data for each experimental group are presented as a mean relative response index (RRI). This was necessary to enable large numbers of sera to be screened simultaneously and to enable comparison of values from different experiments. Briefly, ELISA microtitre plates were coated with 50 μl/well of worm antigen (5μg/ml). Alkaline phosphatase-conjugated sheep-anti-mouse IgG1 (Serotec) was used to measure subclass specific responses. After addition of the substrate (*p*-nitrophenylphosphatase) colour changes were read at 410 nm on a Dynatech MR700 Microplate Reader. Sera were assayed individually in triplicate after storage at −40 °C and a mean optical density (O.D.) value was obtained for each serum. Each plate included control hyperimmune serum (HIM) which was raised as described by Behnke & Parish (1979) and control serum from age and sex matched naive mice and these were used to calculate the RRI for individual sera using the following formula:

$$\text{RRI} = \frac{\text{O.D. of experimental serum} - \text{O.D. of control serum}}{\text{O.D. of HIM} - \text{O.D. of control serum}} \times 100.$$

Thus the mean O.D. for individual sera was expressed as a percentage of the O.D. obtained with the hyperimmune reference serum, after subtraction of the value for control serum. RRIs measured on individual sera on different occasions showed little variation and the results were consistently reproducible. The same stocks of aliquoted HIM and control serum were used throughout the study. Group mean RRIs were calculated from individual RRIs.

Statistical analysis of results

Worm counts and antibody responses are presented as group mean value ± standard error (S.E.M.). Non-parametric statistical procedures were used to analyse the data sets (Sokal & Rohlf, 1969), because normal distribution of data could not be assumed. When more than two groups required comparison at a single time-point the Kruskal Wallis statistic H was calculated to determine whether there was a strain effect across experimental groups. If significant, specific groups were compared to the control group (or as stated) by the Mann–Whitney U test. Probabilities were calculated from statistics tables and $P = 0{\cdot}05$ was taken as the cut-off point for significance unless multiple analyses were undertaken when the cut-off value for significance was lowered to $P = 0{\cdot}025$ or 0·01, depending on the number of comparisons, in order to avoid Type II errors.

RESULTS

Comparison of the course of infection with H. polygyrus *in (SWR × SJL)F1 hybrid mice and in parental strains*

Experiments were carried out comparing the time-course of infection in (SWR × SJL)F1 hybrid mice with that in the two parental strains. The results from two such experiments are illustrated in Fig. 1. Similar results were obtained in 4 additional experiments, not illustrated here. In Exp. 1, B10 mice were also included as a reference strain in which no loss of worms was anticipated in the period examined. As can be seen SWR and SJL lost 69% and 84% respectively of their initial worm burden by week 6, as had been reported previously (Wahid *et al.* 1989). In the second experiment, worm loss commenced by week 4 in SJL mice (27% reduction compared with week 2) but was delayed in SWR with no significant reduction even by week 6, although 2 out of 6 mice had lower worm burdens at this time indicating that loss had already started. By comparison, in Exps 1 and 2, F1 mice lost 86% and 62% of their worm burden by week 4 and 100% and 98% by week 6, respectively. Mean worm recoveries from F1 mice in week 6 were 0·3 and 0·9, respectively compared with

