Parasite Immunology, 1993: **15**: 401–413

Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): parasite specific IgG1 antibody responses and primary response phenotype

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SUMMARY

IgG1 antibody responses to Heligmosomoides polygyrus *were measured in eight mouse strains supporting acute (<8 weeks, SJL, SWR), intermediate (10–20 weeks, NIH, BALB/c) or chronic (>25 weeks, C57BL/0, CBA, C3H, AKR) primary infections. Mice supporting acute or intermediate infections produced more intense antibody responses and total serum IgG1 concentrations were higher than in mice tolerating chronic infections. Positive correlations across mouse strains between the intensity of the antibody response and the percentage loss of worms in weeks 6 and 10 were established. No correlation was found between the response within mouse strains and loss of worms by individual mice. Heavy infections gave marginally higher antibody titres than low intensity infections, but few significant differences were detected and it was concluded that infection intensity did not markedly influence the magnitude of the antibody response. Male and female mice responded similarly despite the earlier loss of worms from females. No association was found between the primary response phenotype and recognition of particular antigens in Western blot analysis, nor did intensity of infection or host gender affect recognition. The possibility that immunomodulatory properties of adult worms may have had a differential influence on ability of strains of contrasting response phenotype to mount IgG1 responses was discussed.*

Keywords *nematode,* Heligmosomoides polygyrus, *mouse, SWR, SJL, NIH, BALB/c, C57BL/10, CBA, C3H, AKR, IgG, IgG1, antibody, immunoglobulin, resistance, worm expulsion, chronic infection*

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Received: 16 September 1992
Accepted for publication: 2 February 1993

INTRODUCTION

Parasitic nematodes responsible for chronic infections, are of considerable importance both in human and veterinary medicine (See reviews Behnke 1987, Behnke, Barnard & Wakelin 1992a, Monroy & Enriquez 1992). Despite their economic and medical significance, the mechanisms exploited by nematodes in surviving within otherwise immunocompetent hosts are still poorly understood.

Heligmosomoides polygyrus is a murine intestinal species which has been studied in an effort to determine the factors enabling chronic infections to persist (Behnke 1987, Monroy & Enriquez 1992). Following primary exposure many mouse strains harbour adult worms for more than 30 and often up to 40 weeks (Robinson *et al.* 1989), whereas others curtail primary infections within eight weeks of exposure (Wahid, Robinson & Behnke 1989), an observation which is of particular significance because it implies that certain mouse genotypes are resistant to the evasive strategies employed successfully by adult *H. polygyrus* during chronic infections in susceptible strains. Although the immunological processes accompanying loss of *H. polygyrus* during primary infections are not understood *H. polygyrus* is known to be susceptible to expulsion in mice in which intestinal inflammation has been induced non-specifically (Behnke, Cabaj & Wakelin 1992b). Recently Urban, Katona & Finkelman (1991) have produced data indicating that in BALB/c mice (Intermediate responders), $CD4^+$ cells are required to control primary infections and that depletion of these cells through treatment with specific antibody results in a depression of serum IgE and an increase in survival and fecundity of adult worms. Collectively these data suggest that some mouse strains can reject the worm through the mediation of $CD4^+$, Th2 lymphocytes which are known to control inflammatory responses to other GI nematodes (Finkelman *et al.* 1991a, Grencis, Hultner & Else 1991, Else & Grencis 1991).

The data necessary to substantiate this hypothesis are difficult to obtain because of the long time frame over which relevant experiments have to be conducted and a role for Th2 cells can be questioned because even fast responding strains fail to show a prominent mastocytosis during primary infections (Dehlawi, Wakelin & Behnke 1987). On the other hand *H. polygyrus* elicits prominent parasite specific IgG1 responses and these also depend on help provided by Th2 cells (Stevens *et al.* 1988, Snapper, Finkelman & Paul 1988, Mosmann & Coffman 1989). Originally IL-4 was considered to be necessary for IgG1 responses but the recent demonstration that *in vivo* depletion of IL-4 abrogated IgE responses but did not affect IgG1 implies that other Th2 cytokines must be involved (Finkelman *et al.* 1991).

IgG1 clearly has an important role to play in the host-parasite relationship of *H. polygyrus* although the involvement of IgG1 in mediating host protective immunity during primary infections is still debatable. Whilst some studies have shown that primary infection IgG1 neither mediates nor blocks protective immunity (Williams & Behnke 1983, Pritchard, Behnke & Williams 1984) others have attributed a protective function to this isotype (Dobson 1982). Since detailed information on the factors determining the intensity, rapidity and specificity of IgG1 responses to *H. polygyrus* and the relevance of these to worm survival are still lacking, we report here the results of a series of experiments which focus specifically on this aspect of the host-parasite relationship. These experiments examined the hypothesis that an inverse relationship between the duration of primary infection and parasite specific IgG1 responses should exist across strains supporting varying durations of infection, if IgG1 responses constiute or accompany an essential host component limiting the survival of adult worms. In addition we have correlated IgG1 responses and the duration of adult worm survival among individual mice within strains and compared both low and high intensity infections and infections in male and female mice within strains. Both sets of variables have a marked influence on the duration of infection (Robinson *et al.* 1989) and the intensity and rapidity of IgG1 responses should therefore vary accordingly.

MATERIALS AND METHODS

Animals

Syngeneic NIH, SWR, SJL, C57BL/10, CBA, C3H, AKR, BALB/c and outbred CFLP mice were either purchased from Harlan Olac Ltd., Bicester, Oxon., UK, or were bred in the departmental animal house under conventional conditions. All animals 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 ten times in ice cold sterile phosphate-buffered saline (PBS) and were homogenized in minimal volume of PBS using a glass tissue homogenizer held in an ice bath. The resulting suspension was centrifuged at 10 000 *g* for one h at 4°C to remove coarse particulate matter. The supernatant 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 (5 μg ml^{-1}). 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 (OD) 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 naïve mice and these were used to calculate the RRI for individual sera using the following formula:

$$RRI = \frac{\text{OD of experimental serum} - \text{OD of Control serum}}{\text{OD of HIM} - \text{OD Control serum}} \times 100$$

Thus the mean OD for individual sera was expressed as a percentage of the OD obtained with the hyperimmune reference serum, after subtraction of the value for naïve 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 naive serum were used throughout the study. Group mean RRIs were calculated from individual RRIs.

