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SUSCEPTIBILITY OF ADULT *HELIGMOSOMOIDES POLYGYRUS* TO INTESTINAL INFLAMMATORY RESPONSES INDUCED BY HETEROLOGOUS INFECTION

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Abstract—BEHNKE J. M., CABAJ W. and WAKELIN D. 1992. Susceptibility of adult *Heligmosomoides polygyrus* to intestinal inflammatory responses induced by heterologous infection. *International Journal for Parasitology* **22**: 75–86. Adult *H. polygyrus* are capable of surviving for many months after primary exposure of mice to infective larvae, raising the possibility that worms of this species have inherent resistance to intestinal immune responses. Accordingly experiments were carried out to determine whether *H. polygyrus* are resistant to the inflammatory changes elicited during the acute phase of the intestinal response to *Trichinella spiralis*. Adult worms were expelled from mice when their presence coincided with the most intense phase of inflammation elicited by *T. spiralis*. The effect was dose-dependent with more intense *T. spiralis* challenge resulting in a correspondingly greater loss of *H. polygyrus*. Even the less pathogenic species *T. pseudospiralis* elicited a response of sufficient intensity in NIH mice to cause the expulsion of *H. polygyrus* from concurrently infected animals. Tissue larval stages of *H. polygyrus* were protected from expulsion by their location deep in the intestinal walls and the maximum detrimental effect against *H. polygyrus* was observed during the adult phase or during the establishment of L3 larvae. Acceleration of the response to *T. spiralis* in immune challenged mice resulted in earlier loss of *H. polygyrus*. When the expulsion of *T. spiralis* was delayed (e.g. from slow responder C57BL/10 mice) the loss of *H. polygyrus* took place correspondingly later. These experiments demonstrate unequivocally that mouse strains which normally tolerate chronic infections with *H. polygyrus* have the capacity to mount intestinal inflammatory responses of sufficient vigour to remove the worms but that this potential is not normally realized. However, the observation that some *H. polygyrus* always survived even when the response induced by *T. spiralis* was of the rapid secondary type suggests that the parasites are resilient in the face of the inflammatory response capable of removing most of the worms. It is suggested that in addition to the immunomodulatory strategy employed by adult worms to prevent the intestinal response being elicited, the worms have a second line of defence which is reflected in their resilience to responses which they have been unable to prevent.

INDEX KEY WORDS: *Heligmosomoides polygyrus*; *Nematospiroides dubius*; *Trichinella spiralis*; *Trichinella pseudospiralis*; Nematoda; mouse; intestinal inflammation; immunity; evasion of immunity.

INTRODUCTION

THE intestinal inflammatory response elicited by *Nippostrongylus brasiliensis* and *Trichinella spiralis* has been the focus of attention for well over 4 decades and the immunological control of this response is well documented (Rothwell, 1989; Moqbel & MacDonald, 1990). It is established that the induction of the response is specific, requiring the sensitization of $CD4^+$ T_H lymphocytes (Crook, 1990; Grencis, Riedlinger & Wakelin, 1985), however, the final effectors remain as controversial as ever. Among recent additions to the list of possible effectors are leukotrienes (Douch, Harrison, Buchanan & Greer, 1983), platelet activating factor (Moqbel & MacDonald, 1990), tumour necrosis factor (Ovington, 1987), mast cell protease II (McKean & Pritchard, 1989) and oxygen radicals (Smith & Bryant, 1989a,b). Once induced the specifically triggered effectors act non-specifically with detrimental consequences to unrelated parasites residing in the intestine at the time of expression (Christensen, Nansen, Fagbemi & Monrad, 1987). During the response to *T. spiralis*, totally unrelated nematodes (*Nippostrongylus brasiliensis*, see Kennedy, 1980) and cestodes (*Hymenolepis diminuta*, see Behnke, Bland & Wakelin, 1977; Christie, Wakelin & Wilson, 1979; *H. nana*, see Ferretti, Gabriele, Palmas & Wakelin, 1984) may be rejected and others (*H. microstoma*, see Howard,

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Christie, Wakelin, Wilson & Behnke, 1978), may have their growth severely impaired. None of these show specific immunological cross immunity with *T. spiralis* and their loss from the host is brought about solely through the non-specific effectors of the inflammatory response initiated by *T. spiralis* and the resultant changes in the intestinal environment.

