The relative involvement of Th1 and Th2 associated immune responses in the expulsion of a primary infection of *Heligmosomoides polygyrus* in mice of differing response phenotype

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Abstract

T helper cell (Th1 and Th2) associated responses were examined following a primary infection with the gastrointestinal nematode *Heligmosomoides polygyrus* in five inbred strains of mice with different resistance phenotypes. Levels of (i) mast cell protease, (ii) specific IgE, (iii) nitric oxide and (iv) specific IgG2a, as markers of Th2 and Th1 associated responses, respectively, were determined in sera and intestinal fluids and correlated with worm burdens. The ‘fast’ responder (resistant) strains SWR and SJL produced strong Th2 and Th1 associated responses respectively in a mutually exclusive fashion. The F¹ hybrid (SWR × SJL) F¹, showed rapid expulsion of the parasite and expressed both intense Th1 and Th2 responses, suggesting synergism between Th1 and Th2 activity in these mice. The results indicate that both Th2 and Th1 responses operate in mice following a primary infection with *H. polygyrus* and that each Th response may be involved to a greater or lesser degree within certain strains. Resistance to *H. polygyrus* was found to correlate only to the intensity of either the gut-associated mastocytosis or nitric oxide production in these strains but not to either specific IgE or IgG2a titres. Chronic infections in the ‘slow’ response phenotype mouse strains CBA and C57BL/10, were associated with both poor Th2 and poor Th1-associated responses attributed to a general parasite-mediated immunosuppression of the host immune response to infection.

Introduction

A major advance in our understanding of the regulation of host immune response(s) to parasitic infections, particularly in murine model systems, has been the division of CD4⁺ lymphocytes into distinct T helper cell subsets (Th1 and Th2) based upon cytokine production (Mosmann & Coffman, 1989). Th1 and Th2 cells are thought in mice to act in a reciprocal manner, being mutually inhibitory and/or self-stimulatory in action (Mossman & Moore, 1991). We postulate that there are two pathways, regulated by distinct CD4⁺ Th cell populations that lead to activation of mechanisms responsible for the elimination of parasitic helminth infections.

The first, via Th2 cells, is characterized by an acute immediate hypersensitivity type inflammatory response associated with a mucosal mastocytosis (regulated by a combination of interleukin(IL)-3, IL-4, IL-9 and IL-10
(Urban et al., 1992)), eosinophilia (dependent on IL-3 and IL-5 (Coffman et al., 1989)) mucosis secretion by goblet cells (dependent on IL-13 (Bancroft et al., 1998; Artis et al., 1999)), intestinal epithelial cell function (mediated by IL-4 and IL-13 (Shea-Donohue et al., 2001)) and specific IgE production (via IL-4 modulation of B cell isotype switching (Finkelman et al., 1990)).

A second mechanism, associated with the Th1 arm of the immune response, is characterized by a delayed-type hypersensitivity (DTH)/cell-mediated immune (CMI) reaction, involving macrophage activation, (with release of reactive nitrogen and oxygen intermediates), cytotoxic T cells, and NK cell antibody-dependent cytotoxicity (regulated by interferon (IFN)-γ, IL-2, IL-12, tumour necrosis factor (TNF)-α and TNF-β), increased epithelial cell turnover (dependent on IFN-γ (Bancroft et al., 1998; Artis et al., 1999)), and specific IgG2a production (mediated via IFN-γ inhibition of IL-4 induced switching, and induction of switching to γ2a (Romagnani, 1992)). Such CMI responses however have traditionally been thought to be restricted to immunity to intracellular parasites and to viral and bacterial infections (Liew et al., 1989) or alternatively associated with DTH-associated immunopathology (Garside et al., 2000).

One current hypothesis advocates that expulsion of gastrointestinal nematodes such as Trichinella spiralis, Nippostrongylus brasiliensis, and Haemonchus contortus occurs via a Th2-associated allergic inflammatory response, supported mainly by evidence based upon the correlation between mastocytosis and mast cell proteases levels and worm expulsion, observed in mice of differing response phenotype (Nawa & Miller, 1979; Alizadeh & Wakelin, 1982; Tuohy et al., 1990; Gill et al., 1992; Grencis et al., 1993). The allergic inflammatory response per se, however, is not thought to irreversibly damage or kill the infecting parasite, as expelled T. spiralis, Strongyloides ratti and N. brasiliensis adult worms have been shown to be capable of survival if surgically transplanted into the intestines of naive animals (Rothwell, 1989).

Therefore, in response to gastrointestinal infections, both Th1 and Th2 mediated immune mechanisms could in theory result in a similar outcome, i.e. the generation of an immunologically non-specific inflammatory response following stimulation by a specific antigen/T-cell interaction. Indeed, both arms of the T cell response are thought to be initiated in the early stages of most helminth infections. Subsequently the response may be biased in favour of Th1 or Th2-type responses depending on the infection and the genetic makeup of the host (Nakamura et al., 1997). The inflammation elicited by either system could make the intestine an unsuitable environment for the persistence of the worms, resulting in their expulsion (Wakelin, 1993). However, the main cellular and humoral effector molecules associated with the two T helper cell pathways are distinct.