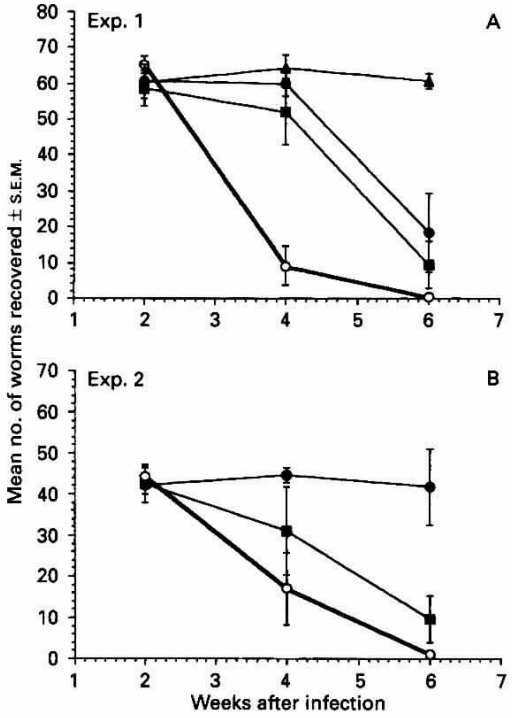

Fig. 1. The course of infection with *Heligmosomoides polygyrus* in B10 (▲), SWR (●), SJL (■) and (SWR × SJL)F1 (○) hybrid mice. (A) For SWR, SJL, B10 and F1 hybrid mice in week 2 $n = 4$, 5, 4 and 5 respectively, the Kruskal–Wallis statistic $H = 1{\cdot}688$, $P =$ N.S. In week 4 $n = 4$, 6, 6 and 10 respectively, $H = 15{\cdot}134$ ($P = 0{\cdot}002$). In week 6 $n = 5$, 10, 6 and 6, $H = 18{\cdot}279$ ($P = 0{\cdot}001$). (B) For SWR, SJL and F1 mice in week 2, $n = 4$ in all cases, $H = 0{\cdot}28$, $P =$ N.S. In week 4, $n = 6$, 5 and 6 respectively, $H = 3{\cdot}7$ ($P =$ N.S.). In week 6, $n = 6$, 6 and 8 respectively, $H = 7{\cdot}55$, $P = 0{\cdot}023$.

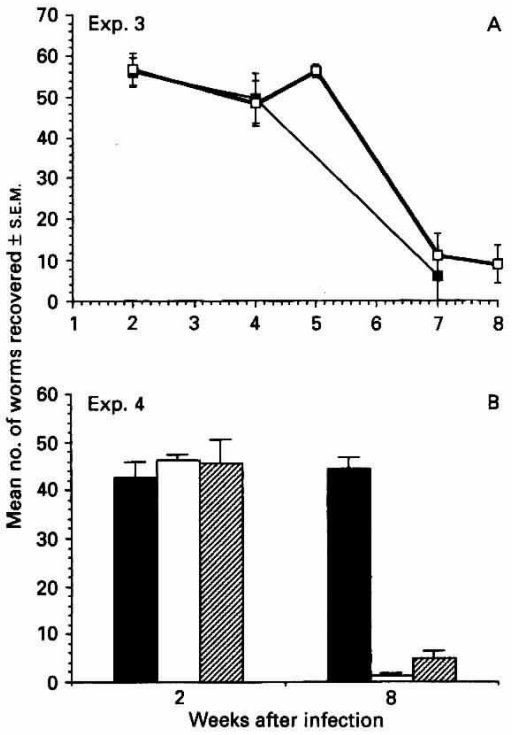

Fig. 2. The course of infection in SJL, B10 and (B10 × SJL)F1 hybrid mice. (A) For SJL (■) and (B10 × SJL)F1 (□) mice in week 2, $n = 4$ and 6, in week 4, $n = 6$ and 9 and in week 7, $n = 6$ and 10 respectively. Worm burdens were not significantly different at any time point. For F1 mice, $n = 10$ in each of weeks 5 and 8. (B) For SJL (□), B10 (■) and F1 (▨) mice in week 2, $n = 5$, 5 and 4 respectively and there is no significant difference between the groups. In week 8, $n = 12$, 9 and 9 respectively. $H = 20{\cdot}957$, $P < 0{\cdot}001$.

SJL mice which had 9·4 and 9·5 respectively. It is evident, therefore, from both data sets that F1 hybrid mice lost worms earlier than either parental strain, although in the second experiment the difference between SJL and (SWR × SJL)F1 mice was less clear cut. In 4 further experiments F1 mice had lower mean worm burdens than parental strains in week 4.

Analysis of the serum IgG1 response to parasite antigens showed that F1 mice produced a more rapid response than either parental strain in week 4 (Fig. 5A, versus SWR $P = 0{\cdot}006$; versus SJL $P = 0{\cdot}003$), although by week 6 there was no significant difference between these strains. B10 mice, in contrast, produced a very weak response during this period.

The influence of slow responder (B10) genes on the course of infection with H. polygyrus *in (B10 × SJL)F1 hybrid mice*

Further experiments examined the influence of the slow responder B10 genes on worm survival in the F1 progeny of crosses with the fast responding SJL strain. Two comparisons were made as illustrated in Fig. 2. In Exp. 3 (B10 × SJL)F1 were compared with SJL mice at intervals during a 7-week period following exposure to 60 L3. The results show that neither strain lost worms in the first 4 weeks and that worm burdens were stable in F1 mice until at least week 5. By week 7 both strains lost the majority of worms (80% and 89% reduction in F1 and SJL, respectively). F1 mice retained a residual burden in week 8.