Total serum IgG1 in the sera from individual mice was determined by the method of Mancini, Carbonara & Heremans (1965) using radial immunodiffusion (RID) kits purchased from The Binding Site, Birmingham. Ring diameters were measured in two directions at 90° and the mean was used to calculate the concentration of immunoglobulins from a calibration curve obtained using appropriate standards.

The spectrum of antigens recognized by antibodies in the sera of infected mice was examined by the Western blotting technique essentially as described by Carr & Pritchard (1987). Phosphate-buffered saline (PBS) soluble adult worm homogenate was electrophoretically transferred to nitrocellulose membranes (Schleicher and Scuell) following separation on 5–20% gradient SDS-PAGE. Strips of membrane were blocked with 5% skimmed milk in PBS-Tween 20 (0·05%) and then incubated overnight at 4°C with appropriate test sera (1:100) using 5% skimmed milk in PBS-Tween 20. After washing in PBS-Tween 20 the strips were incubated with peroxidase conjugated sheep anti-mouse IgGAM or IgG1 (Serotec) or ^{125}I-sheep anti-mouse polyvalent immunoglobulins (0·5 μCi/strip, Amersham, Bucks, UK) for 2 h at room temperature. Following washing in PBS-Tween 20 (3 × 20 min) the strips were immersed in hydrogen peroxide and 4-chloro-1 naphthol chromogen until the reaction developed optimally, rinsed, arranged on a suitable black background and photographed while still wet. In the case of the ^{125}I-labelled IgGAM, the strips were air dried, fixed to the interior of an X-ray cassette and exposed to an X-ray film (Fuji) at −80°C in the presence of an intensifying screen. The molecular weights were estimated with reference to the migration of standard molecular weight markers.

Experimental design

For logistic reasons, it was not possible to conduct large scale experiments with comprehensive comparisons

Table 1 Mouse strains in the experimental combinations in which they were studied

Experiment no.	Strains examined	Sex	Dose of larvae
1	C57BL/10	F	50
2	C57BL/10	F	50
3	C57BL/10	F	50
4	C57BL/10	F	50
5	C57BL/10	M	50
	SWR	F	50
	SWR	F	250
	SJL	F	50
	SJL	F	250
	NIH	F	50
6	SJL	F	50
	C57BL/10	F	50
7	SJL	F	60
	C57BL/10	F	60
8	BALB/c	F	50
	C57BL/10	F	50
9	BALB/c	F	60
10	NIH	F	50
	NIH	F	250
	NIH	M	50
11	CBA	F	50
12	CBA	F	60
	C3H	F	60
	AKR	F	60
13	C57BL/10	F	50
	C57BL/10	F	250
	NIH	F	50
14	BALB/c	F	50
	BALB/c	F	250
	CBA	F	50
	CBA	F	250
15	BALB/c	F	50
	BALB/c	F	250
16	NIH	F	50
17	CBA	F	50
	CBA	M	50
	C57BL/10	F	50
	C57BL/10	M	50
18	SWR	F	50
	SWR	M	50
19	SJL	F	50
20	C57BL/10	F	50
	BALB/c	F	50
	SJL	F	50
21	SJL	F	50
	NIH	F	50
	NIH	M	50
	C57BL/10	F	50
22	NIH	F	60
	SJL	F	60
23	SWR	F	50
24	C57BL/10	F	60
	SWR	F	60

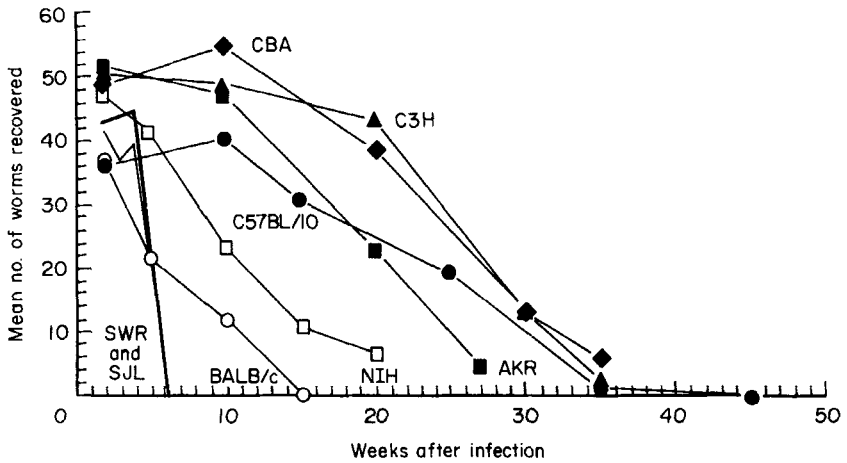

Figure 1 The course of infection with *Heligomosomoides polygyrus* in the eight mouse strains studied. All mice were given low-intensity infections of 50–60 L3 and were killed in groups ($n=6$–12) in the weeks shown. SEMs have been omitted for clarity. The data were obtained from Experiment 10 (NIH), Experiment 12 (CBA, AKR, C3H), Experiment 13 (C57BL/10), Experiment 5 (SWR, SJL) and Experiment 14 (BALB/c). The symbols used to designate strains in this figure have been used consistently throughout the paper. Some of these data have been published previously (See Robinson *et al.* 1989, Wahid *et al.* 1989). —— SWR; —— SJL; —○— BALB/c; —□— NIH; —■— AKR; —●— C57BL/10); —◆— CBA; —▲— C3H.

among all the relevant mouse strains. Combinations of mouse strains were studied in a series of 24 experiments, involving, at most, four strains per experiment. Each experiment focused on particular aspects of the host response and therefore sufficient material was not always available to complete all the possible analyses. However, as far as possible most were repeated, sometimes additional data becoming available through inclusion of necessary controls in experiments investigating other aspects of infection. Table 1 presents a list of the experiments, specifying the strain and sex of mice involved and the infection intensity administered.