Although data obtained from studies using both IL-4 knockout and IL-4 treated mice clearly illustrate the importance of this Th2-associated cytokine in the regulation of worm burdens and susceptibility to infection (reviewed in Finkelman et al., 1997), the specific effector mechanism(s) mediating worm expulsion remain unresolved. There is also a paradox in that H. polygyrus worm expulsion apparently occurs in the absence of a Th2-associated intestinal mastocytosis (Dehlawi & Wakelin, 1988). Similarly, mast cell deficient W/W′ mice have been reported to develop the same level of protective immunity as wild type litter mates to infection with H. polygyrus (Urban et al., 1992) and neutralization of IgE production was not found to inhibit protective immunity in infected mice (Urban et al., 1991).

Moreover, in certain ‘resistant’ strains of inbred mice, such as SJL, H. polygyrus adult worms were rapidly expelled in the absence of either a marked mast cell or specific IgE response (Tuohy, 1992) suggesting that another mechanism(s) operates to remove the parasite in this strain.

In this study, the relative importance of both Th1- and Th2-associated immune responses were assessed following a primary infection with H. polygyrus within selected ‘rapid’ (SWR × SJL,F1, ‘fast’, SJL and SWR), ‘intermediate’ (NHI), and ‘slow’ (CBA and C57BL/10) responder strains of inbred mice in terms of their worm expulsion phenotype (table 1).

Strain-dependent differences in the intensity and kinetics of release of mucosal mast cell protease and parasite-specific IgE responses (as markers of a Th2-associated inflammatory response) and parasite-specific IgG2a and nitric oxide (NO) responses (as indicators of a Th1-associated CMI response) were then evaluated for positive associations with worm expulsion to help provide an insight into the effector mechanism(s) responsible for this phenomenon.

### Materials and methods

#### Animals

Female syngeneic NIH, C57BL/10, CBA/Ca, BALB/c SWR, SJL, and (SWR × SJL,F1 hybrid mice were either purchased from Harlan Olac Ltd (Bicester, Oxon) 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 five mice per group was used in each experiment.

**Heligmosomoides polygyrus**

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

**Heligmosomoides polygyrus excretory/secretory (E/S) products antigen preparation**

Adult worms were recovered from outbred CFLP mice infected 14 days earlier with 400–600 L3 of *H. polygyrus*. The parasites were washed in sterile phosphate buffered saline and cultured aseptically for 24 h at 37°C in RPMI 1640 medium (Gibco) supplemented with 0.1 mM sodium
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 1.5 ml Eppendorf tubes containing 50 µl of Serasieve gel (Hughes and Hughes Ltd), allowed to clot and spun at 13,000 g for 5 min. The resulting serum was then removed and stored at −80°C for 10 min. Thirty microlitres of 100 mM phenylmethylsulphonyl fluoride (PMSF, Sigma) was added; this was added to provide an alternative substrate for any remaining protease in the intestinal secretion. The samples were stored at −40°C until used.

**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 1.5 ml Eppendorf tubes containing 50 µl of Serasieve gel (Hughes and Hughes Ltd), allowed to clot and spun 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⁻¹ penicillin + 100 µg ml⁻¹ streptomycin solution (Gibco), 75 µM monothioglycerol (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 (Millipore, UK), dialysed (22/35 kDa dialysis tubing) against distilled water for 24 h at 4°C, lyophilized and stored at −80°C until used. Each plate included control ‘hyper-immune’ serum (HIS), (Behnke & Parish, 1979) and also serum from naive mice (NS). With regard to IgE levels, 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). Both serum and luminal IgG2a results, however, are presented as OD values, as the intestinal samples contained more IgG2a 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 are presented as mean RRI or OD value ± standard error (SEM) for each group.

**Measurement of H. polygyrus-specific IgG2a and IgE responses**

Standard ELISA assays were used to assess the levels of parasite-specific (E/S products) IgG2a and IgE antibody responses in serum and intestinal fluid samples essentially as described by Wahid & Behnke (1993). Mouse samples were assayed individually; serum was tested at a dilution of 1:50 and intestinal samples were used undiluted. Each plate included control ‘hyper-immune’ serum (HIS), (Behnke & Parish, 1979) and also serum from naive mice (NS). With regard to IgE levels, 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). Both serum and luminal IgG2a results, however, are presented as OD values, as the intestinal samples contained more IgG2a 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 are presented as mean RRI or OD value ± standard error (SEM) for each group.

**Measurement of mucosal mast cell protease (MMCP-1)**

The levels of MMCP-1 present in serum and intestinal fluid were measured using an antigen-capture ELISA technique as described by Huntley et al. (1990). The reagents were provided as a kit from Moredun Animal Health Ltd, Edinburgh. Values of MMCP-1 in serum were expressed as mean µg ml⁻¹ and in intestinal fluid as mean µg mg⁻¹ protein ± standard error (SEM) for each group.