In Exp. 4, following exposure to 50 L3, F1, SJL and B10 mice showed comparable worm burdens in week 2. By week 8 worm burdens remained stable in B10 mice, but SJL and F1 mice had rejected 97% and 90% of their original worm burden, respectively. At this time worm burdens in F1 mice were significantly higher than in SJL mice ($0{\cdot}05 > P > 0{\cdot}025$).

IgG1 antibody responses were similar in SJL and (B10 × SJL)F1 mice in both weeks 4 and 7 (Fig. 5B).

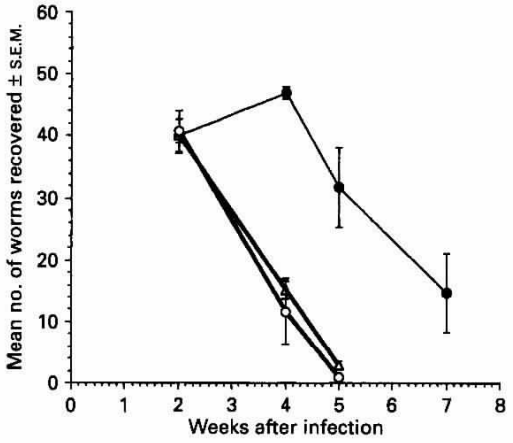

Fig. 3. The course of infection in SWR (●), (B10 × SWR)F1 (△) and (SWR × SJL)F1 (○) hybrid mice. For SWR, (B10 × SWR)F1 and (SWR × SJL)F1 mice in week 2, $n = 5$, 4 and 6 respectively, $H = 0{\cdot}17$, $P =$ N.S.; in week 4 $n = 6$, 5 and 9 respectively, $H = 11{\cdot}486$, $P = 0{\cdot}003$; in week 5 $n = 7$, 6 and 22, $H = 25{\cdot}077$, $P < 0{\cdot}001$. For SWR mice $n = 8$ in week 7.

The influence of slow responder (B10) genes on the survival of H. polygyrus *in (B10 × SWR)F1 hybrid mice*

Exp. 5 compared the course of infection in (B10 × SWR)F1 mice with one of the parental strains, i.e. SWR. On this occasion a batch of (SWR × SJL)F1 hybrid mice was also available and these were included in the experiment. The data in Fig. 3 show that as in Exps 1 and 2, (SWR × SJL)F1 mice expelled a significant proportion of their worm burden by week 4 (71 % reduction relative to week 2) at a time when no loss was evident from SWR mice. However, (B10 × SWR)F1 mice lost worms equally rapidly. Thus in contrast to (B10 × SJL)F1s the outcome of crossing B10 mice with SWR resulted in a host with a faster response to *H. polygyrus*.

The specific IgG1 antibody response of (SWR × SJL)F1 hybrids was more intense compared to the parental strain SWR in week 4 ($P = 0{\cdot}005$) and 5 ($P = 0{\cdot}005$, Fig. 5C), as had been found earlier in Exp. 1. (B10 × SWR)F1 mice also had a higher mean antibody titre than SWR mice but this was not significantly different.

The influence of H-2 haplotype on the survival of H. polygyrus *in F1 hybrids of B10 congenic and SJL mice*

In the final experiment (Exp. 6) SJL mice were crossed with B10 (H-2^b), B10.G (H-2^q) and B10.S (H-2^s) congenic strains to determine whether in (B10 × SJL)F1 the H-2^b component (from B10 parents) of the H-2^{bs} haplotype slowed the rate of worm loss. As is evident from Fig. 4, the inoculum