Not all nematodes parasitizing the intestines of vertebrates elicit acute inflammatory responses of the sort associated with the rejection of *T. spiralis* and *N. brasiliensis*. Some cause long-term chronic infections in which there is little/no evidence of host-protective immunity. For example, *Trichostrongylus tenuis* accumulates in wild grouse and survives for over 600 days (Shaw & Moss, 1989), *Necator americanus* lives for up to 17 years in man (Palmer, 1955; Beaver, 1988) and *Heligmosomoides polygyrus* (*Nematospiroides dubius*) causes infections which last for 8–10 months in mice (Robinson, Wahid, Behnke & Gilbert, 1989). Little is known about the survival strategies of the former species, but *H. polygyrus* has been intensively investigated and it is generally accepted that this species is immunomodulatory, downregulating intestinal immunity to enable its own survival (Behnke, 1987). One of the indicators of intestinal inflammation, the mast cell response, is hardly detectable in mice carrying *H. polygyrus* and even in mice concurrently infected with *T. spiralis* mastocytosis is greatly depressed (Dehlawi, Wakelin & Behnke, 1987). This in turn is associated with prolonged infection with *T. spiralis* (Behnke, Wakelin & Wilson, 1978) indicating that *H. polygyrus* not only downregulates the inflammatory response in relation to its own survival but that the mechanism it invokes acts non-specifically and that it can benefit unrelated species resident in concurrently infected animals (Behnke, 1987; Christensen *et al.*, 1987).

The effect of *H. polygyrus* in prolonging concurrent infections with heterologous species of parasites is well documented (Behnke, 1987; Christensen *et al.*, 1987; Cabaj, 1989). However, the effect of an intense inflammatory response on *H. polygyrus* has never been studied in detail, although the worms are known to be resistant to free oxygen radical damage *in vitro* (Smith & Bryant, 1986). Furthermore, in comparison with nematodes causing acute infections, *H. polygyrus* have relatively high levels of oxygen radical scavenging enzymes and it has been suggested that in consequence the worms are more resilient to host-generated free oxygen radical-mediated damage, which may be a component of the non-specifically acting effectors of intestinal expulsion (Smith & Bryant, 1986). Thus in addition to showing immunomodulatory properties *H. polygyrus* may have a second line of defence to safeguard their survival in the host.

In the present paper we report the results of experiments which extend our earlier studies but this time examine the effect of the intestinal inflammatory response elicited by *T. spiralis* on concurrent infection with *H. polygyrus*. *T. pseudospiralis*, which is reputedly less pathogenic and causes longer lasting infections in mice than *T. spiralis* but also elicits an acute intestinal response (Przyjalkowski, Starzynski, Pykalo & Cabaj, 1981; Przyjalkowski & Pykalo, 1988; Stewart, Wood & Boley, 1985; Stewart, Mann, Ubelaker, McCarthy & Wood, 1988), was exploited as a further source of intestinal inflammation. The experiments were designed to minimize the possible immunodepressive influence of *H. polygyrus*, by keeping infections with the latter species low, worm burdens being just sufficient to allow any reductions to be recognized and analysed statistically, but not high enough to cause a major downregulation of the intestinal response to *Trichinella* spp. In contrast *Trichinella* infections were kept high to ensure maximum establishment and the induction of the host protective intestinal response. It is shown conclusively that adult *H. polygyrus* are lost when the parasites are caught in an inflammatory response which they have been unable to prevent. However, the same experiments again emphasize the tremendous resilience of this parasite, since expulsion of *H. polygyrus* was never complete and often only minimal, supporting the existence of a second line of defence against host immunity, as suggested by Smith & Bryant (1986).

MATERIALS AND METHODS

Mice. The mice used in this study were purchased from Harlan Olac Ltd and were bred in the departmental animal house. Unless otherwise stated, male mice were used throughout. All animals were housed under conventional animal house conditions with access to food and water *ad libitum*.

