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The relationship between circulating and intestinal *Heligmosomoides polygyrus*-specific IgG_1 and IgA and resistance to primary infection

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SUMMARY

Specific serum and intestinal immunoglobulin (Ig)G_1 and IgA responses to Heligmosomoides polygyrus *were measured in a panel of seven inbred mouse strains which exhibit 'rapid' (< 6 weeks (SWR × SJL)F_1), 'fast' (< 8 weeks, SJL and SWR), 'intermediate' (10–20 weeks, NIH and BALB/c) or 'slow' (> 25 weeks, C57BL/10 and CBA) resolution of primary infections. Mice with 'rapid', 'fast' or 'intermediate' response phenotypes produced greater serum and intestinal antibody responses than those with 'slow' phenotypes. The F_1 hybrids ((SWR × SJL)F_1) of two 'fast' responder strains showed the earliest antibody response with maximum titres evident within 6 weeks of infection. There was a negative correlation between the serum IgG_1 responses and worm burdens in individual mice within a number of mouse strains, and also between serum IgG_1 and IgA responses and worm burdens in the 'rapid' ((SWR × SJL)F_1) responder strain. The presence of IgG_1 in the gut was found to be due to local secretion rather than plasma leakage. Using Western immunoblotting, serum IgG_1 from 'rapid' and 'fast' responder but not 'slow' responder mice was found to react with low molecular weight antigens (16–18 kDa) in adult worm excretory/secretory products.*

Keywords Heligmosomoides polygyrus, *nematoda*, *mice*, *IgG antibody*, *IgA antibody*

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INTRODUCTION

The trichostrongyloid nematode *Heligmosomoides polygyrus*, a parasite of the small intestine of the mouse, has been used extensively as an experimental model for studying host–parasite interactions during chronic infections.

Marked strain-dependent variations in the kinetics of worm expulsion have been observed in both primary and secondary infections (Behnke & Robinson 1985, Enriquez *et al*. 1988, Robinson *et al*. 1989, Wahid *et al.* 1989). Many syngeneic strains harbour adult worms for up to 40 weeks after a primary infection, probably reflecting the natural lifespan of the parasite, whereas others curtail primary infections within 6 weeks. These strain variations are thought to reflect the influence of genetic regulation of components of the host response (Liu 1966, Behnke & Robinson 1985, Behnke 1987) and imply that in certain genotypes a successful protective immune response can be mounted during the course of a primary infection to eliminate the parasite.

The process by which immunity is generated in resistant strains, or the mechanism which enables the parasite to evade host immunity in susceptible strains and hence to cause a chronic infection, is still incompletely understood, notably, in relation to the exact role of parasite-specific antibodies. *H. polygyrus* is known to stimulate a dramatic increase in total serum immunoglobulin (Ig)G_1 during infection, particularly during secondary exposure of immune mice (Crandall *et al.* 1974). Although much of the IgG_1 is not specific, parasite-specific antibodies are also generated, and in immune-challenged animals these are host-protective. Passive immunization with purified IgG_1 from immune mice resulted in delayed larval emergence, stunting in adult worms and reduced worm burden and fecundity (Pritchard *et al*. 1983, Pritchard *et al.* 1984, Monroy & Enriquez 1992), clearly demonstrating that in immune animals responding to a challenge infection, parasite-specific IgG_1 contributes to host-protective immunity. Not surprisingly therefore significant correlations have been reported between the effectiveness of the secondary

response in clearing worms and the intensity of the parasite-specific IgG_1 response across strains (Zhong & Dobson 1996).

However, controversy continues to centre around the role of nonspecific and parasite-specific IgG_1 during primary infections. It was originally proposed that the elevated levels of total serum IgG_1 elicited during primary infections with *H. polygyrus* protected the parasite because IgG_1 was most elevated in those strains that exhibited chronic infections (Chapman *et al*. 1979a,b, Williams & Behnke 1983). However, it was subsequently shown that IgG_1 from primary infection sera did not block protective immunity (Pritchard *et al*. 1984); moreover, nearly 50% of the total serum IgG_1 in *H. polygyrus* infected mice was found to be parasite-specific (Pritchard *et al*. 1983). One study reported varied effects of specific serum antibodies on infection in mice following passive transfer of immunity, depending on the quantity and quality of serum injected (Monroy & Enriquez 1992).

With regard to local immune responses at the site of *H. polygyrus* infection, Monroy & Enriquez (1992) found that although susceptible strains had higher serum levels of specific antibodies than resistant strains, the latter had more specific IgM, IgA and IgG antibody-producing cells in their Peyers patches and mesenteric lymph nodes. Moreover, IgG_1 has been previously reported to be elevated in the intestinal lumen of immunized mice (Prowse 1981). Also, high serum IgA levels have been associated with resistance to *H. polygyrus* infections (Molinari *et al.* 1978).

In an earlier paper we reported a negative correlation between the intensity of the serum adult worm-specific IgG_1 antibody response and worm burden across a number of inbred mouse strains experiencing primary infection, suggesting that those strains which mounted rapid and intense IgG_1 responses also lost worms quickly (Wahid & Behnke 1993). However, we were unable to detect any significant negative correlation between serum parasite-specific IgG_1 and worm burdens within strains, as would have been expected if parasite-specific IgG_1 had had a protective role during primary infections. Following on from this earlier study, we examined the relationship between host response phenotype and intestinal parasite-specific antibody activity to test the hypothesis that if antibody plays a significant role in curtailing primary infections in mice, the rapidity and intensity of the local mucosal parasite-specific antibody might be a better measure of the response phenotype than the accompanying serum response. Thus, the intensity and onset of the parasite-specific antibody response should show the following relationship across mouse strains of varying response phenotype: most intense and earliest in 'rapid' > 'fast' > 'intermediate' > 'slow' responders. We selected IgG_1 and IgA as the most likely isotypes to be involved (Crandall *et al*. 1974, Molinari *et al*. 1978, Mazanec *et al*. 1993) and we also analysed the sera and mucosal extracts for evidence that response phenotype is linked to the ability to recognize and respond to particular subsets of excretory/secretory (E/S) antigens of *H. polygyrus.*

MATERIALS AND METHODS

Animals

Female syngeneic NIH, SWR, SJL, C57BL/10, CBA/Ca, BALB/c and (SWR × SJL)F_1 hybrid mice were either purchased from Harlan Olac Ltd. (Bicester, UK) as specific pathogen free, or were bred in the departmental animal unit. All the animals were used at approximately 8–10 weeks of age and maintained under conventional conditions with food and water provided ad libitum. A minimum of 5 mice per group were used in each experiment.