Table 1. Resistance ranking of inbred strains of mice to a primary infection with Heligmosomoides polygyrus

| Strain haplotype | H-2 genes | Background genes | Immunity to infection | Responder status |
|------------------|-----------|------------------|----------------------|-----------------|
| CBA             | k         | CBA              | –                    | Slow            |
| C3H             | k         | C3H              | –                    | Slow            |
| C57BL/10        | b         | B10              | –                    | Slow            |
| B10G            | q         | B10              | –                    | Slow            |
| B10S            | s         | B10              | +                    | Intermediate    |
| B10D2/n         | d         | BALB             | +                    | Intermediate    |
| BALB/c          | d         | BALB             | +                    | Intermediate    |
| NIH             | q         | NIH              | +                    | Intermediate    |
| DBA/1           | q         | DBA/1            | +                    | Intermediate    |
| DBA/2           | d         | DBA/2            | +                    | Intermediate    |
| (NIH x C57BL10)F₁ | bq       | NIH/B10         | +                    | Intermediate    |
| (NIH x B10G)F₁ | q         | NIH/B10         | +                    | Intermediate    |
| SWR             | q         | SWR              | ++                   | Rapid           |
| SJL             | s         | SJL              | ++                   | Rapid           |
| (SWR x SJL)F₁  | q         | SWR/SJL         | +++                  | Extremely rapid |

The relative ranking of the strains was arranged on the basis of the data in Behnke & Robinson (1985) and on experiments conducted over recent years. The responder status, assessed on the basis of time to loss of adult worms following primary exposure, varies from experiment to experiment, but despite this, inter-experiment variation, the relative ranking of the strains remains constant. – slow or very weak; +, intermediate; ++, rapid; +++, extremely rapid and strong.
Measurement of nitric oxide (Greiss reagent)

The Griess reaction assay as described by Green et al. (1982) was used to assay for nitrite in both intestinal fluids and in serum; however, as serum contains greater amounts of nitrate than nitrite, the nitrate was first converted to nitrite using a nitrate reductase reaction. All solutions were made up in double-deionized (nitrate-free) water. Results are expressed as mean levels (μM) of nitrite ± standard error (SEM) for each group.

Conversion of serum nitrate to nitrite

Equal volumes of serum were incubated with a reaction mixture which consisted of equal proportions of: NADPH, 5 mM, FAD, 41.5 mg ml⁻¹, KH₂PO₄, 30 mM, Aspergillus nitrate reductase, 3.4 mg ml⁻¹ and distilled water. The serum mix was then incubated at 37°C for 2–4 h prior to assessment in the nitrite (Greiss reaction) assay.

Experimental design

Three sets of experiments were undertaken to examine the relationship between host resistance, as determined by worm expulsion, and Th1/Th2-mediated immune responses elicited in selected mouse strains following a primary infection with H. polygyrus primary infection. The worm recoveries over the time course of the experiment are shown in fig. 1a. All three strains examined expelled true to their predicted phenotype ranges, i.e. SWR mice rapidly expelled their worms between weeks 4–6 p.i., NIH between weeks 6–10 p.i. and CBA mice maintained their burdens over the 10 weeks examined, with significant differences seen with respect to both the mouse strain and the timing of expulsion (strain: χ² = 14, df = 2, P < 0.001; time: χ² = 82. df = 3, P < 0.001).

Both serum and luminal MMCP-I levels declined in all three strains from peak values at week 2 over the subsequent 6–10 weeks p.i. With regard to serum MMCP-I levels (fig. 1b), in the slow responder strain (CBA) these were significantly lower than those detected in the other two responder strains of mice at weeks 6 and 10 p.i. (F = 7, df = 2, P = 0.002; week 6: CBA 0.46 ± 0.04 μg ml⁻¹, NIH 0.94 ± 0.08 μg ml⁻¹, SWR 0.81 ± 0.05 μg ml⁻¹; week 10: CBA 0.54 ± 0.1 μg ml⁻¹, NIH 0.66 ± 0.04 μg ml⁻¹, SWR 0.87 ± 0.09 μg ml⁻¹). There was also a significant difference in the kinetics of production of serum MMCP-I between the three strains of mice (F = 69, df = 2, P < 0.001). However, there was no significant difference in the levels of MMCP-I between the three strains examined in the intestinal fluids (fig. 1c; week 2: 0.21 ± 0.04 μg ml⁻¹ protein (CBA) to 0.28 ± 0.01 μg ml⁻¹ protein (SWR); week 10: 0.05 ± 0.01 μg ml⁻¹ protein (CBA) to 0.06 ± 0.01 μg ml⁻¹ protein (SWR)).

IgE levels in both serum and gut increased throughout the infection in all the three strains examined. However, both the ‘fast’ responder (SWR) and the ‘intermediate’ responder (NIH) mice produced significantly greater levels of serum IgE with faster kinetics than the ‘slow’ responder (CBA) mice at weeks 6 (RRI of 90.5 ± 11.77 in SWR and 51.26 ± 11.73 in NIH, compared to 0.59 ± 11.0 in CBA) and 10 p.i. (RRI of 142.13 ± 7.77 in SWR and 98.72 ± 9.5 in NIH, compared to 24.36 ± 7.87 in CBA) (fig. 1d, strain: F = 38, df = 2, P < 0.001; time: F = 56, df = 3, P < 0.001). This pattern of higher and more rapid IgE responses in the ‘fast’ and ‘intermediate’ responder strains than observed in the slow responder strain was mirrored in the intestine (week 6 p.i., RRI of 234.23 ± 42.52 in SWR, 232.62 ± 40.54 in NIH, 54.45 ± 35.31 in CBA; week 10 p.i., RRI of 240.88 ± 46.34 in SWR, 235.49 ± 90.45 in NIH, 107.81 ± 26.94 in CBA; fig. 1d).