Parasite. T. spiralis was originally obtained in 1975 from the Wellcome Research Laboratories (Beckenham, Kent) since when it has been passaged regularly in CFLP mice. Our stain corresponds to *H. polygyrus bakeri* as reported by Durette-Desset, Kinsella & Forester (1972). The methods used for maintenance and infection have been reported previously (Jenkins & Behnke, 1977). Adult parasites were recovered by a 6 h incubation at 37°C of small intestines, suspended in a gauze, in 50 ml beakers containing Hanks's saline as described. Faecal egg counts were carried out by the method of Behnke & Parish (1979). The strain of *T. spiralis* and the methods used for the infection of mice and recovery of worms have been described previously (Wakelin & Lloyd, 1976). *T. pseudospiralis* was obtained from the Polish Academy of Sciences and was maintained identically to *T. spiralis*.

Statistical analysis of results. The results are presented as group mean values (*MWR*) ± standard error (S.E.M.). Non-parametric statistical procedures were used to analyse the data sets, because of small sizes (Sokal & Rohlf, 1969). When more than two groups required comparison at a single time

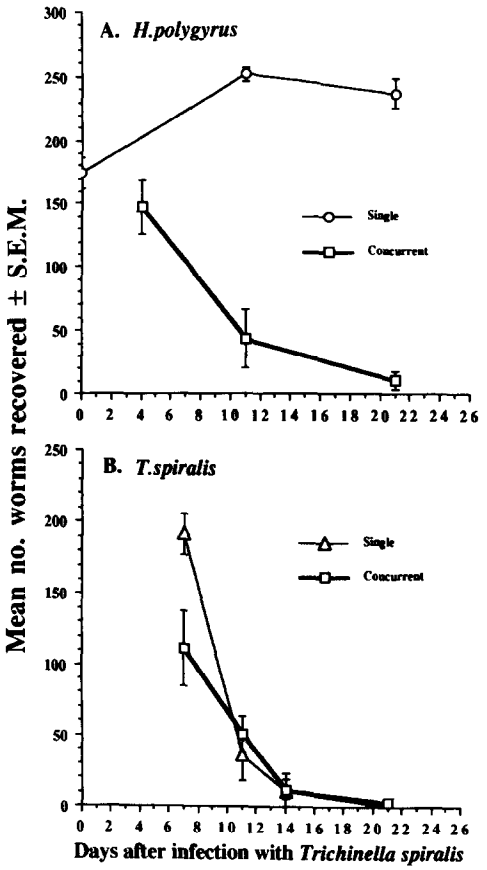

FIG. 1. Loss of adult *H. polygyrus* during the intestinal inflammatory phase of the response to concurrent *T. spiralis* infection (Expt 1). Three groups of male NIH mice were infected with 250 L3 of *H. polygyrus* (single infection) 14 days earlier (day −14), 330 muscle larvae of *T. spiralis* (single infection) on day 0 or both species (concurrent infection) and were killed for worm recovery on the days shown (n = 5 on each occasion).

point the Kruskal–Wallis statistic H was calculated to determine whether there was a significant treatment effect. 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 are presented as follows: * $P = 0.05$; ** $0.05 > P \geqslant 0.02$; *** $0.02 > P \geqslant 0.01$; **** $0.01 > P \geqslant 0.001$; ***** $P < 0.001$.

RESULTS

Expulsion of adult H. polygyrus *following superimposed challenge with* Trichinella spiralis (*Expt* 1)

A preliminary experiment was carried out in which NIH mice were infected with *H. polygyrus*, *T. spiralis* or with both species so that *T. spiralis* was superimposed on an existing adult population of *H. polygyrus* in the concurrently infected groups. It can be seen from the data which are summarized in Fig. 1 that the majority of *T. spiralis* were expelled at exactly the same time by mice carrying single and concurrent infections, although worms persisted longer in concurrently infected mice with 2.8 ± 3.8 being recovered on day 21 compared with none in the single infection group (*T. spiralis* only). It is also evident from Fig. 1 that whilst *H. polygyrus* persisted without loss to day 21 post-challenge in mice infected only with this species, there was a considerable loss of worms from concurrently infected animals with an 82.9 and 95.6% reduction by days 11 and 21, respectively.