H. polygyrus

The parasite used was *Heligmosomoides polygyrus bakeri* (Behnke *et al.* 1991). The methods used for parasite maintenance, infection of mice and recovery of adult worms at autopsy were essentially as described by Jenkins & Behnke (1977).

H. polygyrus excretory/secretory (E/S) products antigen preparation

Adult worms were recovered from outbred CFLP mice infected 14 days earlier with 400–600 L_3 of *H. polygyrus*. The parasites were washed in phosphate buffered saline and cultured aseptically for 24 h at 37°C in RPMI 1640 medium (Gibco, UK) supplemented with 0·1 mM sodium pyruvate (Sigma, UK), 100 U/ml Penicillin + 100 μg/ml Streptomycin solution (Gibco), 75 μM monothiolglycerol (Sigma) and 10% foetal calf serum (Gibco). The supernatant was filtered through a 0·22 μm filter (Millipore, UK) dialysed (22/35 kDa dialysis tubing) against distilled water for 24 h at 4°C, lyophilized and resuspended in a smaller volume. The sample was analysed for protein using the Bio-Rad protein assay kit (Bio-Rad, UK) and stored at − 40°C.

Serum collection

Mice were killed with an overdose of ether, their thoracic cavities opened and their hearts punctured. The blood that accumulated in the cavity was collected into Eppendorf tubes containing 50 μl of Serasieve gel (Hughes and Hughes Ltd, UK), allowed to clot and centrifuged at 13 000 ***g*** for 5 min. The resulting serum was then removed and stored at −40°C.

Intestinal lavage

Intestinal secretions were collected by a modification of the method of Elson *et al.* (1984). Mice were killed and the small intestine removed. Three ml of a solution of 0·1 mg/ml soybean trypsin-chymotrypsin inhibitor (Sigma) in 50 mM ethylenediaminetetracetic acid (Sigma) was flushed through the intestine which was massaged gently before recovery of the fluid. The samples were then centrifuged at 650 ***g*** for 10 min before addition of 30 μl of 100 mM phenylmethylsulphonyl fluoride (PMSF, Sigma) in 95% ethanol. The samples were further clarified by centrifugation at 27 000 ***g*** at 4°C for 20 min, after which 20 μl of PMSF and 20 μl of 1% sodium azide (Sigma) and 100 μl foetal calf serum were added to the supernatant. The samples were then stored at −40°C.

Measurement of *H. polygyrus*-specific IgG_1 and IgA responses

A standard ELISA assay was used to assess the levels of parasite-specific (E/S products) IgG_1 and IgA antibody responses in the serum and intestinal fluid samples, essentially as described by Wahid & Behnke (1993).

Mouse samples were assayed individually; serum was tested at a dilution of 1 : 100 and intestinal samples were used undiluted. Each plate included control 'hyper-immune' serum (HIS, Behnke & Parish 1979) and also serum from naive mice (NS). The optical density (OD) for each sample, minus the OD of the NS, was expressed as a percentage of the OD of the HIS minus the NS, giving a relative response index (RRI). Luminal IgA results, however, were presented as OD values as the intestinal samples contained more IgA than the HIS samples, which led to very high RRI values, so that small changes in OD values gave very large differences in the corresponding RRI values. Nevertheless, the pattern of response was found to be similar whichever way the results were expressed. Results were presented as mean RRI or OD value ± standard error for each group.

Measurement of total IgG

Total IgG (mg/ml) in serum and intestinal samples was determined by the method of Mancini *et al.* (1965) using radial immunodiffusion kits (Binding Site, UK). Three prediluted calibrators (120, 720 and 1120 mg/l IgG) were included to produce a standard curve. Serum from infected mice was tested at 1 : 30 dilution, serum from naive mice at 1 : 10 dilution and intestinal secretions were assayed undiluted.

Measurement of albumin

The levels of serum and intestinal albumin were assessed by a modification of the method of Doumas *et al.* (1971) using the albumin reagent bromocresol green (BCG, Sigma Diagnostics Albumin Reagent, Sigma, UK), essentially as described in the procedure sheet. A protein standard solution, consisting of 50 mg/ml albumin and 30 mg/ml globulin (Sigma), was used to derive a linear calibration curve from a range of albumin concentrations between 0 and 50 mg/ml.

Western immunoblotting

H. polygyrus antigen recognition profiles of different responder strains of mice were examined by the technique of western immunoblotting. The component proteins from adult worm E/S products were separated by 12% SDS-PAGE, essentially as described by Laemmli (1970), and transferred onto nitrocellulose by electrophoresis for 90 min at 10°C at 1 A according to the method of Burnette (1981). Briefly, the nitrocellulose was cut into strips and blocked for 1 h at RT with 3% bovine serum albumin (Sigma, UK) in PBS containing 0·05% Tween 20 (Sigma). The strips were washed three times and incubated with the test sera (pooled samples for each group) at a 1 : 50 dilution overnight at 4°C. The strips were washed again and incubated in a 1 : 1000 solution of alkaline phosphatase-conjugated polyvalent anti-mouse IgG_1 (Binding Site, UK) for 2 h at RT. They were then washed 6 × 20 min and developed in a 1 : 1 molar ratio of 5-bromo-4-chloro-3-indolyl phosphate (BCIP, p-toluidine salt (Sigma) at 50 mg/ml in 100% dimethylformamide) and nitro blue tetrazolium (NBT) (Sigma) at 75 mg/ml in 70% dimethylformamide made up in Tris-buffered saline, pH 9·5. The reaction was stopped by rinsing the strips in distilled water and the blot photographed whilst still wet.

Experimental design

Four experiments were carried out using the following combinations of mouse strains: Experiment 1—CBA, NIH and SWR infected with 50 L_3 larvae; Experiment 2—BALB/c and SWR infected with 50 L_3 larvae; Experiment 3—C57BL/10, SWR and (SWR × SJL)F_1 infected with 100 L_3 larvae; Experiment 4—SWR, SJL and (SWR × SJL)F_1 infected with 50 L_3 larvae.

Statistical analysis

Results are presented as mean value ± standard error of mean. As normal distribution of the data could not be assumed, each data set was subjected to statistical analysis of variance using a generalized linear model (Crawley 1993, Wilson & Grenfell 1997) which tolerates non-normally distributed data and also allows analysis of variance in

nonbalanced data sets to assess whether there was a significant overall effect of worm burden and antibody levels with respect to time and strain. Where a significant effect of strain was found, individual analyses were performed at each time point. Results are presented as probability values (P). A probability of $\leqslant 0{\cdot}05$ was considered significant.