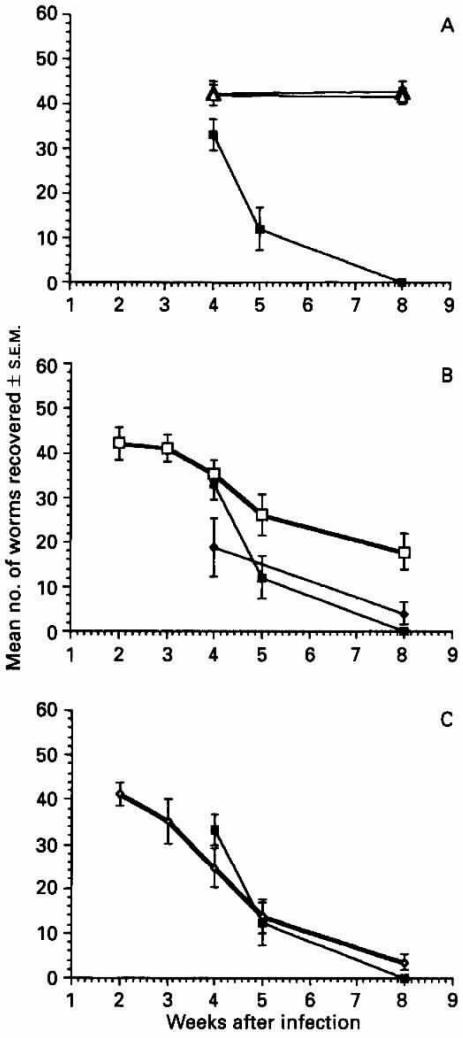

Fig. 4. The course of infection in SJL, B10 and B10.G mice and various F1 hybrid crosses of SJL with B10 MHC congenic strains. In weeks 2 and 3 for (B10 × SJL)F1 (□) and (B10.G × SJL)F1 (◇) mice $n = 6$ and 6, and 10 and 10 respectively. In week 4 for B10 (▲), B10.G (△), (B10 × SJL)F1, (B10.S × SJL)F1 (◆) and (B10.G × SJL)F1 mice $n = 8$, 8, 12, 16, 9 and 14 respectively, $H = 13{\cdot}919$, $P = 0{\cdot}016$. In week 5 for SJL (■), (B10 × SJL)F1 and (B10.G × SJL)F1 mice $n = 9$, 12 and 12 respectively, $H = 5{\cdot}811$, $P = 0{\cdot}05$. In week 8 for B10, B10.G, SJL, (B10 × SJL)F1, (B10.S × SJL)F1 and (B10.G × SJL)F1 mice $n = 13$, 13, 9, 15, 8 and 14 respectively, $H = 52{\cdot}932$, $P < 0{\cdot}001$.

was about 80 % infective and worm burdens in (B10 × SJL)F1 (Fig. 4B) and (B10.G × SJL)F1 (Fig. 4C) mice did not differ in week 2 and were similar to those of B10 and B10.G mice in week 4 (Fig. 4A). There were insufficient numbers of mice available to enable representative groups from all strains to be killed in week 2 but no differences in establishment had been detected earlier in any such comparisons at this time. The B10 and B10.G parental strains did not lose worms during the 8 week period of infection