Low levels of parasite-specific IgG2a (as measured by OD units) were detected in the serum of all three
Mechanisms of expulsion of primary infections of *Heligmosomoides polygyrus* in mice

Fig. 1. Worm burdens (a), serum (b) and intestinal (c) MMCP-I levels and parasite-specific serum (d) and intestinal (e) IgE in ‘fast’ responder (SWR △), ‘intermediate’ responder (NIH ■) and ‘slow’ responder (CBA ●) strains of mice after a primary infection with 50 L3 larvae of *Heligmosomoides polygyrus*. MMCP-I levels are expressed as mean values (μg ml⁻¹ (serum) or μg mg⁻¹ protein (intestine)) of the infected minus naïve animals; IgE levels are expressed as mean index relative to naïve and hyper-immune serum (RRI, see methods) of infected minus naïve animals.

137
strains of mice; however, NIH mice produced significantly more IgG2a than the other two strains at week 10 p.i. (fig. 2a; strain: \( F = 46, \ df = 2, \ P < 0.001 \); time: \( F = 86, \ df = 3, \ P < 0.001 \)). In intestinal samples, relatively high levels of IgG2a (as measured by OD units) were detected in all three strains of mice compared to levels in the serum, suggesting local production of this antibody isotype (fig. 2b). Greatest IgG2a levels were produced by the SWR mice in a biphasic response, peaking at weeks 4 (0.79 ± 0.08 OD units) and 10 p.i. (0.29 ± 0.09 OD units), peaked at week 4 p.i. (0.40 ± 0.03 OD units) before falling to control values by week 6 p.i. By comparison, in CBA mice, intestinal levels of IgG2a rose steadily from basal levels at week 2 p.i., peaking at week 6 p.i. (0.30 ± 0.08 OD units) and then declining slightly by week 10 p.i. (0.14 ± 0.03 OD units) (fig. 2b).

Analysis of serum samples from the three strains of mice for the presence of nitrite showed that levels in all three strains rose rapidly after week 2 p.i. to peak at week 6 p.i. for SWR (week 2, 0.2 ± 0.02 μM; week 6, 15.29 ± 1.38 μM) and NIH (week 2, 0.2 ± 0.6 μM; week 6, 13.38 ± 2.21 μM) mice and at week 10 for CBA mice (week 2, 0 ± 2.14 μM; week 10, 13.35 ± 1.87 μM). No significant difference was seen between the peak levels of serum nitrate detected in the three strains, although CBA mice were found to have a significantly slower response (\( F = 6, \ df = 2, \ P = 0.004 \), fig. 2c). In contrast, within intestinal samples, there was a significant difference between the levels of nitrate detected in the three strains of mice (\( F = 67, \ df = 2, \ P < 0.001 \), fig. 2d). Little nitrite was detected in SWR mice, although their levels peaked at week 6 p.i. to 4.35 ± 1.59 μM. In contrast, levels in NIH mice rose from week 2 (5.4 ± 2.12 μM) to a maximum level at week 6 (11.42 ± 3.0 μM), which then fell to background levels at week 10 p.i. Nitrate titres in CBA mice fell to background levels at week 4 p.i. from 5.11 ± 1.62 μM at week 2 and then rose again to peak at week 10 p.i. (8.82 ± 1.5 μM).

**Experiment 2: rapid, fast and slow expelling infections**

In this set of experiments, three strains representing ‘rapid’ ((SWR × SJL)F1), ‘fast’ (SWR) and ‘slow’ (C57BL/10) responder strains of mice were examined for their response phenotype to a primary infection with 100 L3. The worm recoveries over a six week experimental
Mechanisms of expulsion of primary infections of *Heligmosomoides polygyrus* in mice

period are shown in fig. 3a. The ‘rapid’ responder (SWR × SJL)F1 strain quickly expelled their worms within 6 weeks, whereas worm burdens were maintained by the ‘slow’ responder C57BL/10 mice over this period. In the ‘fast’ responder SWR mice, however, an unexpectedly small decline in parasite burden occurred by week 6 p.i. (<14%), although greater expulsion may have been predicted to occur if the experiment had been continued until week 8 p.i. Statistical analysis showed that the differences observed in expulsion were significant with respect to both the strain and the kinetics of expulsion (strain: $\chi^2 = 32.5$, df = 2, $P < 0.001$; time: $\chi^2 = 13.45$, df = 2, $P = 0.005$) (fig. 3a).