Statistical analysis of results

Antibody responses are presented as group mean RRI value ± standard error (SEM). Non-parametric statistical procedures were used to analyse the data sets, because normal distribution of data could not be assumed (Sokal & Rohlf 1969). 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. Correlations between variables were tested by the Spearman Rank Order Correlation Test and the statistic r_s is given, as appropriate. 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 $P=0{\cdot}01$ in order to avoid Type II errors.

RESULTS

The kinetics of low-intensity primary infection in fast, intermediate and slow responder mouse strains

The kinetics of primary infection with *H. polygyrus* in mouse strains tolerating long-lasting chronic infections (Robinson *et al.* 1989) and those limiting infection earlier (Wahid *et al.* 1989) have been described previously. The present work is based on a range of mouse strains representing three primary response phenotypes, slow (resulting in chronic infections > 25 weeks, e.g., C57BL/10, CBA, C3H and AKR), intermediate (10–20 weeks, e.g., NIH and BALB/c) and fast (resulting in acute infections < 8 weeks, e.g., SJL and SWR) and, for reference, representative examples of the time-course of infection in each of these strains are summarized in Figure 1.

Serum IgG1 responses to parasite antigens during the course of acute, intermediate and chronic infections

Data from 15 experiments are illustrated in Figure 2, each experiment measuring the response in only one or at most three strains of mice. Mice were infected with 50–60 L3 unless otherwise stated and the infectivity of the inocula used was in the range 54% (Experiment 15) to 100% (Experiment 5), although the poor establishment in Experiment 15 was unusually low. It is evident that the response of C57BL/10 mice was extremely poor, in some experiments virtually no detectable antibody being observed in the first 10 week period of infection (Figures 2a, b, c & e). At most the RRI rose to 25% in this strain (Experiment 3, Figure 2a). Likewise the other strains

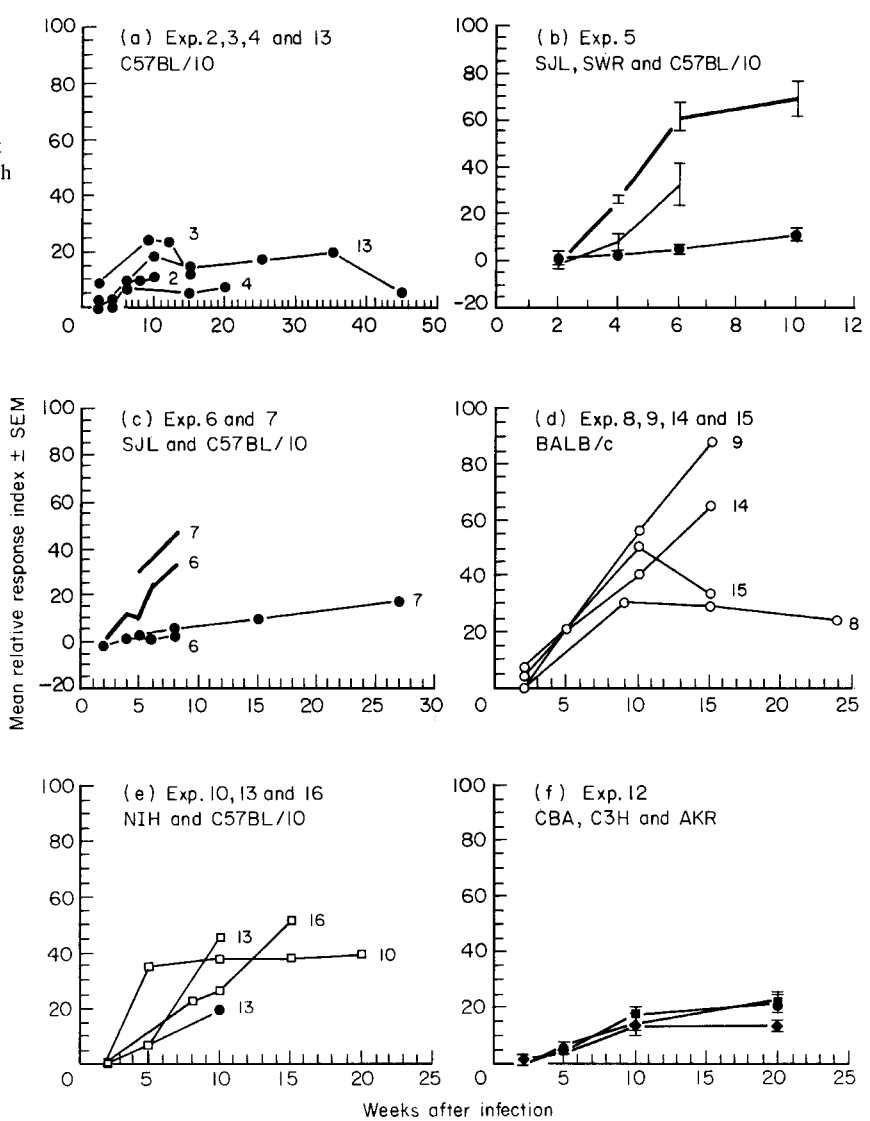

Figure 2 The IgG1 antibody response to *Heligmosomoides polygyrus* during primary infections in female mice of strains representing slow, intermediate and fast responder phenotype. Data from 15 experiments are summarized (SEMs are not given in (a), (c), (d) & (e) for clarity), in each of which mice were infected with 50–60 L3 larvae on day 0 in the combinations shown and were killed for worm counts and antibody assays in groups of (n = 6–12) in the weeks indicated. For key to symbols see Figure 1. Integers beside each set give the experiment no.

Statistical analysis.