Relationship between intensity of T. spiralis (*Expts* 2–5) *and* T. pseudospiralis (*Expts* 6–7) *infections and the proportion of* H. polygyrus *lost during intestinal inflammation*

Four separate experiments were carried out to ascertain the importance of infection intensity with *T. spiralis* on the proportion of adult *H. polygyrus* lost during the ensuing inflammatory response in concurrently infected mice. All the experiments were carried out in NIH mice, each included control groups to monitor the establishment of *T. spiralis* in single and concurrent infection groups. In each, *H. polygyrus* was given 14 days before *T. spiralis* and the mice were killed 3 weeks after the latter infection. The results are summarized in Fig. 2A, in which the mean worm burdens recovered at autopsy are expressed as a percentage of the single infection control group (*H. polygyrus* only). Rank order correlation coefficients were also calculated and these are presented in Table 1.

TABLE 1—STATISTICAL ANALYSIS OF THE RELATIONSHIP BETWEEN THE INTENSITY OF *T. spiralis* AND *T. pseudospiralis* INFECTION AND THE PROPORTION OF *H. polygyrus* SURVIVING THE INTESTINAL INFLAMMATORY RESPONSE IN CONCURRENTLY INFECTED MICE

Experiment	Number of mice	r_s	P
Effect of *T. spiralis*			
2	40	−0.792	<0.001
3	17	−0.777	<0.001
4	30	−0.919	<0.001
5	30	−0.718	<0.001
Effect of *T. pseudospiralis*			
6	25	−0.815	<0.001
7	41	−0.872	<0.001

Spearman Rank-Order Correlation Coefficients were calculated using standard procedures and probabilities were read from tables.

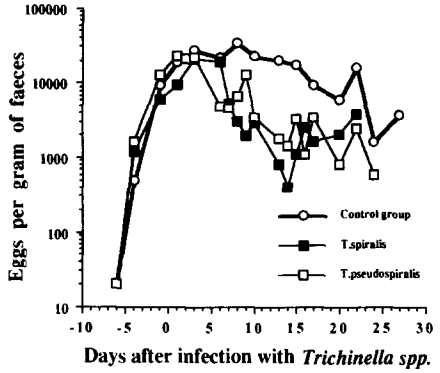

FIG. 3. The effect of the inflammatory response generated by *Trichinella* on the fecundity of *H. polygyrus* in concurrently infected mice (Expts 5 and 7). These experiments were carried out simultaneously and were based on the same batch of mice and the same infective larvae of *H. polygyrus*. The establishment of *T. spiralis* and *T. pseudospiralis* varied significantly following administration of identical doses of infective larvae. The figure therefore compares faecal egg counts in groups of concurrently infected mice (n = 6) in which establishment of *Trichinella spiralis* was 667.0 ± 20 and *T. pseudospiralis* 465 ± 10. The control group (n = 6) was the same for both experiments.