Serum MMCP-I levels were already raised by week 2 p.i. in both the (SWR × SJL)F1 (0.78 ± 0.13 µg ml$^{-1}$) and SWR (1.04 ± 0.10 µg ml$^{-1}$) mice, peaking at week 4 (F1: 1.32 ± 0.16 µg ml$^{-1}$; SWR: 1.41 ± 0.06 µg ml$^{-1}$) and then declining slightly by week 6 p.i. (fig. 3b). Overall, there was no significant difference between the levels of serum MMCP-I in the ‘fast’ responder compared to the ‘rapid’ responder mice. In the ‘slow’ responder (C57BL/10) mice, MMCP-I levels also rose slightly by week 4 p.i. (to 0.24 ± 0.08 µg ml$^{-1}$) and were maintained by week 6 p.i., but were very much lower than in either of the other two strains examined (fig. 3b). There was a significant statistical difference in the level and kinetics of serum MMCP-I secretion between the fast and slow responder strains of mice (strain: $F = 66$, df = 2, $P < 0.001$; time: $F = 8$, df = 2, $P = 0.001$) (fig. 3b). The pattern of secretion of luminal MMCP-I was similar to that of the serum, although MMCP-I titres increased at week 6 p.i. in SWR mice (from 0.21 ± 0.03 µg mg$^{-1}$ protein at week 4 to 0.26 ± 0.02 µg mg$^{-1}$ protein at week 6) and in (SWR × SJL)F1 mice (from 0.20 ± 0.04 µg mg$^{-1}$ protein at week 4 to 0.14 ± 0.06 µg mg$^{-1}$ protein at week 6; fig. 3c).

Again, levels of MMCP-I were significantly lower in the ‘slow’ compared to the ‘rapid’ and ‘fast’ responder strains of mice ($F = 29$, df = 2, $P < 0.001$).

With regard to specific serum IgG, levels rose rapidly in (SWR × SJL)F1 mice, peaking at week 4 (RRI = 52.19 ± 5.53) and then declining by week 6 p.i. (RRI = 22.41 ± 5.87), whereas IgE levels in SWR mice rose less dramatically but were still increasing by week 6 p.i. (week 4, RRI = 16.61 ± 5.0; week 6, RRI = 40.95 ± 12.67; fig. 3d). In C57BL/10 mice, there was a significantly slower rise in IgE levels ($F = 7$, df = 2, $P = 0.003$), with levels rising from basal at week 2 to an index of 8.86 ± 2.37 at week 4 p.i. which was then maintained at week 6 p.i., but were lower than levels seen in the ‘rapid’ and ‘fast’ responder strains (fig. 3d). A similar pattern of response was reflected in intestinal IgE levels, except that levels in the F1 hybrid mice were maintained at week 6 p.i. (fig. 3e). There was significant difference with regard to strain and kinetics in the levels of intestinal IgE observed (strain: $F = 3$, df = 2, $P = 0.048$; time: $F = 13$, df = 2, $P < 0.001$).

Serum IgG2a levels followed a similar pattern of production in both (SWR × SJL)F1 and C57BL/10 mice, rising from basal levels at week 2 p.i. peaking at week 4 (OD in F1 hybrid, 0.03 ± 0.02; OD in C57BL/10, 0.2 ± 0.4) and then declining at week 6 p.i. (fig. 4a). Levels in the F1 hybrid mice were, however, greater than in C57BL/10 mice at week 4 p.i. SWR mice, in contrast, showed a small decline in IgG2a levels between weeks 2 and 4 p.i. (OD at week 2, 0.01 ± 0.001; OD at week 4, 0.05 ± 0.002), then rose markedly to week 6 (0.04 ± 0.009, fig. 4a). The differences in both the levels and kinetics of serum IgG2a among the three strains of mice were found to be statistically significant (strain: $F = 138$, df = 2, $P < 0.001$; time: $F = 100$, df = 2, $P < 0.001$). An increase in intestinal IgG2a levels was observed in the F1 hybrid mice between weeks 2 and 6 p.i., mirroring a similar, albeit smaller, rise in SWR mice. Levels in C57BL/10 titre conversely, fell slightly over this period (fig. 4b). None of these differences in intestinal IgG2a levels reached statistical significance.

In the serum samples, high background levels of nitrite were detected in all the three strains of mice examined such that infection with *H. polygyrus* added no detectable increase in nitrite levels (results not shown). Within the intestine, in comparison to the other strains, levels of nitrite in the ‘rapid’ responder ((SWR × SJL)F1) mice increased rapidly throughout the experimental period from a mean level of 3.05 ± 1.0 µM at week 2 p.i., to 17.1 ± 2.57 µM at week 6 p.i. (strain: $F = 34$, df = 2, $P < 0.001$; time: $F = 12$, df = 2, $P < 0.001$). By contrast, only minimal levels were detected in both the ‘fast’ responder (SWR) and ‘slow’ responder (C57BL/10) strains of mice (fig. 4c).

**Experiment 3: rapid and fast expelling infections**

In this set of experiments three strains, representing ‘rapid’ responder ((SWR × SJL)F1) mice and their respective ‘fast’ responder parental strains (SWR and SJL) were examined for their response phenotype to a primary infection of *H. polygyrus*. Again, worm recoveries (fig. 5a) did not conform exactly to the expected response pattern, in that, although the (SWR × SJL)F1 and SJL mice expelled rapidly over 4–6 weeks p.i. as predicted, worm burdens were maintained in the SWR mice. In spite of this delay in expulsion in the SWR mice, there was a statistically significant difference in the work burdens and kinetics of expulsion among the three strains of mice examined (strain: $\chi^2 = 31$, df = 2, $P < 0.001$; time: $\chi^2 = 45$, df = 2, $P < 0.001$).