(b)
Week
2, $H = 5{\cdot}97$ (P = NS)
4, $H = 8{\cdot}006$ ($P = 0{\cdot}018$)
6, $H = 13{\cdot}639$ ($P = 0{\cdot}001$)

(c) Experiment 6,
Week
2, $0{\cdot}05 > P > 0{\cdot}025$
4, $0{\cdot}01 > P > 0{\cdot}001$
6, $P < 0{\cdot}001$
8, $P < 0{\cdot}001$

Experiment 7,
Week
5, $0{\cdot}01 > P > 0{\cdot}001$
8, $0{\cdot}01 > P > 0{\cdot}001$

(e) Experiment 13,
Week
2, P = NS
10, $0{\cdot}01 > P > 0{\cdot}01$

(f) Experiment 12,
Week
2, $H = 7{\cdot}28$ ($P = 0{\cdot}026$)
20, $H = 5{\cdot}481$ ($P = 0{\cdot}05$)
5 and 10 (P = NS)

supporting chronic infections hardly produced any *H. polygyrus* specific IgG1 serum antibody (Figure 2f). In addition to the data in Figure 2f, two experiments, not illustrated here, showed much the same pattern. In the first of these (Experiment 11) the RRI in CBA did not rise above 10% during 18 weeks post infection (the response was measured in weeks 2, 6, 8, 14 and 18 in a group of nine female mice infected with 50 L3). In the second (Experiment 17), groups of 10–11 CBA and 7–8 C57BL/10 mice were killed 20 and 31 weeks after infection with 50 L3. In week 20 the RRI was $20{\cdot}3 \pm 2{\cdot}6$ and $13{\cdot}5 \pm 3{\cdot}2$ for CBA and C57BL/10 mice respectively (P = NS) and in week 31, $28 \pm 4{\cdot}9$ and $14{\cdot}4 \pm 4{\cdot}3$ ($0{\cdot}05 > P > 0{\cdot}025$) respectively. Both experiments therefore confirm the low intensity IgG1 response of CBA mice to *H. polygyrus* infection.

In marked contrast SJL and SWR mice both showed a rapid IgG1 response with significantly enhanced levels being detected within the first five-week period of infection. In all direct comparisons SJL and SWR mice responded more vigorously than C57BL/10 mice (Figures 2b & c).

Both intermediate responder strains, NIH and BALB/c, also had more intense IgG1 responses than the slow responding strains when compared within or across experiments. For example in Experiment 13 the responses of NIH and C57BL/10 mice were compared directly and

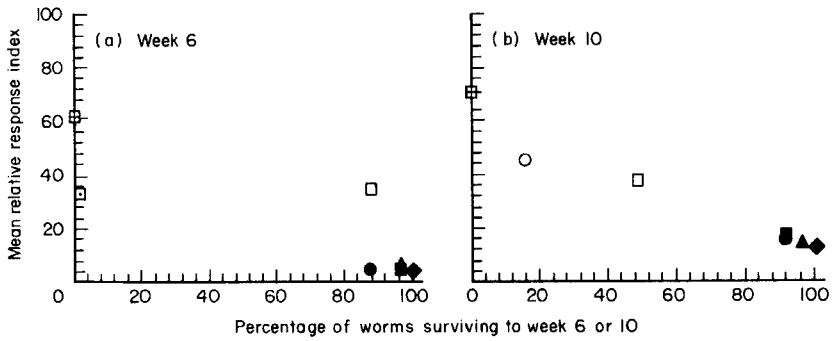

Figure 3 Correlation between response phenotype, as reflected in worm survival, and the intensity of the specific IgG1 antibody responses. Each point represents a mouse strain and the value was obtained by averaging all mean values available for the week specified.
Data for (a) (week 6) were obtained from C57BL/10 (Experiment 5, $n=6$); CBA, C3H and AKR (Experiment 12, $n=6$); NIH (Experiment 10, $n=7$); SJL and SWR (Experiment 5, $n=9$, 7 respectively).
Data for (b) (week 10) were obtained from C57BL/10 (Experiment 5 and 13, $n=7$, 10 respectively); CBA, C3H and AKR (Experiment 12, $n=8$); NIH (Experiment 10, $n=10$); BALB/c (Experiment 14 and 15, $n=8$, 10 respectively); SJL (Experiment 5, $n=6$).
Statistical analysis.
(a) $rs=-0{\cdot}8571 (P=0{\cdot}019)$
(b) $rs=-0{\cdot}9641 (P=0{\cdot}018)$
The symbols used to designate strains have been described in Fig. 1. Additional symbols were also used to denote SWR and SJL strains: ⊡ SWR; ⊞ SJL.

Table 2 Relationship between intensity of the specific anti *H. polygyrus* IgG1 antibody response and the percentage reduction in worms in individual mice

Experiment	Strain	Week	*n*	*Rs*	*P*
Slow responder strains					
13	C57BL/10	25	12	−0·298	Not significant
17	C57BL/10	20	7	+0·0357	Not significant
12 and 17 (pooled)	CBA	20	18	+0·202	Not significant
Intermediate responder strains					
9	BALB/c	10	6	+0·0294	Not significant
14 and 15	BALB/c	5	17	−0·259	Not significant
14	BALB/c	10	7	−0·333	Not significant
9 and 14 (pooled)	BALB/c	10	13	−0·001	Not significant
5	NIH	10	6	−0·543	Not significant
10	NIH	15	9	−0·025	Not significant
13	NIH	10	16	−0·084	Not significant
16	NIH	10	7	−0·45	Not significant
16	NIH	15	8	+0·479	Not significant
21	NIH	8	9	−0·56	Not significant
22	NIH	7	7	+0·051	Not significant
5, 13 and 16 (pooled)	NIH	10	29	−0·263	Not significant
Fast responder strains					
6	SJL	5	9	−0·0766	Not significant
23	SWR	6	8	−0·2036	Not significant

the difference in week ten was highly significant ($0{\cdot}01 > P > 0{\cdot}001$). Likewise in Experiment 21 (data not illustrated) the RRI in week eight was higher in NIH mice ($43{\cdot}6 \pm 6{\cdot}0$, $n=9$) than in C57BL/10 ($18{\cdot}7 \pm 4{\cdot}4$, $n=8$, $P=0{\cdot}03$). In another experiment (Experiment 20—data not illustrated) BALB/c mice ($n=6$) were compared directly to C57BL/10 eight weeks after infection. The RRIs were $15{\cdot}2 \pm 3{\cdot}5$ and $-1{\cdot}4 \pm 3{\cdot}1$ respectively. In this same experiment the RRI of SJL mice ($n=6$) was $25{\cdot}7 \pm 4{\cdot}6$.