Correlations between variables were assessed by Spearman's Rank Order Correlation test where r_s is Spearman's correlation coefficient and $z = r_s\sqrt{(n-1)}$. A probability of $\leqslant 0{\cdot}05$ was considered significant.

RESULTS

Kinetics of parasite-specific serum and intestinal IgG_1 and IgA isotype responses

The kinetics of parasite-specific IgG_1 and IgA responses in serum and intestinal fluid samples of mouse strains tolerating long-lasting chronic infections and in those limiting their infections earlier were examined in one hybrid and six inbred strains of mice. This work was based on a range of mouse strains representing four primary response phenotypes, 'slow' (resulting in chronic infections > 25 weeks, e.g. C57BL/10 and CBA), 'intermediate' (10–20 weeks, e.g. NIH and BALB/c), 'fast' (resulting in acute infections < 8 weeks, e.g. SJL and SWR) and 'rapid' responders (resulting in infections of > 6 weeks $(SWR \times SJL)F_1$ hybrids).

Data from four experiments are illustrated in Figures 1 to 4, each experiment measuring the response in only two or three strains. The worm burden data from Experiment 1 has previously been published (Wahid *et al.* 1994)

Experiment 1: acute, intermediate and chronic infections

Figure 1 illustrates the specific isotype response pattern obtained between mice with 'fast' (SWR), 'intermediate' (NIH) and 'slow' (CBA) response phenotypes following a primary infection with *H. polygyrus*.

The worm burdens recovered are illustrated in Figure 1(a). The inoculum (50 L_3 larvae) gave an infectivity rate of 91% in the NIH mice at week 2. The worm burdens of SWR and CBA mice, however, were unusually low at this time, showing only 72% and 43% establishment, respectively. At week 4, worm burdens were higher in CBA mice and similar to those in NIH mice, suggesting that the lower recovery of worms from CBA mice in week 2 may have been due to incomplete recovery of the inoculum, perhaps due to the late emergence of worms into the gut lumen. SWR mice lost worms rapidly, with a 73% reduction in worm burdens by week 4 and 96% by week 6 ($P < 0{\cdot}001$). NIH mice had stable worm burdens until week 6 and then lost 99% of their worms by week 10 ($P < 0{\cdot}001$). In contrast, there was no loss of worms in the CBA mice over this time period.

Mice with an 'intermediate' response phenotype (NIH) produced a more intense serum and intestinal IgG_1 response than those with a 'fast' (SWR) response phenotype (Figure 1b,c), although the serum response occurred much later than in the SWR mice (week 4, $P < 0{\cdot}001$). CBA mice produced lower and later serum and intestinal IgG_1 responses than either the SWR or NIH mice in accordance with their 'slow' expulsion primary response phenotype (serum IgG_1, $p \leq 0{\cdot}02$; intestinal IgG_1, $P < 0{\cdot}001$). SWR mice also produced an earlier serum IgA response at week 4 than either NIH (week 6; $P < 0{\cdot}001$) or CBA (week 10; $P < 0{\cdot}001$) mice (Figure 1c). Initially, CBA mice produced a lower intestinal IgA response than SWR mice (week 4, $P < 0{\cdot}001$) or NIH mice (week 6, $P = 0{\cdot}04$), however, levels in the CBA mice continued to rise to similar levels by 10 weeks postinfection (Figure 1e).

Experiment 2: acute and intermediate infections

The specific isotype responses of mice with 'fast' (SWR) and 'intermediate' (BALB/c) response phenotypes were compared following a primary infection with *H. polygyrus* (Figure 2).

In this experiment, SWR mice showed rapid expulsion, with 46% loss of worms by week 4 postinfection and complete expulsion by week 8 postinfection. Loss of worms begun much later in mice with 'intermediate' (BALB/c) response phenotype (week 8, $P < 0{\cdot}001$) with 75% lost at week 10 postinfection (Figure 2a).

Over the time course of the infection, SWR mice showed a more intense serum and intestinal IgG_1 and IgA response than BALB/c mice (Figure 2b–e). At week 4, however, when worm expulsion was occurring in the SWR mice, antibody titres did not differ significantly between the two strains.

Experiment 3: rapid, acute and chronic infections

In this experiment three strains of mice with 'rapid' $((SWR \times SJL)F_1)$ 'fast' (SWR) and 'slow' (C57BL/10) response phenotypes with regard to primary infections to *H. polygyrus* were autopsied in groups at 2, 4 and 6 weeks postinfection and their respective worm burdens as well as serum and intestinal IgG_1 and IgA responses determined (Figure 3).

The infectivity of the inoculum (100 L_3 larvae) was 85–95% in all three strains of mice. Mice with a 'rapid' response phenotype $((SWR \times SJL)F_1)$ lost 47% of their worms by week 4 and all their worms by week 6 post-