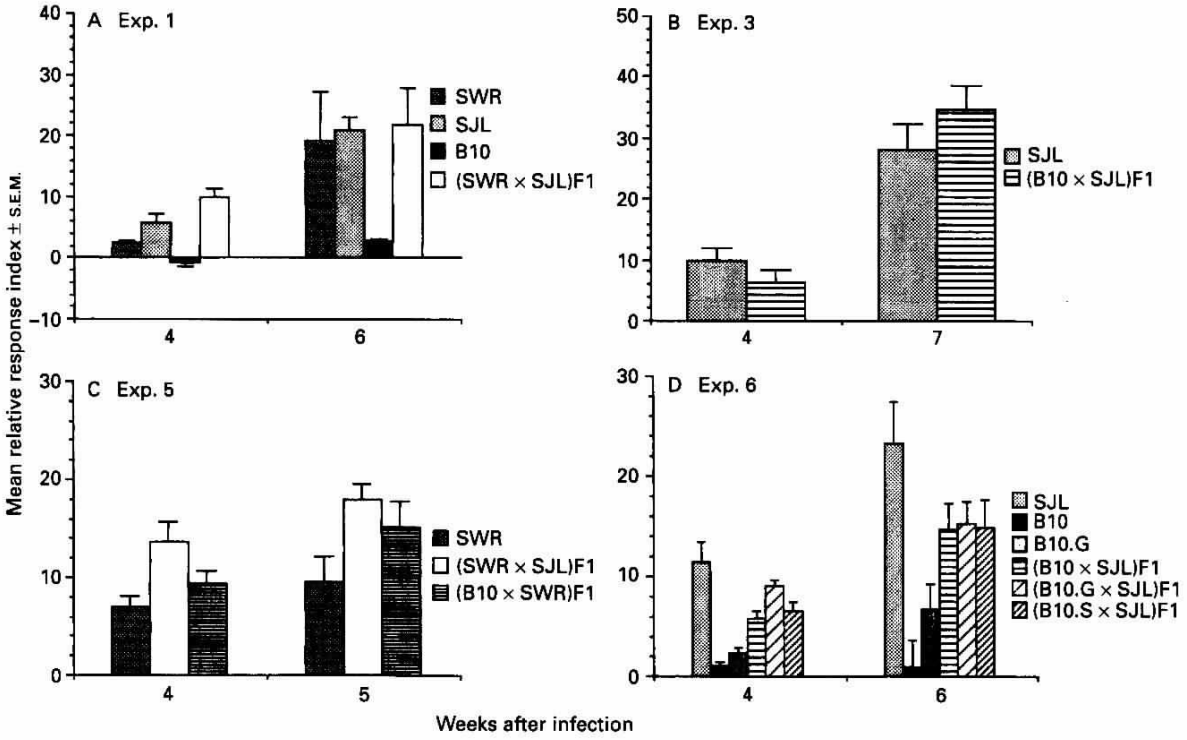

Fig. 5. Parasite-specific IgG1 antibody responses in mice representing fast and slow responder phenotypes and in their F1 hybrid progeny. (A) In week 4 $H = 17{\cdot}362$, $P < 0{\cdot}001$; in week 6 $H = 13{\cdot}649$ ($P = 0{\cdot}0034$). (B) No significant difference between groups in weeks 4 and 7. (C) In week 4 $H = 6{\cdot}737$, $P = 0{\cdot}034$, in week 5 $H = 9{\cdot}078$, $P = 0{\cdot}028$. (D) In week 4 $H = 35{\cdot}682$, $P < 0{\cdot}001$, in week 6 $H = 19{\cdot}163$, $P = 0{\cdot}002$.

(Fig. 4A) whereas SJL parental mice lost 71% of their worm burden by week 5 and only a single worm was recovered from the 9 mice examined in week 8.

As in Exp. 3 (B10 × SJL)F1 mice did not lose a significant proportion of the worm burden by week 4 (13·9% relative to week 3, P = N.S.), although on this occasion 37·9% loss (relative to week 2) was evident by week 5 ($P = 0{\cdot}025$). However, as earlier in this strain, loss was incomplete and 42% of the initial worm burden was still evident in week 8. In contrast the H-2^{qs}-bearing (B10.G × SJL)F1s (Week 5, $0{\cdot}05 > P > 0{\cdot}025$) and H-$2^{s}$-bearing (B10.S × SJL)F1 (Week 4, $P = 0{\cdot}025$) showed significantly earlier loss of worms than the H-2^{bs}-bearing (B10 × SJL)F1 hybrids. Worm burdens in (B10.G × SJL)F1 and (B10.S × SJL)F1 hybrids were not significantly different from those of SJL mice in weeks 4, 5 nor in week 8 despite the higher mean worm recoveries in both strains on the latter occasion (MWR = $3{\cdot}6 \pm 1{\cdot}6$, $4{\cdot}0 \pm 2{\cdot}4$ and $0{\cdot}1 \pm 0{\cdot}1$, respectively), attributable to 5 out of 14 and 4 out of 8 mice respectively still carrying some worms in comparison to only 1 SJL mouse with 1 worm at this time. In contrast 13 out of 15 (B10 × SJL)F1 hybrids still had worms in week 8 and the MWR ($17{\cdot}8 \pm 4{\cdot}1$) was significantly higher than that of SJL mice ($P < 0{\cdot}001$).

As in Exp. 1, SJL mice produced a vigorous, and B10 mice a very weak, specific IgG1 antibody response in weeks 4 and 6 p.i. B10.G mice responded a little more vigorously than B10 mice, but the difference between the groups was not significant (Fig. 5D). In both weeks 4 and 6 all hybrid strains had significantly higher antibody titres than B10 mice ($P < 0{\cdot}01$). In week 4 only (B10 × SJL)F1s among the hybrid strains had a significantly lower antibody response than SJL ($0{\cdot}025 > P > 0{\cdot}01$) whereas in week 6 despite lower mean titres there was no significant difference between any hybrid strain and SJL mice ($P = 0{\cdot}065$).