The difference between the fast responders (SJL & SWR) and the moderate responders (NIH & BALB/c) was less clear-cut. As can be seen in Figure 2d BALB/c mice showed considerable variation between experiments, on occasion responding as vigorously as SJL mice (Figure 2d, Experiment 9) although when high titres were recorded from this strain it was always several weeks later than in SJL mice. Likewise NIH mice responded variably, often with a peak RRI in excess of 40% (Figure 2e, Experiment 16). In some experiments no convincing

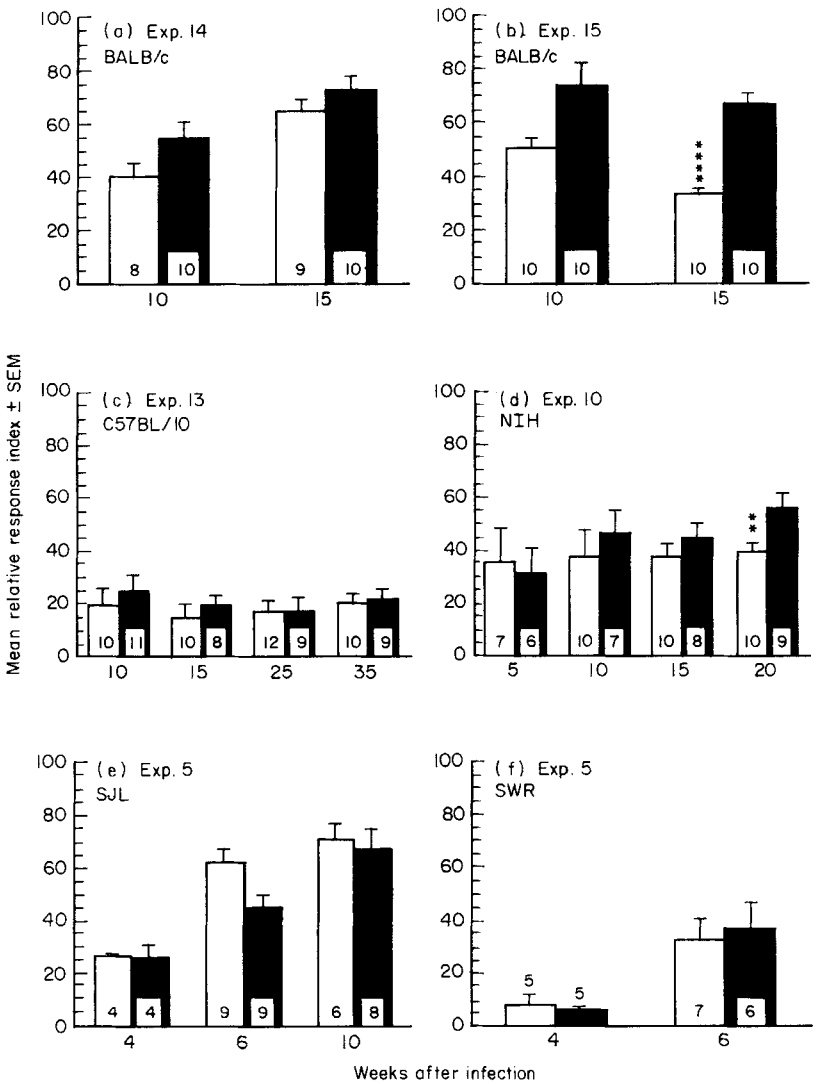

Figure 4 The relationship between the specific IgG1 antibody response to *Heligmosomoides polygyrus* and the intensity of infection. Open columns = low intensity; Filled columns = high intensity. The integers on columns give *n* in each case.
Statistical analysis. Groups were compared by the Mann-Whitney *U* test and the key to the symbols used is as following:
$^{**}0{\cdot}05 > P \geqslant 0{\cdot}02$; $^{****}0{\cdot}01 > P \geqslant 0{\cdot}001$.

difference was detected between NIH and SJL mice. For example in Experiment 21 (data not illustrated) the RRI of SJL mice ($n = 8$) in week eight was $58{\cdot}7 \pm 7{\cdot}8$ whilst that of NIH mice ($n = 9$) was $43{\cdot}6 \pm 6{\cdot}0$ (P = NS). Occasionally NIH produced more intense IgG1 responses (e.g., Experiment 22, week seven, SJL RRI = $28{\cdot}1 \pm 4{\cdot}3$ [$n = 6$], NIH RRI = $45{\cdot}8 \pm 6{\cdot}0$ [$n = 7$], $0{\cdot}05 > P > 0{\cdot}025$). On balance SJL and SWR mice responded faster than NIH and BALB/c mice although the latter strains were capable of achieving comparable titres later in the infection.

Relationship between antibody response and worm loss at times following infection

Two types of analyses were undertaken to relate the intensity of antibody responses to loss of worms. The first analysis addressed directly the prediction that there should exist an inverse relationship across mouse strains between the intensity and rapidity of the IgG1 responses and the survival of worms. In order to arrive at a single measure of worm expulsion and RRI for each strain, data from relevant experiments were averaged. Figure 3 summarizes the results of this analysis. As can be seen there was a significant negative correlation between the RRI for IgG1 and worm survival across the seven mouse strains studied in both week six and week ten.