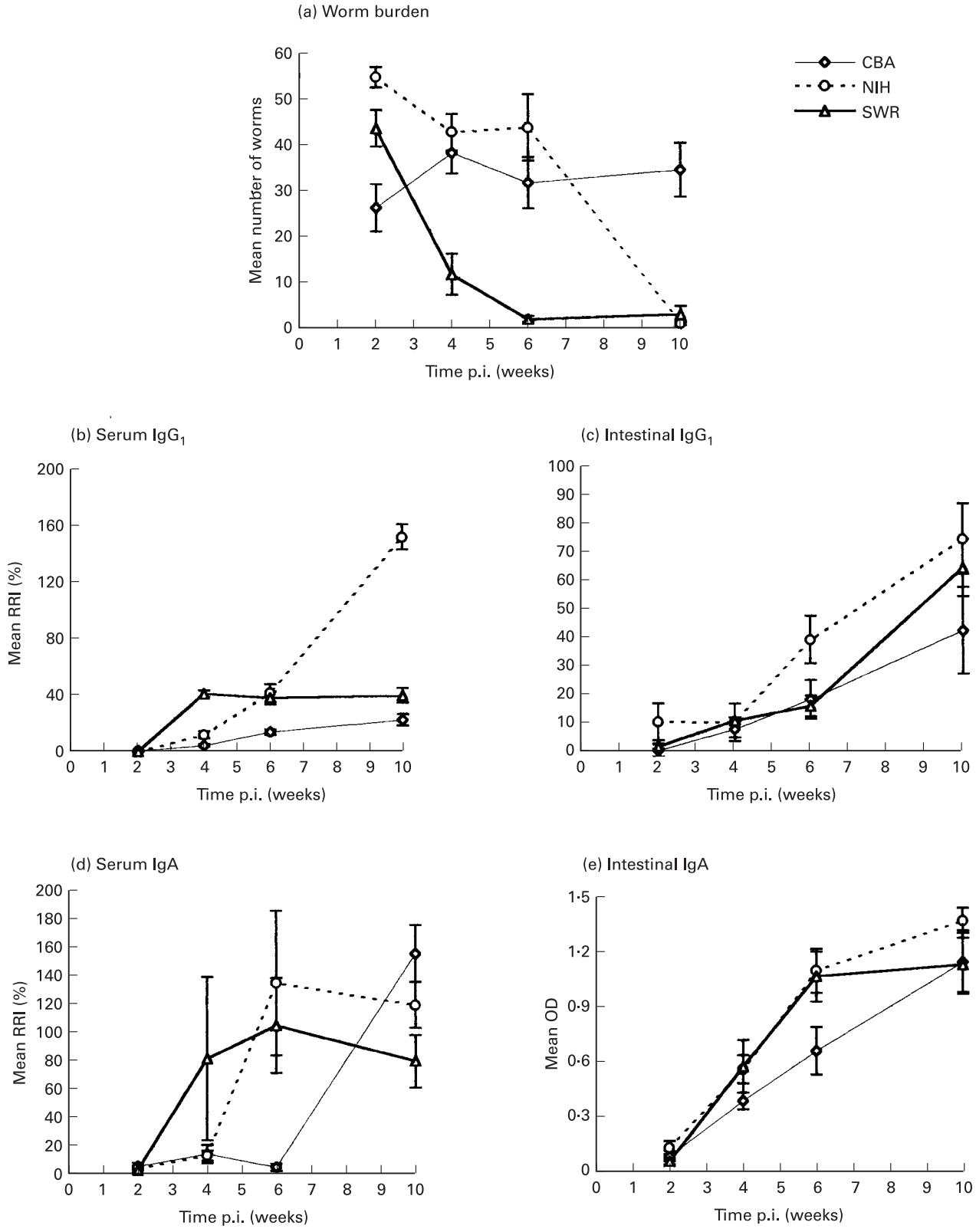

Figure 1 Experiment 1. The primary response phenotype in 'fast' responder (SWR), 'intermediate' responder (NIH) and 'slow' responder (CBA) strains of mice after infection with 50 L_3 *H. polygyrus*. (a) worm burden; (b) parasite-specific serum IgG_1; (c) parasite-specific intestinal IgG_1; (d) parasite-specific serum IgA and (e) parasite-specific intestinal IgA.

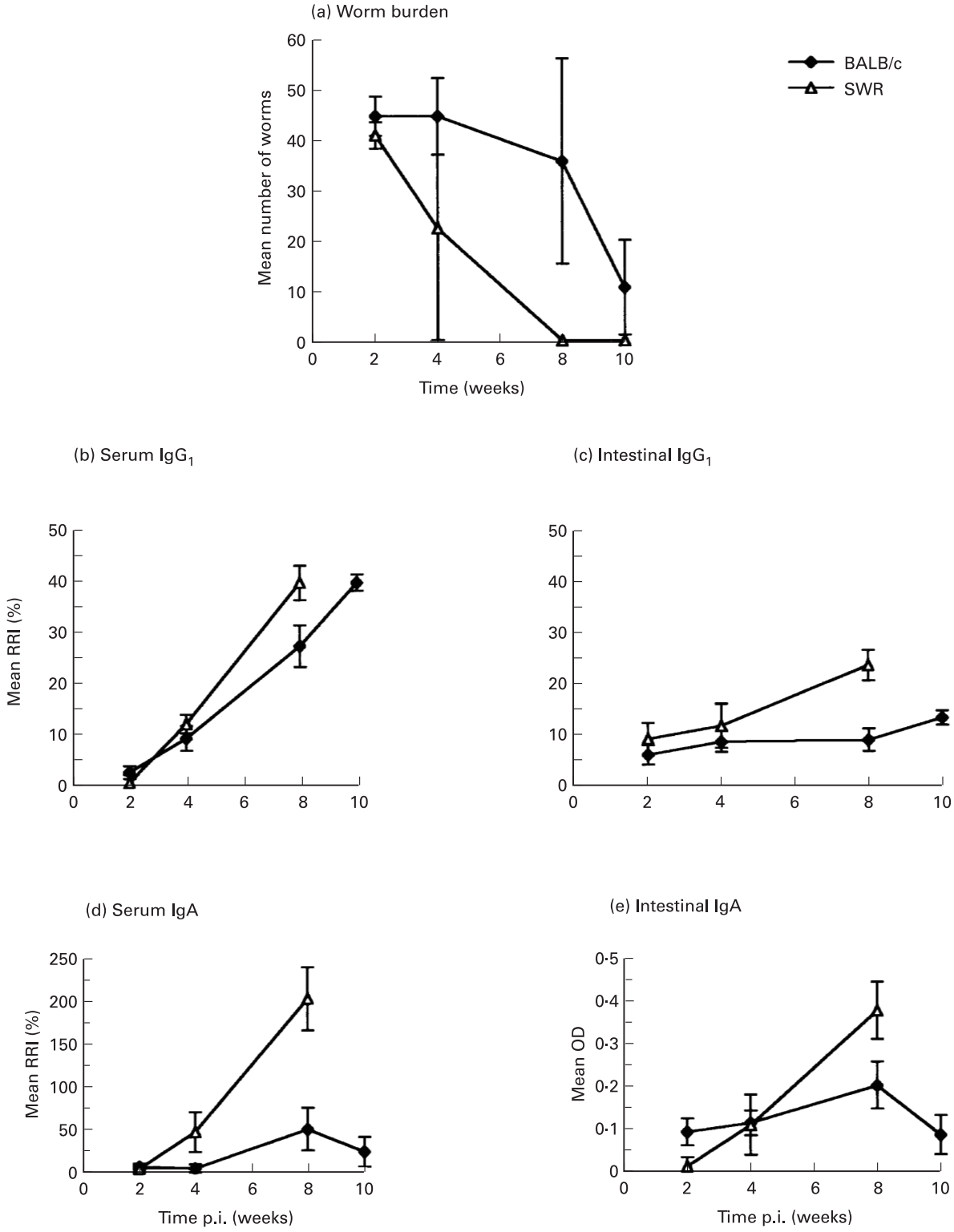

Figure 2 Experiment 2. The worm recoveries (a) and variation in parasite-specific serum IgG_1 (b), intestinal IgG_1 (c), serum IgA (d) intestinal IgA (e) in 'fast' responder (SWR) and 'intermediate' responder (BALB/c) strains of mice after infection with 50 L_3 *H. polygyrus*.