DISCUSSION

The data in this paper strongly support the hypothesis that more than a single gene is responsible for endowing mice with the fast response phenotype to *H. polygyrus*, as exemplified by SJL and SWR mice. When these two strains were crossed, the F1 hybrids showed an enhanced ability to terminate primary infections. More than 50% of the worms were lost by week 4, at a time when both parental strains still supported stable worm burdens or were just beginning to lose worms. Such an accelerated response would not have been expected if a single gene, possessed by both parental strains, was solely

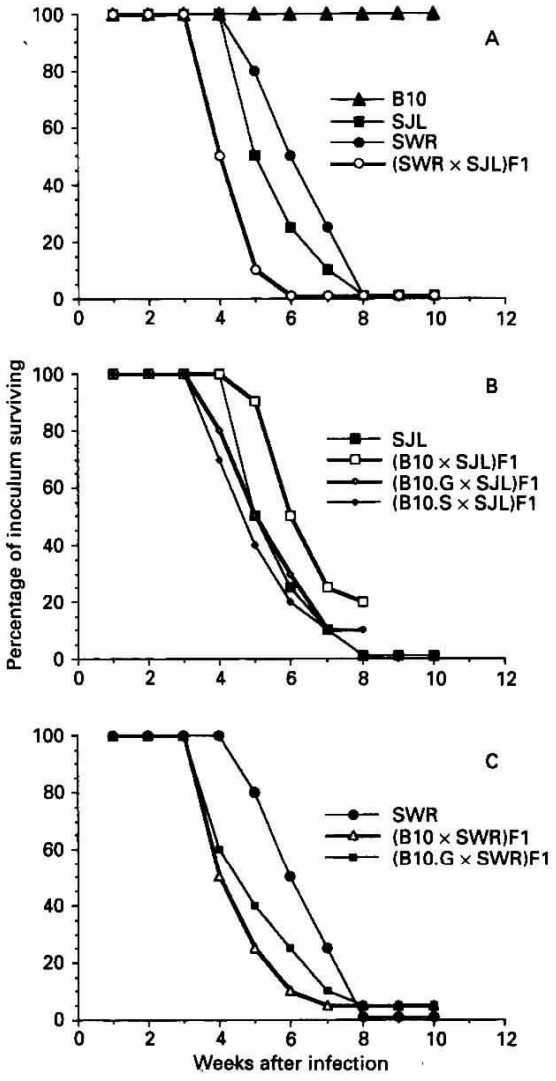

Fig. 6. A schematic summary of the course of infection with *Heligmosomoides polygyrus* in parental (except B10.G and B10.S which have an identical course of infection to B10 mice in the first 8 weeks of infection) and hybrid strains examined. Data for SJL mice are repeated in (A) and (B) and that for SWR in (A) and (C) to enable comparison with other strains across the data-sets.

responsible, unless there are two alleles of the same gene capable of enhancing resistance and their co-expression in heterozygotes results in complementary activity.

Further data in this paper support the existence of a multigenic system (Fig. 6). Firstly when SWR and SJL mice were crossed with the slow responding B10 mice, their progeny responded in different ways. (B10 × SJL)F1 mice lost worms at the same rate, occasionally slower than their SJL parent. (B10 × SWR)F1s showed an accelerated response relative to SWR mice, comparable in fact to that seen in (SWR × SJL)F1 hybrids. Thus the SWR response can be complemented either by SJL or B10 genes to give worm loss within 4 weeks of infection whereas the SJL genome cannot be enhanced by heterozygosity with B10 genes. This suggests that B10 mice, despite their inability to curtail infections early, have a gene/s which complements other genotypes in accelerating worm loss and raises the possibility that SJL and B10 mice may share this gene.

Secondly, on the basis of earlier studies we considered it likely that the H-2^b haplotype alleles from B10 mice may have been responsible for limiting the onset of worm loss in (B10 × SJL)F1s mice. Therefore we compared worm loss in (B10 × SJL)F1, (B10.G × SJL)F1 and (B10.S × SJL)F1 hybrids which bear H-2^{bs}, H-2^{qs} and H-2^s haplotypes respectively but do not differ otherwise in their background genes. Both (B10.S × SJL)F1 and (B10.G × SJL)F1 mice showed accelerated worm loss relative to (B10 × SJL)F1 hybrids with a reduction in worm burdens evident by weeks 4 and 5, respectively. Thus the delayed onset of worm expulsion seen only in (B10 × SJL)F1 mice was not due to B10 background genes, as all hybrids shared these genes, and probably reflected the influence of the H-2^b haplotype.