MMCP-I serum levels were again raised in (SWR × SJL)F1 and both parental strains at week 2 p.i. (F1 hybrid, 1.09 ± 0.03 µg ml$^{-1}$; SWR, 1.05 ± 0.05 µg ml$^{-1}$; SJL, 0.43 ± 0.02 µg ml$^{-1}$), with levels in all three strains peaking at week 4 (F1 hybrid, 1.14 ± 0.07 µg ml$^{-1}$; SWR, 1.32 ± 0.07 µg ml$^{-1}$; SJL, 0.62 ± 0.06 µg ml$^{-1}$) and declining by week 6 p.i. (F1 hybrid, 0.53 ± 0.09 µg ml$^{-1}$; SWR, 0.79 ± 0.08 µg ml$^{-1}$; SJL, 0.38 ± 0.13 µg ml$^{-1}$) (fig. 5b). SJL MMCP-I levels, however, were significantly lower than those in either SWR or (SWR × SJL)F1 mice ($F = 42$, df = 2, $P < 0.001$) and could be ranked in order of intensity as SWR > F1 > SJL, although there was no statistical difference between levels in SWR and F1 hybrid mice (fig. 5b). A similar pattern of response was observed in the intestinal fluid (fig. 5c), with levels of MMCP-I significantly lower in SJL mice than SWR or F1 hybrid mice ($F = 13$, df = 2, $P < 0.001$) and control levels of nitrite input to the experimental fluid were similar to those from week 2 to 6 p.i. Serum IgE levels rose rapidly from basal levels at week 1 in (SWR × SJL)F1 mice and peaked at week 4 p.i. (RRI = 68.86 ± 2.36) before declining at week 6 p.i.
Fig. 3. Experiment 2. Worm burdens (a), serum (b) and intestinal (c) MMCP-I levels and parasite-specific serum (d) and intestinal (e) IgE in ‘rapid’ responder ((SWR × SJL)F1 ▲), ‘fast’ responder (SWR △) and ‘slow’ responder (C57BL/10 ○) strains of mice after a primary infection with 100 L3 larvae of Heligmosomoides polygyrus. MMCP-I levels are expressed as mean values (μg ml⁻¹ (serum) or μg mg⁻¹ protein (intestine)) of the infected minus naïve animals; IgE levels are expressed as mean index relative to naïve and hyper-immune serum (RRI) of infected minus naïve animals.
Mechanisms of expulsion of primary infections of *Heligmosomoides polygyrus* in mice

and continued to increase by week 6 (RRI = 192.33 ± 24.63). The levels of specific mucosal IgE in SJL mice, however, remained minimal and were again significantly lower than those observed among the other two strains of mice (F = 11, df = 2, P < 0.001; fig. 5e).

IgG2a responses in serum samples of SWR mice were significantly different from those observed among the other strains of mice (F = 11, df = 2, P < 0.001) and showed a marked rise at weeks 4 and 6 p.i., with levels at week 6 greater than those observed among the other two strains of mice (OD at week 6: SWR = 0.17 ± 0.03; SJL = 0.02 ± 0.01; F1 = 0.06 ± 0.02, fig. 6a). Levels in (SWR × SJL)F1 mice rose steadily but more slowly than in the SWR mice, whereas levels in SJL mice dropped from week 2 (OD = 0.06 ± 0.01) to minimal values by week 6 p.i. (fig. 6a). Levels of luminal IgG2a again showed a sharp increase in SWR mice, with levels in the F1 hybrids rising to week 4 p.i. and maintained at week 6 p.i.; SJL mice produced only minimal levels of parasite-specific serum IgG2a (fig. 6b). Again, there was significant statistical difference in the levels and also the kinetics of IgG2a observed among the three strains of mice (strain: F = 17, df = 2, P < 0.001; time: F = 9, df = 2, P = 0.001).

Within sera, significantly greater levels of nitrite were detected in (SWR × SJL)F1 samples at all times after infection compared to SWR and SJL mice, peaking at week 6 p.i. (23.57 ± 3.53 μM; F = 4, df = 2, P = 0.048). Levels in SJL mice rose from background at week 2 p.i. to peak at week 6 p.i. (11.45 ± 5.33 μM), whereas levels in SWR mice rose from basal at week 2 to peak values at week 4 (6.93 ± 1.16 μM) and were then maintained at week 6 p.i. (fig. 6c). In intestinal secretions, levels of nitrite rose markedly in both SJL and (SWR × SJL)F1 mice at week 4 (SJL: 14.45 ± 2.38 μM; F1: 13.33 ± 0.98 μM) and then fell at week 6 p.i. (SJL: 5.38 ± 1.41 μM; F1: 12.41 ± 1.17 μM). Levels of nitrite detected in SWR mucosal samples were low throughout the infection period (fig. 6d). There was significant statistical difference with regard to level and kinetics of luminal nitrite observed between the three strains of mice (strain: F = 14, df = 2, P < 0.001; time: F = 17, df = 2, P < 0.001).