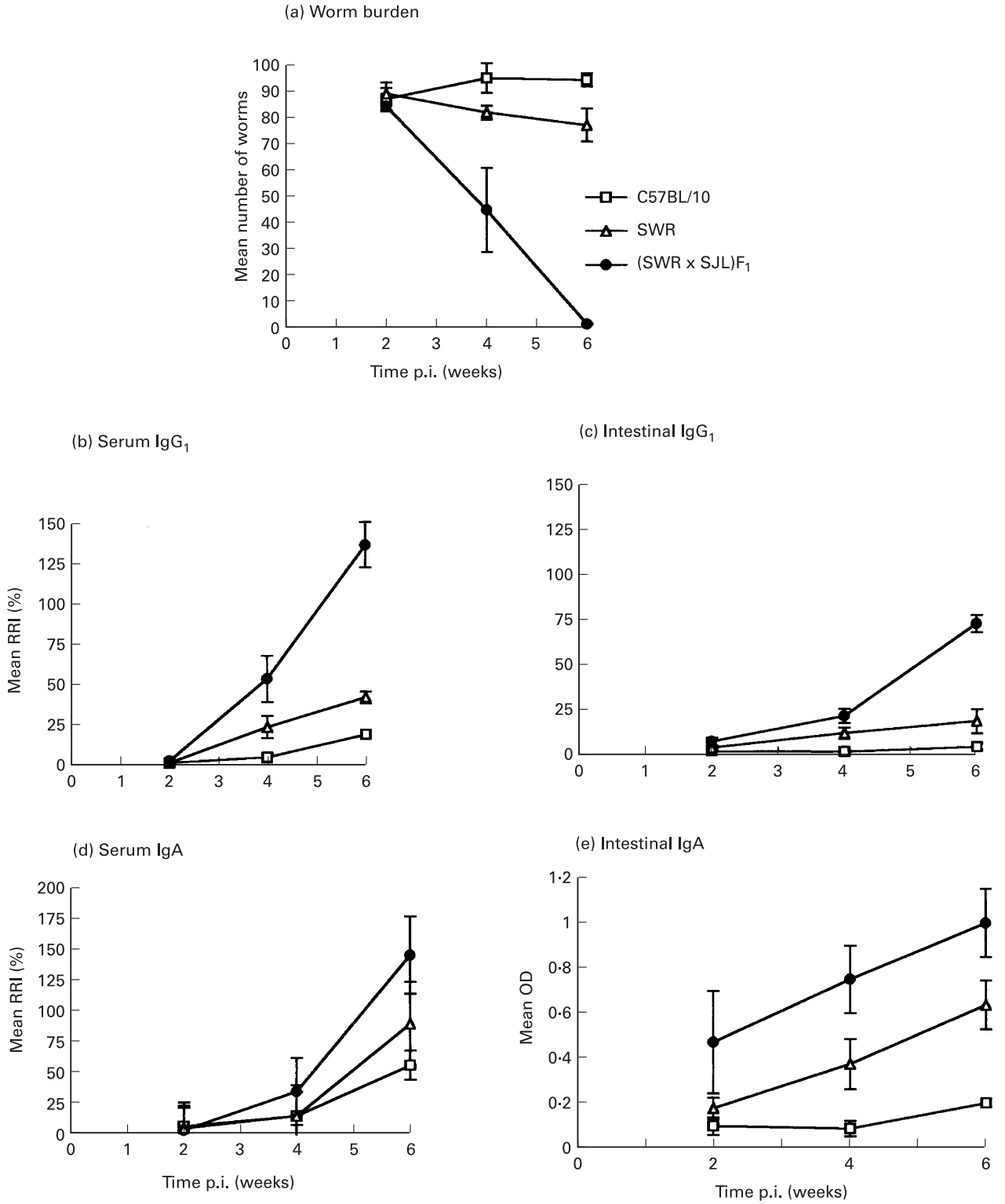

Figure 3 Experiment 3. The primary response phenotype in 'rapid' responder ((SWR × SJL)F_1 hybrids), 'fast' responder (SWR) and 'slow' responder (C57BL/10) strains of mice after infection with 100 L_3 *H. polygyrus*.(a) worm recoveries; (b) parasite-specific serum IgG_1; (c) parasite-specific intestinal IgG_1; (d) parasite-specific serum IgA and (e) parasite-specific intestinal IgA.