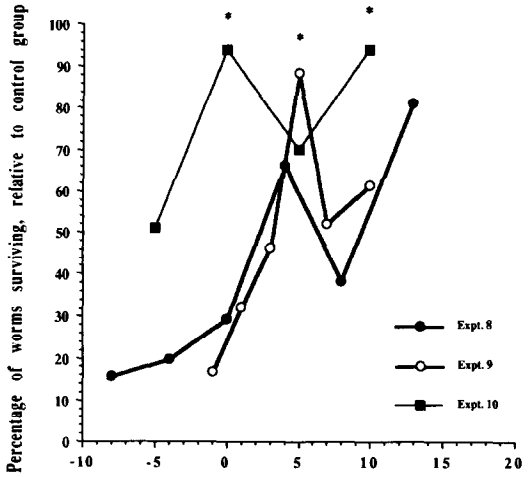

FIG. 4. The effect of varying the time interval between infections and the sequence of infections with *H. polygyrus* and *T. spiralis* on the survival of *H. polygyrus* in concurrently infected mice (Expts 8–10). Three experiments were carried out in male NIH mice; 250 (Expts 9 and 10) or 400 muscle larvae (Expts 8) of *T. spiralis* were administered on day 0. Groups of five to six mice were infected with 100 L3 of *H. polygyrus* before, on the same day or after *T. spiralis* on the days shown and on each occasion a group of naive control mice was also given *H. polygyrus*. All the mice were killed for worm counts on day 28 post-*T. spiralis* infection. The establishment of *T. spiralis* in single infection mice killed before expulsion (days, 3, 6 and 6, respectively) was as follows: Expt 8, 247.3 ± 19.6; Expt 9, 169 ± 17; Expt 10, 204.3 ± 16.3. The mean establishment of *H. polygyrus* in single infection control mice ranged from 73.2 to 80.7% (Expt 8), 80.5 to 91.8% (Expt 9) and 77.6 to 100% (Expt 10) of the inoculum administered. Statistical analysis: *MWR*s from concurrently infected groups were compared with their respective single infection control group by the Mann–Whitney U test and points marked* were not significantly different from control groups ($P > 0.05$).

declined. The reduction in eggs per gram of faeces corresponded to the onset of the inflammatory response to *Trichinella*.

Consequence of varying the interval between and order of infection with H. polygyrus *and* T. spiralis (*Expts* 8–11)

All of the experiments described so far were based on a primary infection with *H. polygyrus* followed 14 days later by challenge with *T. spiralis* or *T. pseudospiralis*. The inflammatory response generated by *Trichinella* spp. will therefore have coincided in all cases with the presence of adult *H. polygyrus*. It was necessary to extend these observations to encompass the effect of inflammation on the larval stages of *H. polygyrus*. Four experiments were carried out in which the sequence of primary/challenge infection and the interval between primary and challenge were varied. The results from three such experiments are summarized in Fig. 4.

The trend is very similar in all three experiments, with almost identical results for two (Expts 8 and 9) and a slightly earlier sequence of events but a similarly shaped graph for Expt 10. The most impressive effect was always when *H. polygyrus* was administered well before *T. spiralis* such that the inflammatory response coincided with the presence of adult worms. As the interval between primary infection with *H. polygyrus* and challenge with *T. spiralis* was reduced, so the proportion of *H. polygyrus* surviving increased. When *H. polygyrus* was given 5 days after *T. spiralis* (Expts 8 and 9) or on the day of *T. spiralis* infection (Expt 10) relatively few worms were lost. However, as the interval between a primary *T. spiralis* infection and a challenge with *H. polygyrus* increased, so the proportion of worms surviving again decreased. Finally when the interval was 13 days (Expt 8), there was again relatively little loss of *H. polygyrus*.

A fourth experiment (Expt 11) examined the effect of administering the two species at the same time (day 0) and this confirmed the data from Expts 8 and 9, indicating that only 26% of the worms survived to day 21 (*MWR* control group = 114.8 ± 13.4, n = 5; *MWR* concurrently infected group = 29.8 ± 9.3, n = 5).

TABLE 2—COMPARISON OF THE EFFECT OF PRIMARY AND SECONDARY RESPONSES TO *Trichinella spiralis* ON *Heligmosomoides polygyrus* (EXPERIMENT 12)

Treatment			*n*	Mean no. worms recovered ± S.E.M. on day 7	
Primary † *T. spiralis*	*H. polygyrus*‡	Secondary § *T. spiralis*		*H. polygyrus*‖	*T. spiralis*¶
No	Yes	No	6	74.3 ± 1.8	—
Yes	Yes	No	8	77.8 ± 1.8	—
No	Yes	Yes	6	29.3 ± 6.0****	111.5 ± 19.8
Yes	Yes	Yes	8	17.6 ± 4.2****	2.4 ± 1.5
No	No	Yes	4	—	170.5 ± 22.5
Yes	No	Yes	5	—	12.6 ± 12.6

All the mice were male NIH.

† 440 larvae of *T. spiralis* were given on day −34.

‡ 100 larvae of *H. polygyrus* were administered on day −14 and all the mice were killed on day 7, i.e. 7 days after challenge with *T. spiralis*.