Thirdly, in complete contrast to SJL, the H-2^b haplotype did not impair worm loss in mice with SWR background genes. Indeed (B10 × SWR)F1 hybrids began to lose worms as rapidly as (SWR × SJL)F1 hybrids and it is pertinent that (B10 × NIH)F1 hybrids also lose worms rapidly (Robinson *et al.* 1989). Thus if there is an MHC effect associated with the H-2^b haplotype it is expressed only in (B10 × SJL)F1 hybrids and not in hybrids with SWR or NIH backgrounds, reinforcing the earlier suggestion that SJL and B10 mice share an important gene controlling the processes governing worm loss but susceptible to moderation in mice carrying H-2^b alleles, and presumably silent in B10 mice.

Another aspect of our data deserving attention concerns the failure of all combinations of hybrids with a B10 background parent to lose all their worms by week 8. Whilst SJL, SWR and (SWR × SJL)F1 mice always lost > 98% of their worm burden once loss had begun, in all B10 hybrids, irrespective of MHC haplotype, the rate of worm loss was slower with a residual worm burden persisting until week 8 in a proportion of individuals (see Wahid *et al.* 1989). Even (B10.G × SJL)F1 and (B10.S × SJL)F1 hybrids, which began to lose worms in week 4, did not entirely clear all worms by week 8, although there was no statistical difference when MWRs were compared to those of SJL mice. On balance we attribute the failure of hybrids involving a B10 parent to completely remove all worms once loss has been initiated, to the influence of a B10 background gene/s. It is conceivable that the mechanisms

responsible for loss of worms were down-regulated prematurely in hybrid strains carrying B10 background genes. This could reflect the existence of a gene/s which evolved to save on the host resources that would have been required to sustain the response necessary to ensure total clearance of parasites once a sufficient proportion of the parasite burden had been lost and no longer posed a threat to the health of the host (see Behnke, Barnard & Wakelin, 1992).

Specific IgG1 antibody responses were monitored in most of the experiments but only selected data have been presented. These show that SWR and SJL mice consistently produced vigorous antibody responses in the period preceding worm loss, but (SWR × SJL)F1 hybrids which lost worms earlier generated more intense antibody responses. In contrast, B10 mice produced very weak responses which seldom rose much above the baseline values signified by control sera from naive mice. Such poor IgG1 antibody responses to *H. polygyrus* may reflect the susceptibility of this strain (Wahid & Behnke, 1993), and more specifically, the H-2^b haplotype (Behnke & Wahid, 1991) to down-regulation by parasite immunomodulatory factors. When SJL mice were crossed with B10 or B10 congenic strains, the response of the resulting hybrids was weaker (Exp. 6) or indistinguishable (Exps 3 and 5) from that of the fast responding SJL or SWR parental strain and always faster and more intense than that of the B10 parent. Therefore SJL and SWR mice have dominant genes which enhance the IgG1 antibody response in hybrid combinations with mice of poor response phenotype and synergize with each other to generate a more responsive phenotype.

Finally, we propose that the fast responses of SJL and SWR mice are mediated by dominant genes, or alleles of a common gene, which can complement each other in F1 hybrids to produce a mouse strain which shows worm loss by the fourth week of infection, clears all parasites by week 6 and produces faster parasite-specific IgG1 antibody responses than either parent. The response phenotype mediated by SJL and SWR genomes can be moderated but not suppressed in hybrid combinations involving mice with B10 background genes and carrying the H-2^b haplotype. The genome of SWR is not down-regulated by heterozygosity with H-2^b and its ability to mediate worm loss may even be enhanced. The response of SJL mice, however, is slowed in hybrids carrying H-2^b alleles but can be restored to that of the SJL parent by heterozygosity with H-2^q or homozygosity (H-2^s) despite heterozygosity with B10 background genes, indicating that it is the H-2^b-associated genes and not the B10 background genes which slow the response of (B10 × SJL)F1 hybrids relative to SJL mice. However, in all hybrids involving heterozygosity with B10 background genes, worm loss is not complete and a residual worm burden is tolerated for several weeks after clearance of worms from parental strains.

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