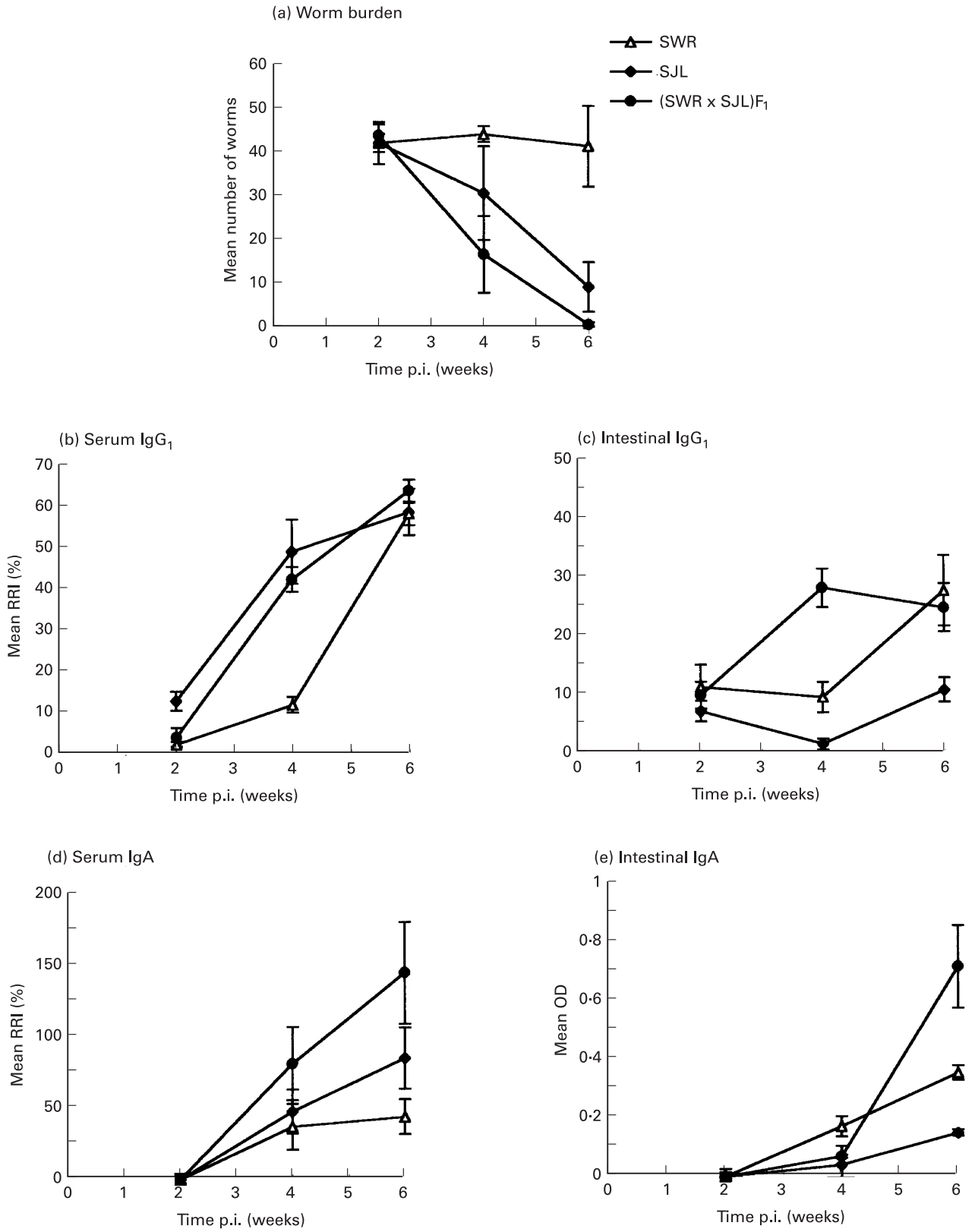

Figure 4 Experiment 4. The primary response phenotype in 'fast' responder parental strains SWR and SJL and their 'rapid' responder F_1 hybrid strains of mice after infection with 50 L_3 *H. polygyrus*.(a) worm recoveries; (b) parasite-specific serum IgG_1; (c) parasite-specific intestinal IgG_1; (d) parasite-specific serum IgA and (e) parasite-specific intestinal IgA.

infection. There was a significant difference between the number of worms in the SWR and C57BL/10 mice at week 6 postinfection ($P < 0{\cdot}03$), with some worm loss occurring in the former strain whilst the numbers of worms were maintained in the latter strain (Figure 3a).

A strain-dependent specific antibody response was found to occur in both serum and intestinal fluid and in both antibody isotypes (Figure 3b–e). The $(SWR \times SJL)F_1$ mice showed the most rapid IgG_1 response with enhanced antibody levels being detected within the first 4 weeks of infection and reaching a maximal level at week 6 ($P < 0{\cdot}001$), corresponding to the timing of worm expulsion (Figure 3b,c). SWR mice in turn responded more vigorously than C57BL/10 mice; the antibody response of the latter being extremely poor. The kinetics of serum and luminal IgA was similar to that of IgG_1, as can be seen from Figure 3(d,e).

Experiment 4: rapid and acute infections

In this experiment, the isotype response between a hybrid strain of mouse exhibiting a 'rapid' response phenotype ($(SWR \times SJL)F_1$) was compared to that of its two parental strains, both with 'fast' response phenotypes (Figure 4).

The kinetics of worm loss indicate that whilst the rate of expulsion was greater in the F1 hybrid with almost complete worm loss by week 6 postinfection, as in Experiment 3, the SJL mice also rapidly lost their worms, with 77% lost by week 6. In contrast, the SWR mice retained most of their worms throughout the experiment, although some worm loss was evident in individual mice at week 6 postinfection, later than would normally have been expected for this strain of mice (Figure 4a).

The serum IgG_1 and IgA levels reflected the pattern of worm expulsion in that the F_1 hybrids and SJL mice produced greater amounts of antibody than the SWR mice (Figure 4b,d). However, although the F1 mice produced more luminal IgG_1 at week 4 and IgA at week 6, SWR mice had similar levels of luminal IgG_1 at week 6 postinfection and produced more antibody (both IgG_1 and IgA) than the SJL mice (Figure 4c,e).

The relationship between antibody response and worm loss at times following infection

An attempt was made to correlate the antibody responses of individual mice within strains with their corresponding worm burdens at time points prior to and during expulsion. Correlations were evaluated for all four phenotype response strains, but were only possible at times where worm burdens on the day of autopsy spanned a sufficiently wide range, that is, analysis was only possible at time points during, but not following worm expulsion.

Negative correlations were observed between the intensity of serum IgG_1 response and worm burden in 'rapid' responder $(SWR \times SJL)F_1$ mice at week 4 postinfection (Experiment 3, $r_s = -0{\cdot}94$, $P = 0{\cdot}025$; expt 4, $r_s = -0{\cdot}899$, $P = 0{\cdot}025$), in 'fast' responder SJL mice at week 4 (Experiment 4, $r_s = -0{\cdot}9$, $P = 0{\cdot}05$) and in 'intermediate' responder BALB/c mice at week 10 (Experiment 2, $r_s = -1$ $P = 0{\cdot}05$). Negative correlations were also detected between the intensity of the serum IgA response and worm burden in

Table 1 The relationship between IgG and albumin in serum and intestinal fluid

Strain	Week (mg/ml)	Infected/naïve (mg/ml)	Gut IgG (mg/ml)	Gut albumin (mg/ml)	Serum IgG	Serum albumin
Experiment 1						
C57BL/10	2	Naïve	*	1·04	4·86	36·97
C57BL/10	6	Infected	*	1·43	11·25	34·16
$(SWR \times SJL)F_1$	2	Naïve	*	1·36	10·84	36·78
$(SWR \times SJL)F_1$	6	Infected	*	1·87	37·08	44·15
Experiment 4						
CBA	2	Naïve	*	0·15	*	34·01
CBA	6	Naïve	*	0	0·27	46·53
CBA	2	Infected	*	0·3	10·63	34·48
CBA	6	Infected	*	0	11·33	35·4
SWR	2	Naïve	*	0	2·64	40·68
SWR	6	Naïve	*	0·34	4·72	37·47
SWR	2	Infected	*	0	13·39	35·62
SWR	6	Infected	*	0	11·62	35·71

* Levels were below that of lowest standard (0·12 mg/ml) and therefore unable to be measured.