In the second analysis an attempt was made to correlate the RRI of individual mice within strains with worm survival assessed as the proportion of the group mean worm burden on day 14 (reflecting worm establishment) surviving in individual mouse until the day of autopsy. Correlations between RRI and percent worm survival were attempted for slow, intermediate and fast responder

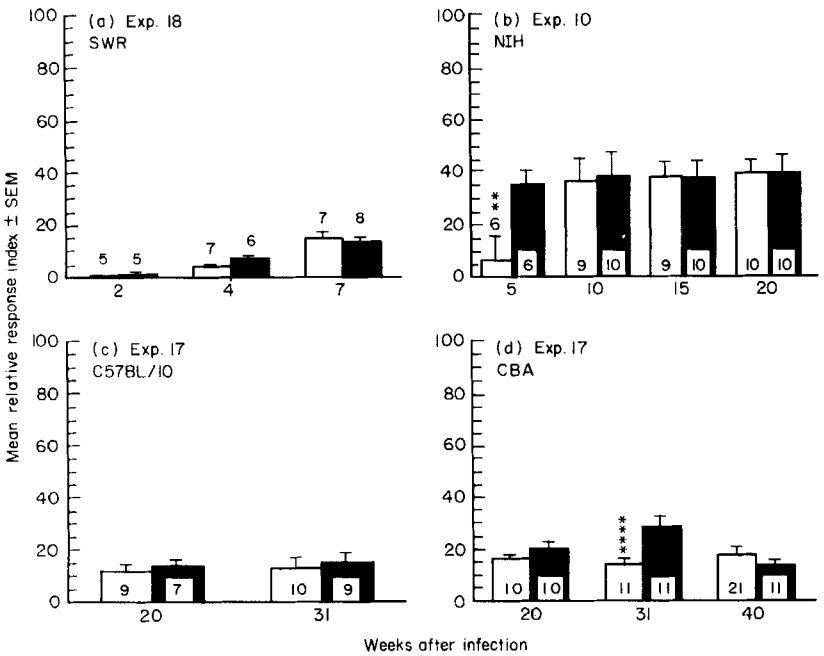

Figure 5 The relationship between the specific IgG1 antibody response to *Heligmosomoides polygyrus* and host gender. Open columns = male mice; Filled columns = female mice. The integers on columns give *n* in each case. Statistical analysis. Groups were compared by the Mann-Whitney *U* test and the key to the symbols used is as following: **0·05 > $P \geqslant 0{\cdot}02$; ****0·01 > $P \geqslant 0{\cdot}001$.

Table 3 Total serum IgG1 concentration during infection with *H. polygyrus* in mice of contrasting response phenotype

		Weeks after infection			
Strain	Experiment	0	6	8	10
Slow responder strains					
C57BL/10	20	2·32 ± 0·22	Nd	4·66 ± 0·56	Nd
C57BL/10	24	Nd	2·89 ± 0·23	Nd	Nd
CBA	12	Nd	Nd	Nd	1·37 ± 0·15
AKR	12	Nd	Nd	Nd	3·94 ± 0·40
C3H	12	Nd	Nd	Nd	3·35 ± 0·47
Intermediate responder strains					
BALB/c	20	2·26 ± 0·13	Nd	7·09 ± 0·48	Nd
NIH	10	Nd	Nd	Nd	6·0 ± 0·36
NIH	22	Nd	Nd	10·76 ± 0·70	Nd
Fast responder strains					
SJL	20	1·82 ± 0·61	Nd	5·01 ± 0·55	Nd
SJL	24	Nd	5·05 ± 0·19	Nd	Nd
SWR	24	Nd	6·74 ± 0·23	Nd	Nd

Nd = not done.

strains but were only possible in cases where worm burdens on the day of autopsy spanned a sufficiently wide range to enable a correlational analysis to be undertaken. With SJL and SWR mice, in most cases worm expulsion occurred too quickly for the correlation to be attempted: all the worms were present in the early stages of infection, whereas later autopsies revealed almost complete expulsion. The results are shown in Table 2. There were no detectable relationships among any of the strains irrespective of their response phenotype (slow, intermediate or fast).

Effect of intensity of infection on antibody response

Earlier published data showed that worm loss was dependent on the infection intensity in some strains,

notably in BALB/c mice (Robinson *et al.* 1989) in which high intensity worm burdens were lost later than low intensity infections. Therefore, it was of interest to examine the relationship between intensity of infection and antibody response to *H. polygyrus*. Accordingly sera from relevant experiments were analysed for IgG1 specific antibody activity. Antibody responses in mice subjected to high and low intensity infections were also studied in BALB/c, NIH, SWR, SJL, C57BL/10 and CBA. The infectivity of the inocula used for these experiments varied in the range 54% (Experiment 15) to 95% (Experiment 10). The results are presented in Figure 4.

Overall there was little difference in the IgG1 antibody response between groups given low or high intensity primary infections. Among the intermediate responder strains, BALB/c mice, in which worm loss is significantly accelerated during low intensity infections, averaged higher antibody titres following the more intense exposure although a significant difference was detected on only one occasion (week 15, Figure 4b). NIH behaved similarly (Figure 4d), mice given heavier inocula having higher mean titres but a significant difference was only detected in week 20.

There was no significant difference in low responder C57BL/10 (Figure 5c) nor in CBA mice (Experiment 14, not illustrated). In the latter strain the RRI was measured in mice with low and high intensity infections in weeks 6 ($5{\cdot}7 \pm 1{\cdot}1$, $11{\cdot}9 \pm 3{\cdot}5$), 10 ($16{\cdot}9 \pm 1{\cdot}7$, $23{\cdot}6 \pm 2{\cdot}3$) and 15 ($31{\cdot}7 \pm 7{\cdot}3$, $21{\cdot}1 \pm 3{\cdot}0$) respectively and on no occasion was a significant difference identified.

Fast responder SWR mice likewise showed no significant difference (Figure 4f). Interestingly however, SJL mice, in which there appears to be no infection-intensity effects on the timing of expulsion (Wahid *et al.* 1989), had higher average titres when exposed to low intensity infections, but these were not significantly different from the group given the higher intensity exposure.

Effect of host gender on antibody response

Following low intensity exposure, female mice generally lose adult *H. polygyrus* sooner than males (Robinson *et al.* 1989). It was of interest therefore, to determine whether host gender-dependent loss of worms would be reflected in differences in antibody activity between the sexes. Figure 5 summarizes the four experiments in which relevant comparisons were made, incorporating examples from fast (SWR), intermediate (NIH) and slow (C57BL/10 and CBA) responder strains. The infectivity of the inocula used in these experiments varied from 78% (Experiment 16) to 99% (Experiment 17).