In summary, the results of the three experiments suggest that *H. polygyrus* infection in ‘slow’ responder strains such as CBA and C57BL/10 stimulates neither a Th1 (as assessed by intestinal IgG2a and NO) nor a Th2 (as assessed by intestinal MMCP-I and IgE) response. The ‘intermediate’ responder strain, NIH, however, are capable of generating both responses (as assessed by intestinal IgE and NO). ‘Fast’ responder SJL mice, produce minimal IgE and MMCP-I responses and very little IgG2a, but show a rapid and strong intestinal NO reaction. On the other hand, SWR mice, also ‘fast’ responders, exhibit high MMCP-I and IgE responses but low NO reactions to the parasite. The ‘rapid’ responder (SWR × SJL)F1 hybrids in contrast show both strong Th1 and Th2 effector responses in terms of MMCP-I, specific IgE and NO production, to a primary infection with *H. polygyrus*.

**Discussion**

*Heligmosomoides polygyrus* is believed to give rise to chronic infections in mice by the release of immunomodulatory factors that down-regulate T cell responses...
which promote the development of Th2-associated reactions such as mucosal mastocytosis and eosinophilia (Behnke, 1987; Dehlawi et al., 1987; Dehlawi & Wakelin, 1988). In spite of such effects, some inbred strains of mice are still able to expel the parasite within a relatively short period of time after infection, which in part undoubtedly reflects genetic influences upon the host response to parasite-mediated suppression (Behnke et al., 2000).

This study was undertaken to assess the relative roles of Th2-associated immediate and Th1-associated delayed hypersensitivity responses in resistance to infection with *H. polygyrus* and to look for evidence of differential modulation of these T cell-mediated reactions within such inbred strains following parasite-mediated immunosuppression.

In this study, mast cell protease levels in sera and luminal secretions, as a marker of a Th2-associated...
immediate hypersensitivity response, were generally shown to exhibit a temporal association with worm expulsion. In (SWR×SJL)F1 mice, the level of mast cell protease peaked at week 4 p.i. which correlated with the onset of expulsion while ‘slow’ responder strains CBA and C57BL/10 produced consistently low levels of MMCP-I. There was a notable exception to this general pattern in that in the ‘fast’ responder strain SJL, rapid worm expulsion was observed where little or no MMCP-I release was detected in either serum or intestinal fluids. Therefore, overall, the MMCP-1 results obtained in this study support a role for a Th2 inflammatory response being involved in immunity to a primary infection with *H. polygyrus* in most of the strains examined although this appears to be a strain-dependent response.

The induction of intestinal IgE has been proposed to be an important protective mechanism against certain gastrointestinal nematodes particularly those that penetrate the duodenum mucosa, such as *Strongyloides venezuelensis*, *T. spiralis* and *H. polygyrus* (Negrao-Correa, 2001). However, the prevailing theory is that IgE is not thought to play a major role in immune worm expulsion as IgE deficient mice have been reported to be capable of expelling their worms (Onah & Nawa, 2000). In addition, treatment of *H. polygyrus*-infected mice with an anti-mouse IgE mAb was also found not to inhibit protective immunity (Urban et al., 1992). From the results presented in this study, the role of IgE in the expulsion of *H. polygyrus* remains equivocal although in ‘fast’ responder SWR mice, primary infection always resulted in a strong specific IgE response. However, in two out of the three experiments performed, with this strain they failed to expel their worms within 6 weeks p.i. in spite of a marked IgE response. In contrast, SJL, another ‘fast’ responder strain, also expelled their worms rapidly, but in the virtual absence of a specific IgE response. NIH mice, classed as ‘intermediate’ responders, however also produced high IgE levels comparable to those of the SWR mice, but generally expelled their worms later than the SWR mice. Interestingly, the ‘rapid’ expelling hybrid of the two ‘fast’ expelling parental strains, (SWR×SJL)F1, also produced a strong specific IgE response which temporally correlated with expulsion whereas both ‘slow’ expelling strains, CBA and C57BL/10, mounted only poor specific IgE responses. The data suggest that the relative importance of any specific IgE response like the MMCP-1 response is also strain-dependent.

In mice, where NO was detected using the Griess reagent method, higher levels of nitrite were found in the mucosal washings of infected mice than in sera. This suggests that NO is produced locally and may have a role in the mucosal immune response to infection with *H. polygyrus*. Moreover, serum titres of nitrite did not mirror mucosal levels, possibly attributable to high concentrations of naturally occurring nitrate in blood masking the kinetics of local NO production in the gut in response to infection. In addition, high levels of mucosal nitrite, hence NO, were also associated with both ‘rapid’ (SWR×SJL)F1 and certain ‘fast’ responder (SJL) strains.
of mice. In this study, relatively high mucosal levels of NO were detected early in infection in the latter strain, in the virtual absence of either a Th2-associated MMCP-1 or specific IgE response, but were also not associated with a comparable Th1-associated specific IgG2a response. Indeed, the general finding from this work was that specific IgG2a responses did not appear to correlate with either worm expulsion or Th2-associated responses (e.g. MMCP-1) or to other Th1 associated indicators such as serum or mucosal NO production. However, there was a trend for specific IgG2a responses to parallel specific IgE levels in their respective strains, suggesting that such responses are more closely linked with particular genotypes rather than resistance phenotypes of the host strains. This goes against current dogma which suggests that IFN-g and IL-4 act as reciprocal agents in the determination of IgG2a and IgE isotype switching (Coffman et al., 1988) and lends support to a dual mechanism of Th1 and Th2 responses operating in tandem.