(SWR×SJL)F_1 mice at week 4 (Experiment 3, $r_s = -0{\cdot}77$, $P = 0{\cdot}05$; expt 4, $r_s = -0{\cdot}899$, $P = 0{\cdot}025$) and in 'fast' responder SWR mice at week 4 (Experiment 2, $r_s = -0{\cdot}9$, $P = 0{\cdot}05$). Moreover, a number of negative correlations were observed between worm burden and intestinal IgG_1(Experiment 2, week 4, $r_s = 0{\cdot}87$, $P = 0{\cdot}05$; Experiment 3, week 6, $r_s = -0{\cdot}857$, $P = 0{\cdot}025$) and IgA (Experiment 2, week 4, $r_s = -0{\cdot}1$, $P = 0{\cdot}025$; Experiment 3, week 6, $r_s = -0{\cdot}857$, $P = 0{\cdot}025$) responses in SWR mice.

Negative correlations were only observed at time points during worm expulsion and not at time points prior to or following expulsion in each experiment. Moreover, in other strains examined in which no such correlations were observed, i.e. NIH, C57BL/10 and CBA, either worm expulsion was completed (NIH) or had not been initiated (C57BL/10 and CBA) within the time points examined (results not shown).

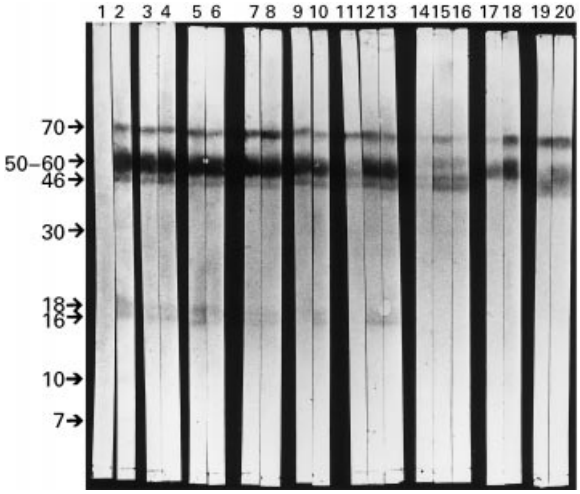

Figure 5 Immunoblot analysis of the serum-specific IgG_1 recognition pattern of various strains of mice to *H. polygyrus* E/S products. Serum samples from individual mice were pooled and tested at a dilution of 1 : 50. Lane 1, naive serum; lane 2, hyper-immune serum; lane 3, (SWR×SJL)F_1 week 4 p.i. serum (Experiment 4); lane 4, (SWR×SJL)F_1 week 6 p.i. serum (Experiment 4); lane 5, (SWR×SJL)F_1 week 4 p.i. serum (Experiment 3); lane 6, (SWR×SJL)F_1 week 6 p.i. serum (Experiment 3); lane 7, SJL week 4 p.i. serum (Experiment 4); lane 8, SJL week 6 p.i. serum (Experiment 4); lane 9, SWR week 4 p.i. serum (Experiment 1); lane 10, SWR week 6 p.i. serum (Experiment 1); lane 11, NIH week 4 p.i. serum (Experiment 1); lane 12, NIH week 6 p.i. serum (Experiment 1); lane 13, NIH week 10 p.i. serum (Experiment 1); lane 14, BALB/c week 4 p.i. serum (Experiment 2); lane 15, BALB/c week 8 p.i. serum (Experiment 2); lane 16, BALB/c week 10 p.i. serum (Experiment 2); lane 17, C57BL/10 week 4 p.i. serum (Experiment 3); lane 18, C57BL/10 week 6 p.i. serum (Experiment 3); lane 19, CBA week 4 p.i. serum (Experiment 1); lane 20, CBA week 6 p.i. serum (Experiment 1).

Leakage of serum proteins into the intestinal lumen

The appearance of parasite-specific IgG_1 in intestinal fluid and its similarity to the levels and pattern of IgG_1 in the serum suggested a possible humoral origin for this intestinal antibody. An evaluation of whether there was leakage of IgG into the gut or whether the intestinal IgG was secreted locally was therefore undertaken. Albumin is an abundant serum (but not gut) protein, so an increased level of albumin in the gut secretions would indicate increased intestinal permeability or leakage of serum proteins into the intestinal lumen (Tomasi & Grey 1972, Snider & Underwood 1986). Consequently, an assessment of albumin levels in both serum and intestinal fluids was carried out in both naive and infected animals in expts 1 and 4, as well as an evaluation of the total IgG levels in those mice in both fluid compartments. The results are shown in Table 1.

In many cases, gut IgG levels (and sometimes serum IgG levels in naive animals) could not be determined as these values were below that of the lowest standard in the assay (0·12 mg/ml). Moreover, very little or no albumin was detectable in the intestinal samples.

The results of two experiments (Table 1) showed that there was no evidence of leakage of IgG from the serum into the gut even at week 6 postinfection, when highest levels of IgG_1 were detected, and therefore suggest that the specific immunoglobulin isotypes detected in intestinal fluid were present either because of local secretion or active transport processes.

Antigen recognition

Western immunoblot analysis of the serum specific IgG_1 antigen recognition profiles of various responder phenotype strains to *H. polygyrus* E/S products is illustrated in Figure 5. It can be seen that mice with a 'rapid' response phenotype ((SWR×SJL)F_1) and, to a lesser degree, both the 'fast' response phenotype parental strains (SJL and SWR) recognized two low molecular weight bands (18 and 16 kDa) at weeks 4 and 6 postinfection (lanes 3–10). Neither antigens were recognized by mice with an 'intermediate' response phenotype (BALB/c) at weeks 4, 6 or 10 postinfection (lanes 14–16), nor by mice exhibiting a 'slow' response phenotype (C57BL/10 (lanes 17 and 18) and CBA (lanes 19 and 20)) at weeks 4 and 6 postinfection However, in another strain of mice with an 'intermediate' response phenotype (NIH), the two low molecular weight antigens were recognized, although here the intensity of recognition increased with time, being detectable at week 6, and optimal at week 10 (lanes 11–13). Both low molecular weight bands were recognized by hyper-immune but not naive sera (lanes 1 and 2).

DISCUSSION

In order to resolve the role of antibody in host-protective immunity on initial exposure of mice to *H. polygyrus*, we examined the relationship between worm burden and parasite-specific serum and intestinal IgG_1 and IgA responses during the course of primary infections in various mouse strains known to conform to four distinct primary response phenotypes ('rapid' ((SWR × SJL)F_1), 'fast' (SWR and SJL), 'intermediate' (NIH and BALB/c) and 'slow' (CBA and C57BL/10)). We tested the hypothesis that if parasite-specific antibody plays a crucial role in limiting the duration of primary infections, strains that reject worms quickly ('rapid' and 'fast' response phenotypes) should show more rapid and intense parasite-specific antibody responses than strains which tolerate the parasite for longer ('intermediate' and 'slow' response phenotypes). Our specific prediction was that serum and intestinal parasite-specific IgG_1 and IgA would show the following relationship: greater in strains with 'rapid' than 'fast' than 'intermediate' than 'slow' response phenotypes.