§ 300 larvae of *T. spiralis* were given on day 0.

Statistical analysis of results:

‖ For *H. polygyrus* $H = 21.706$, $P < 0.001$.

¶ For *T. spiralis* $H = 16.83$, $P < 0.001$.

Additional comparisons were carried out with the Mann–Whitney *U* test using the values for control mice infected only with *H. polygyrus* as the reference group.

****$0.01 > P \geqslant 0.001$.

Comparison of the effect on H. polygyrus *of primary and secondary responses to* T. spiralis (*Expts* 12–14)

The secondary response to *T. spiralis* occurs earlier in immune NIH mice and is associated with more intense inflammation of the intestine than takes place during the primary response. If the principal reason for loss of *H. polygyrus* in concurrently infected mice is through the non-specifically acting mediators of the inflammatory response specifically elicited by *T. spiralis*, secondary responses to *T. spiralis* occurring in concurrently infected mice would be expected to exert a significantly more potent effect against *H. polygyrus*. This prediction was tested in three experiments. All were carried out in male NIH mice. The basic design involved four groups of mice to control for possible specific as well as non-specific interactions. Thus each experiment had one group of mice which received only *H. polygyrus* (day −14) and therefore controlled for infectivity of this species (infectivity control). A second group received a primary infection with *T. spiralis* (days −34 or −35) and a second with *H. polygyrus* (day −14) to control for possible cross immunity between the species as well as residual activity persisting after expulsion of the primary *T. spiralis* infection (cross immunity control). A third group received a primary infection with *H. polygyrus* (day −14) followed by challenge with *T. spiralis* (day 0) as in earlier experiments. The important experimental group comprised mice which received a primary infection with *T. spiralis* (day −34 or −35), followed by a second infection with *H. polygyrus* (day −14) and a third infection with *T. spiralis* (day 0). Additional control groups monitored the infectivity of *T. spiralis* in both infections and the expression of acquired resistance in the absence of concurrent infection.

The results from Expt 12 are summarized in Table 2. It is quite clear that there was no residual activity persisting from the primary *T. spiralis* and no cross immunity between the species, since the establishment of *H. polygyrus* was not impaired when *T. spiralis* was given 3 weeks earlier. However, both of the *H. polygyrus* infected groups challenged with *T. spiralis* on day 0 had significantly fewer *H. polygyrus* on day 7 and the *MWR* was lower in mice which had been primed with *T. spiralis*. As can be seen, control groups indicated that the primary *T. spiralis* infection had sensitized the mice and an accelerated expulsion of *T. spiralis* ensued when both single-species and concurrently infected mice were subjected to homologous challenge.

A second experiment (Expt 13) also monitored changes in both species on two occasions following challenge with *T. spiralis* (days 5, *H. polygyrus*, $H = 4.718$, $P =$ not significant and 28, *H. polygyrus*, $H = 7.463$, $P = 0.024$). Control groups again established that the mice receiving the primary infection with *T. spiralis* on day −35 developed strong

immunity to challenge administered on day 0. *H. polygyrus* established unimpaired in mice which had received *T. spiralis* on day −35 and were challenged with *H. polygyrus* 3 weeks later (day −14). On day +5 (i.e. day 19 of the *H. polygyrus* infection) these mice had 84.3 ± 4.3 worms ($n = 6$) and on day +28 (42 days after infection with *H. polygyrus*) 80.3 ± 6.6 ($n = 6$). The *T. spiralis* infection given on day 0 comprised 250 muscle larvae of which 144.5 ± 4.1 established and on this occasion, by day 5, there was no effect on *H. polygyrus* in mice which had not been primed with *T. spiralis* ($MWR = 83.3 \pm 2.7$, $n = 6$) although a small reduction was evident by day 28 ($MWR = 72.3 \pm 6.7$, not significant). However, mice primed with *T. spiralis* on day −35, challenged with *H. polygyrus* on day −14 and then with *T. spiralis* on day 0 only had 57.5 ± 9.8 *H. polygyrus* on day 5 and 43.8 ± 7.9 on day 28 ($P = 0.008$).