Little nitrite was detected in either the sera or intestinal samples of ‘slow’ expelling strains, CBA or C57BL/10 suggesting that neither a strong Th2 nor Th1-associated immune response are elicited in these susceptible strains following infection with H. polygyrus. The overall mast cell protease/IgE (Th2-associated) and nitric oxide/IgG2a (Th1-associated) responses obtained in this study portray a complex pattern of responses between strains of differing resistance phenotype. These patterns can be interpreted as illustrating that two expulsion mechanisms may be operating in the immune response of certain strains of mice to infection with H. polygyrus. In the ‘fast’ responder, SWR, strain, expulsion appears to be linked to Th2-associated MMCP-1 responses, whereas, in the ‘intermediate’ responder, NIH, strain, lack of early expulsion appears to be associated with the eliciting of a reciprocal Th1-associated NO response. This latter interpretation is supported by the findings that vaccination of NIH with Freunds’ adjuvant appears to stimulate a reciprocal Th1-associated NO response. This latter observation is supported by the finding that vaccination with NIH and Freund’s adjuvant appears to stimulate an inhibitory CMI response which acts to inhibit worm expulsion in this strain (Ben-Smith, unpublished observation).

In the ‘slow’ responder strains of mice, C57BL/10 and CBA, susceptibility appears to reflect both a poor Th2 and a poor Th1 response phenotype. However, unlike SWR, the ‘fast’ expulsion phenotype of SJL mice is associated with a strong Th1-associated NO response. Moreover, in the ‘rapid’ expelling F1 hybrid of both ‘fast’ responder parental strains (SWR X SJL)F1 expulsion appeared to correlate with both a strong Th1- (NO) and Th2- (MMCP-1) associated responses, suggesting that the F1 hybrid has inherited both mechanisms of immune expulsion from its respective parents which can act synergistically.

Although in this study nitric oxide production is proposed to reflect a Th1-mediated response, the data presented may prove compatible with the current dogma regarding the apparent central role of IL-4 in the control of H. polygyrus infection should the NO-associated responses prove to result from IL-4 regulated release of TNFα as has been previously reported for T. spiralis infections (Garside et al., 2000). It would therefore be of interest to repeat these studies utilizing TNFα/TNFαR gene deficient mice and gnotobiotic mice to investigate these latter possibilities. The protective mechanisms involved in the immune expulsion of H. polygyrus, therefore remain to be fully characterized.

In conclusion, the data presented in this study would indicate that both Th2- and Th1-associated effector processes may occur in primary H. polygyrus infections in mice, resulting in worm expulsion. While expulsion is most commonly associated with a Th2-type pattern of mediator response, in certain strains Th1-type responses predominate and may mediate expulsion. Chronic H. polygyrus infections may then result from a general parasite-mediated suppression of both arms of the Th response in susceptible strains.

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References

Alizadeh, H. & Wakelin, D. (1982) Genetic factors controlling the intestinal mast cell response in mice infected with Trichinella spiralis. Clinical and Experimental Immunology 49, 331–337.

Artis, D., Potten, C.S., Else, K.J., Finkelman, F.D. & Grenchis, R.K. (1999) Trichuris muris: Host intestinal epithelial cell hyperproliferation during chronic infection is regulated by interferon-gamma. Experimental Parasitology 92, 144–153.

Bancroft, A.J., McKenzie, A.N.J. & Grenchis, R.K. (1998) A critical role for IL-13 in resistance to intestinal nematode infection. Journal of Immunology 160, 3453–3461.

Behnke, J.M. (1987) Evasion of immunity by nematode parasites causing chronic infections. Advances in Parasitology 26, 1–71.

Behnke, J.M. & Parish, H.A. (1979) Expulsion of Nematospiroides dubius from the intestine of mice treated with immune serum. Parasite Immunology 1, 26–31.

Behnke, J.M. & Robinson, M. (1985) Genetic control of immunity to Nematospiroides dubius: a 9-day anthelmintic abbreviated immunizing regime which separates weak and strong responder strains of mice. Parasite Immunology 7, 235–253.

Behnke, J.M. & Waheed, E.N. (1991) Immunological relationships during primary infection with Heligmosomoides polygyrus (Nematospiroides dubius): H-2 linked
protective immunity to nematodes. *Immunological Reviews* 127, 205–219.

Wahid, F.N. & Behnke, J.M. (1993) Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): parasite specific IgG1 antibody responses and primary response phenotype. *Parasite Immunology* 15, 401–413.

Wahid, F.N., Robinson, M. & Behnke, J.M. (1989) Immunological relationships during primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): expulsion of adult worms from fast responder syngeneic and hybrid strains of mice. *Parasitology* 98, 459–469.

Wakelin, D. (1993) Allergic inflammation as a hypothesis for the expulsion of worms from tissues. *Parasitology Today* 9, 115–116.

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