In general, our expectations were fulfilled in so far as the 'rapid' and 'fast' responder strains generated earlier and more intense parasite-specific IgG_1 and IgA responses than the 'intermediate' and 'slow' responders, and this was consistent in both serum and intestinal lavages, the latter reflecting the local immune response. The serum antibody responses were consistent with our earlier study (Wahid & Behnke 1993), but contrast with those of Zhong & Dobson (1996) who failed to find a significant relationship between response phenotype and serum parasite-specific antibody responses when assaying samples taken 21 days after primary infection from seven mouse strains. However, their study reported a significant relationship between parasite-specific IgG and host resistance following challenge infection of immune mice of various strains. Our results are also contradictory to those reported by Crandall *et al*. (1974), who failed to find significant intestinal IgG_1 or IgA following primary infection with *H. polygyrus*, although they reported a significant increase in intestinal IgG_1 following a challenge infection of immunized mice. However, their observations following primary infection of mice with *H. polygyrus* are not surprising, as antibody levels were examined after only 2 weeks of infection, when both strains of mice used still had significant worm burdens.

One important difference between our study and that of others was that we employed mice showing a greater spectrum of response phenotypes during primary infections, ranging from the 'rapid' response phenotype of (SWR × SJL)F_1 hybrids, which lose worms soon after week 4, through the 'fast', 'intermediate' and 'slow' response phenotypes, the latter tolerating infections of more than 40 weeks duration. Moreover, we assayed both serum and intestinal samples taken at several stages during the course of each experiment. It was of interest that the kinetics and intensity of the specific humoral antibody response generally mirrored those occurring in intestinal fluids, suggesting a possible causal relationship between the two, such as leakage of antibody into the gut from the plasma. However, as very little or no albumin (an abundant serum protein) was detected in the luminal samples, leakage of serum IgG_1 and IgA could be discounted as an explanation for raised intestinal levels of these isotypes. Therefore, the parasite-specific IgG_1 and IgA detected in intestinal fluid were attributed to either local secretion or active transport processes across the blood/gut interface.

If antibody is involved in limiting primary infections then a negative relationship between the intensity of parasite-specific antibody and worm burden should be detectable within mouse strains, and should be most marked at the time when worm loss is actually taking place. We detected such relationships in serum parasite-specific IgG_1 and IgA in the 'rapid' responders (week 4), in serum IgG_1 in the 'fast' responder SJL mice (week 4), in serum IgA (week 4) in 1 of 3 experiments in SWR mice and in serum IgG_1 in the intermediate BALB/c mice at week 10. Comparable relationships were more rare when the intestinal antibodies were analysed, but significant negative relationships were detected among the 'fast' responding SWR strain in weeks 4 and 6. However, detection of significant relationships is necessarily dependent on sufficient spread of worm burdens to enable a correlation test to be applied and this can only happen when worms are actually in the process of being expelled and in the 'rapid' and 'fast' responder strains this can be a very brief period of time which could be easily missed. Nevertheless, it is of interest that all the significant relationships detected were negative, as predicted from our hypothesis.

It has been postulated that although the expression of host-protective (antiparasite) immunity and the immunomodulatory activities of the parasite appear to be in a dynamic equilibrium, there is a threshold above which the parasite can no longer modulate the host protective response against it and the balance tips in favour of the host (Behnke & Parish 1979, Lawrence & Pritchard 1994). These parasite immunomodulatory factors have still not been fully characterized but are thought to be low molecular weight products (Monroy *et al.* 1989). Neutralization of such immunomodulatory factors by antibody, in those strains least affected by the parasite's immunomodulatory strategy (i.e. the 'rapid' and 'fast' responders) may be the explanation for their ability to mount host protective responses, thereby reducing parasite burdens much earlier than other strains. Failure to produce such antibodies would result in chronic

infections, as in our 'slow' responder phenotypes. In this context, it is of interest that among the array of antigens recognized by the sera assayed by western immunoblotting, two antigens of molecular weight 16 and 18 kDa were preferentially recognized by the 'rapid' and 'fast' responder strains, as found earlier by Wahid (1991) in the $(SWR \times SJL)F_1$ hybrids. There was no evidence of specific antibodies against these antigens in serum and intestinal fluids from the 'slow' responders, although one of the 'intermediate' responder strains (NIH) showed some reactivity, but much later during infection than in the faster responding strains. Unfortunately, all our attempts to test intestinal fluids via Western immunoblotting failed to find any reactivity against these molecules. This was probably due to the dilution factor involved in the collection of the intestinal samples. It is of interest that Zhong & Dobson (1996) reported that the parasite-specific antibody responses in several strains of mice were principally directed against three low molecular weight antigens of 18, 21 and 23 kDa which dominated the somatic and E/S components of all adult worm antigen preparations tested, and our results therefore concur with these findings.

Thus, these observations suggest that antibody directed at specific antigens of *H. polygyrus* may have a role to play in mediating parasite expulsion and that the ability to generate both a vigorous and antigen-specific antibody response may be a contributory mechanism facilitating host-protective immunity to *H. polygyrus* during primary infection. This is supported by the finding that primary infection sera from the 'rapid' responder $(SWR \times SJL)F_1$ mice, and to a lesser extent, from the 'fast' responder parental strains of this hybrid have been used to successfully transfer resistance to recipients, while primary infection sera from chronically infected slow responder mice did not (Wahid 1991). However, we do not argue here that parasite-specific antibody is the only host-protective mechanism operating during primary infections with *H. polygyrus*. Conversely, we propose that in those mouse strains which can mount rapid parasite specific antibody responses (whether because they are resistant to the parasite's immunomodulatory factors or because they respond so rapidly that they neutralize these immunomodulatory factors) other immune effector mechanisms can be initiated earlier to curtail infections within weeks rather than months of initial exposure. 'Fast' responder mouse strains such as SWR are known to produce earlier mast cell responses in the intestine (Wahid *et al*. 1994) and adult *H. polygyrus* are susceptible to the effects of such responses (Behnke *et al.* 1992). Moreover, 'fast' responder mice can also generate earlier and greater levels of IgE and Th2-type cytokines such as interleukin (IL)-3, IL-4, IL-5, IL-9 and IL-10 (Wahid *et al*. 1994). Taken together, these results suggest that 'fast' and 'slow' responder mice differ not only in their responsiveness to parasite antigens, but also in their ability to initiate and sustain a Th2-type response to *H. polygyrus*, thus facilitating expulsion of adult worms.

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