These two experiments were repeated on a larger scale with groups of mice from all the above treatments killed at regular intervals throughout the secondary infection with *T. spiralis*. The results are shown in Fig. 5. The data confirm and extend both of the above experiments and show that secondary responses to *T. spiralis* which take place in concurrently infected mice exert a rapid and potent effect against *H. polygyrus* causing earlier loss of worms than when a primary *T. spiralis* infection is superimposed on existing adult *H. polygyrus*.

Comparison of the effect on H. polygyrus *of primary responses to* T. spiralis *in fast and slow responder mouse strains (Expts* 15–17)

The final series of experiments exploited the known difference in the time of onset and subsequent intensity of the intestinal inflammatory response to *T. spiralis* between fast (NIH) and slow (C57BL/10) responder strains (Wakelin, 1978), in a further attempt to link the loss of *H. polygyrus* from concurrently infected mice to the acute inflammatory response elicited by *T. spiralis*. It would be anticipated that concurrently infected NIH mice would lose a greater proportion of *H. polygyrus* and the loss would occur earlier than in C57BL/10 mice.

The predictions of the hypothesis were tested in three experiments, two of which are summarized in Table 3 (Expts 15 and 16). In Expt 15, a single infection dose of *T. spiralis* was used throughout, whereas in Expt 16, groups of concurrently infected mice were challenged with one of two doses as shown. There was also a difference in establishment as shown by control groups infected only with *T. spiralis* and killed before expulsion. Thus in Expt 15 establishment of *T. spiralis* was relatively poor, but despite this a significant reduction in the *H. polygyrus* worm burden was

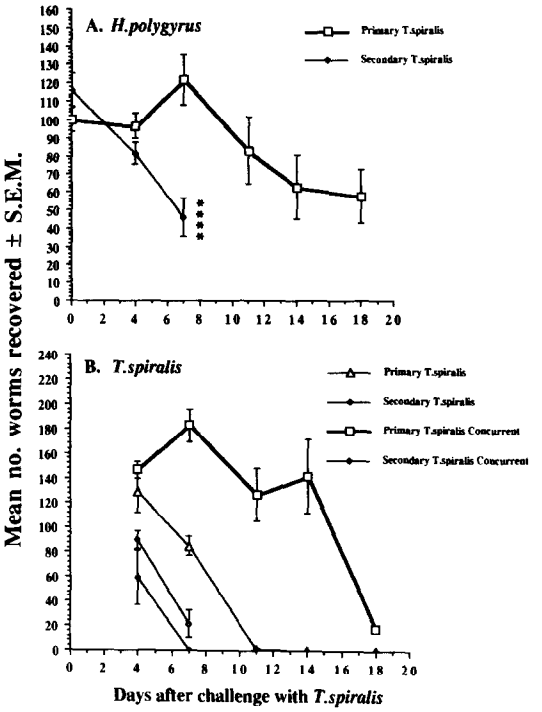

FIG. 5. Comparison of the effect on *H. polygyrus* of primary and secondary responses to *T. spiralis* (Expt 14). A. Groups of male NIH mice received a primary infection with 320 muscle larvae on day −35, followed by 100 L3 of *H. polygyrus* on day −14 and 360 muscle larvae of *T. spiralis* on day 0 and were killed on the days shown (n = 9–10). One group of mice received only *H. polygyrus* and were killed on day 0 to monitor infectivity of the *H. polygyrus* inoculum (represented on the figure as primary infection day 0, n = 9). Another group received the primary *T. spiralis* infection (day −35), followed by *H. polygyrus* on day −14 but was not challenged with *T. spiralis*, to monitor cross immunity between the species (represented on the figure as secondary *T. spiralis*, day 0, n = 10). B. Additional control groups monitored the course of primary and secondary infections with *T. spiralis* in the presence (n = 9–10 on each occasion) and absence (n = 4 on each occasion) of *H. polygyrus*. Statistical analysis of results: The *MWR*s were compared with control groups killed on the same day. For *T. spiralis* day 4, $H = 16.761$, $P = 0.001$ and day 8, $H = 16.97$, $P = 0.001$. Additional comparisons were made using the Mann–Whitney U test and for the group marked **** $0.01 > P \geqslant 0.001$.

observed by day 28 post-challenge with *T. spiralis* in concurrently infected mice of both strains. However, proportionally the loss was marginally greater in NIH relative to C57BL/10 mice (33.9% vs 28.3%). When *T. spiralis* infections were more intense (Expt 16), significant loss of *H. polygyrus* was detected as early as 11 days post-challenge confirming earlier data (Fig. 1). Proportionally the loss was again greater in NIH mice.