Female SWR mice (Experiment 18) expelled worms earlier than males (In week seven females [$n=8$] had $14{\cdot}6 \pm 6{\cdot}4$ worms, males had $34{\cdot}3 \pm 6{\cdot}1$ [$n=7$], $P<0{\cdot}05$), but as can be seen from Figure 5a, IgG1 titres were similar in both sexes on all occasions tested. In Experiment 10 (Figure 5b) female NIH mice responded faster than males as reflected in antibody titres in week five but at subsequent time points there were no significant differences in antibody titres between the sexes. However, differences between the mean worm burdens did not become apparent until week ten (week ten, males $37{\cdot}7 \pm 5{\cdot}9$, females $23{\cdot}2 \pm 6{\cdot}7$; week 15, males $20{\cdot}7 \pm 7{\cdot}6$, females $10{\cdot}7 \pm 4{\cdot}9$; week 20, males $21{\cdot}3 \pm 6{\cdot}1$, females ($6{\cdot}7 \pm 2{\cdot}9$) although these were not significant because of variation in parasite burdens between individuals in each group. In a second comparison involving NIH mice (Experiment 21, data not illustrated) there was a significant difference ($P=0{\cdot}004$) in worm burden in week eight (females $21{\cdot}7 \pm 3{\cdot}7$ and males $34{\cdot}4 \pm 3{\cdot}7$) and the mean RRI of female mice was also higher (females $43{\cdot}6 \pm 6{\cdot}0$, males $25{\cdot}3 \pm 6{\cdot}8$) but not significantly so.

Figure 5 also illustrates results from Experiment 17 in which strains representing the slow responders were studied. Female CBA mice had significantly higher titres than males in week 31 (Figure 5d) but there was no difference in the response of C57BL/10 mice. There were no significant differences in worm burden in either strain (data not shown).

Comparison of total serum IgG1 during low-intensity infections in mice of varying responder phenotype

Total serum IgG1 was measured in selected strains representing the three categories of responder phenotype. Data from 5 experiments allowed such comparisons. The results are summarized in Table 3. Although it was not possible to measure IgG1 concentrations in naïve mice in all experiments, the data which were obtained show that 1–2·5 mg/ml can be expected in uninfected mice and this is consistent with other reports in the literature (Crandall, Crandall & Franco 1974, Pritchard, Behnke & Williams 1984, Williams & Behnke 1983). With this baseline it is quite evident that the slow responder strains had relatively modest changes in total serum IgG1 during the course of the first ten weeks following infection, maximal levels not exceeding 4 mg/ml. In contrast both intermediate and fast responder strains showed greater changes with IgG1 serum levels rising to 5–10 mg/ml.

Comparison of antigen recognition during low-intensity infections in mice of varying responder phenotype

Sera from the experiments described above were used to compare antigen recognition profiles on Western blot

analysis. A large number of gels were run in total. Each gel examined 12–24 individual sera and each was controlled with a pool of naïve sera and a pool of hyperimmune sera at one extremity. Comparisons were made using the sera of individual mice within strains, before worm loss and after loss of worms. Additional comparisons were made across strains, between sexes, and between high and low intensity exposed mice.

After compensation for differences in overall antibody titres of sera under comparison, none of the analyses undertaken revealed any major differences in the reactivity of sera to parasite antigens. There was some variation between individual animals within experimental groups comprising particular strains but no consistent differences which could be attributed to strain specific responses to particular antigens or within strains to the timing of worm loss. Hyperimmune sera consistently showed up three bands of low-molecular weight when worm homogenate antigen was subjected to Western blot analysis. These resolved at 16, 18 and 20 kDa. Only the lower of these bands was identified by our experimental mice subjected to primary infections and there was variation between sera in its recognition but we could not ascribe this to any of the variables which were of interest in the context of the present paper. C57BL/10 mice never recognized this antigen but BALB/c mice did so although not in a totally predictable manner. BALB/c mice which had been subjected to a low level infection (Experiment 15) and had expelled most of their worms by week 15 did not recognize the 16 kDa antigen. However, some (but not all) mice in Experiment 15, given heavier infections and still harbouring substantial worm burdens in week 15 ($109{\cdot}6 \pm 20{\cdot}0$), did so. In Experiment 19, SJL mouse sera taken immediately after expulsion (week eight) recognized the 16 kDa antigen whilst in other experiments with SJL mice we could not detect reactivity against this antigen.

DISCUSSION

The parasitic nematode *H. polygyrus* is unusual for the chronicity of the infections which develop after primary exposure of mice to infective larvae (Keymer & Hiorns 1986, Robinson *et al.* 1989). On repeated exposure, acquired immunity eventually develops and is known to be associated with specific IgG1 antibodies and with an increase in total serum IgG1 often exceeding 20 mg/ml (Chapman *et al.* 1979a, Williams & Behnke 1983, Pritchard *et al.* 1983). In contrast, primary infections seldom cause more than a two-fold increase in total IgG1 (Molinari, Ebersole & Cypress 1978) and specific titres are significantly lower than in hyperimmunized animals (Crandall *et al.* 1974, Williams & Behnke 1983, Pritchard *et al.* 1984). Because the involvement of IgG1 in determining the duration of primary infections is still controversial, a clarification of the function of this isotype is important if the role of Th1/Th2 cells is to be understood in relation to parasite evasive strategies. For this reason we analysed sera collected during primary infections in various mouse strains (Robinson *et al.* 1989, Wahid *et al.* 1989) for antibody responses within this immunoglobulin isotype and we addressed the hypothesis that if IgG1 is involved in limiting primary infections, an inverse relationship should exist between the intensity and rapidity of specific IgG1 responses and parasite survival.

A panel of eight syngeneic mouse strains was employed, representing three responder phenotypes which could be clearly differentiated from each other. Thus chronic infections were sustained in C57BL/10, CBA, C3H and AKR mice, infections of intermediate length (generally 10–20 weeks) in BALB/c and NIH mice and infections never lasted more than eight weeks in SWR and SJL strains. Comparison between strains at different time points during infection revealed that all the fast responder mice generated parasite specific IgG1 antibodies during the first five weeks of infection, peaking during the 6–8 weeks when loss of worms was taking place. BALB/c and NIH mice also developed strong responses but these peaked later corresponding with the relatively delayed onset of worm loss. In marked contrast IgG1 responses in the slow responder strains were without exception of low intensity, in some experiments with C57BL/10 mice no detectable antibody being evident until well after five weeks of infection. We established an inverse correlation across mouse strains between the intensity of their specific IgG1 antibody response to parasite antigens and worm survival (Figure 3). At first sight these data are consistent with the hypothesis that IgG1 plays a role in limiting primary infections.

However, other data reported herein are not consistent with this notion. Firstly, there were no significant correlations between the intensity of the specific IgG1 response within mouse strains and loss of worms by particular individuals (Table 2), as might be expected were IgG1 to play a crucial role in worm expulsion (See Dobson, Brindley & Sitepu 1982). Secondly, in groups of mice subjected to low or heavy infections, among which the former lose worms earlier (Robinson *et al.* 1989), it was the more heavily infected animals which had marginally higher IgG1 titres (See Dobson & Cayzer 1982). Thirdly, there was no significant difference between the sexes in the IgG1 response, despite the earlier loss of worms from female BALB/c and NIH mice in these experiments. The greater resistance of female mice to infection is well

documented in the literature (Dobson 1961, Dobson & Owen 1978) but, in contrast to our findings, more intense antibody responses have been observed in female relative to male mice by other workers (Dobson & Cayzer 1982, Dobson 1982). Lastly we could not detect any difference in antigen recognition profiles which might have indicated that particular mouse strains limited infection through their ability to identify specific antigens. Essentially all strains responded to the same antigens with only minor variations between individuals within strains and a suggestion that some differences existed in respect of recognition of the 16 kDa antigen.

There may be a simple explanation for all of the above findings. The data in Table 3 show that mice which ranked as slow responders produced only modest changes in their total IgG1 serum levels and it is therefore possible that mice which produce strong specific IgG1 responses to *H. polygyrus* may be doing so because they are good IgG1 responders and not because it is a component of their protective response to parasite infection. Our results may simply reflect a secondary association arising from a common mechanism controlling both protective responses to *H. polygyrus* and IgG1 secretion. However, to our knowledge, C57BL/10, CBA and C3H mice are not intrinsically poor producers of IgG1 to other antigens and parasites (Chapman *et al.* 1979b, Lebrun & Spiegelberg 1987, Delgado & McLaren 1990, Lopes *et al.* 1990). AKR mice were found to respond weakly with IgG1 relative to B10BR mice following infection with *Trichinella spiralis* (Pond, Wassom & Hayes 1989) but were later reported to generate mainly IgG1 and IgM responses to group II antigens from the L1 stage of this parasite (Denkers *et al.* 1990). We therefore consider it unlikely that the poor IgG1 responses of the slow responder strains can be attributed solely to a genetically determined constraint on IgG1 responsiveness to relevant antigens.

An alternative possibility is that *H. polygyrus* actively interferes with host IgG1 responses to infection, perhaps as a consequence, or an essential component, of the immunomodulatory strategy which adult worms employ to enable their chronic survival (Lossom, Lloyd & Soulsby 1985, Dobson & Cayzer 1982, Behnke, Hannah & Pritchard 1983). Parasite immunomodulatory factors (IMF) may downregulate IgG1 responses more effectively in mice of particular genotypes (Else & Wakelin 1990). The poor host-protective responses of C57BL/10 and CBA mice to *H. polygyrus* have been reported previously (Ali & Behnker 1985, Robinson *et al.* 1989, Parker & Inchley 1990) and we have hypothesized that IMFs may be more effective in mice bearing the H-2^b and H-2^k haplotypes associated respectively with these strains (Behnke & Wahid 1991). Both mastocytosis and IgG1 responses appear to be suppressed in slow responder mice (Dehlawi *et al.* 1987), whilst in moderate responders (H-2^d and some H-2^q, e.g., BALB/c and NIH) and in fast responders (H-2^s and some H-2^q e.g., SJL and SWR), mastocytosis is downregulated but IgG1 responses are less affected. *H. polygyrus* therefore, may be capable of affecting differentially the products of an initial Th2 response, with a more severe and wide ranging influence in slow responders compared with fast responders. Speculatively, this may be achieved by several distinct IMFs some of which target MHC and others cytokine regulatory pathways or by a single factor with pleiotropic properties, some of which are dependent on interactions with MHC. Indeed, it is likely that IMFs may prove to be molecules much like cytokines (Haig & Miller 1990) with equally complex interactions as suggested by Behnke *et al.* (1992a).

In conclusion, the experiments described in this paper have clearly demonstrated that mouse strains which speedily limit primary infections with *H. polygyrus*, produce more intense specific IgG1 antibody responses to parasite antigens and greater amounts of total serum IgG1 than strains which tolerate chronic infections. This relationship held across eight mouse strains representing three clearly distinguishable primary infection phenotypes. However, other data were not compatible with the view that IgG1 antibodies mediated protection directly and that worm loss was dependent on the intensity of the specific IgG1 response. We suggest that a consequence of the parasite's survival strategy involving IMFs, is more effective downregulation of some mouse genotypes to respond with IgG1 than others. In consequence the intensity and rapidity of IgG1 responses during primary exposure to *H. polygyrus* reflect host response phenotype as assessed by the duration of infection.

ACKNOWLEDGEMENTS

We are grateful to Professors D.Wakelin and P.N.R. Usherwood for the provision of facilities for this study in the Department of Life Science at Nottingham University. The work was supported by the MRC through project grants G8820855/T and G8923735/T to J.M.B. F.N.W. held an Iraqi government studentship during part of this study. We thank Mr K.Cosgrove and D.Fox for supervision over the maintenance of our experimental animals and Mrs J.Brown for technical support.

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