TABLE 3—EXPULSION OF *Heligmosomoides polygyrus* FROM FAST AND SLOW RESPONDER STRAINS CONCURRENTLY INFECTED WITH *Trichinella spiralis*

Strain	Infection ‡ with *T. spiralis*	Mean no of *H. polygyrus* † recovered ± S.E.M. on days after infection with *T. spiralis* (*n*) Expt 15 Day 0	Expt 15 Day 28	Expt 16 Day 11	Expt 16 Day 30
NIH	None	91.5 ± 6.6(6)	112.0 ± 4.6(6)	87.5 ± 2.8(6)	81.2 ± 3.1(12)
NIH	400 larvae	—	**** 74.0 ± 8.0(9)	***** 37.0 ± 5.4(12)	***** 5.7 ± 2.9(6)
NIH	750 larvae	—		**** 51.4 ± 8.7(11)	***** 16.8 ± 6.3(5)
C57BL/10	None	89.0 ± 11.1(5)	88.8 ± 4.1(5)	74.8 ± 5.1(6)	71.9 ± 3.8(12)
C57BL/10	400 larvae	—	**** 63.7 ± 3.1(6)	78.4 ± 2.9(11)	**** 35.5 ± 12.7(6)
C57BL/10	750 larvae	—		**** 56.2 ± 5.8(11)	***** 32.8 ± 6.2(6)

†All the mice were infected with 120 L3 of *H. polygyrus* on day −14 (Expt 15) or with 100 L3 on day −20 (Expt 16).

‡Mice infected with both species of parasites were given the dose of muscle larvae shown, on day 0. Additional control groups were included to monitor the establishment of *T. spiralis* ($n = 3$ in all cases) and the following parasite burdens were recovered:

Expt 15 (day 5), NIH = 121.3 ± 23.2 and C57BL/10 = 111.3 ± 14.8.

Expt 16 (day 4), 400 *T. spiralis* larvae, NIH = 230.3 ± 5.8 and C57BL/10 = 247.3 ± 42.8.

750 *T. spiralis* larvae, NIH = 457.7 ± 6.4 and C57BL/10 = 442.7 ± 29.4.

Statistical analysis of results:

Expt 16, day 11, for NIH mice $H = 12.805$ ($P = 0.002$); for C57BL/10, $H = 9.881$ ($P = 0.007$).

Day 30, for NIH mice $H = 17.245$ ($P < 0.001$); for C57BL/10, $H = 10.707$ ($P = 0.005$).

Additional comparisons were made using the Mann–Whitney *U* test and the single species infection (*H. polygyrus* only) control group for reference.

****$0.01 > P \geqslant 0.001$, *****$P < 0.001$.

By day 30 the differences between the two strains were even further exaggerated with a 93 and 79.3% reduction in *H. polygyrus* in NIH mice compared with the singly infected control group, but a loss of only 50.6 and 54.4% in C57BL/10 mice.

These findings were confirmed in a larger experiment which followed the time course of events more precisely in both mouse strains and the results are summarized in Figs. 6 and 7. Again it is quite evident that *H. polygyrus* burdens already began to decline by day 10 in the fast responder NIH mice, at a time when *T. spiralis* had not yet been lost (Fig. 6c). Then, as *T. spiralis* was expelled, so a major proportion of *H. polygyrus* was also lost. In C57BL/10 mice, inflammation generated by *T. spiralis* was slower to appear and correspondingly loss of both parasites in concurrently infected animals and *T. spiralis* in single infection control groups was delayed. As in the earlier experiments (Expts 15 and 16) the loss from NIH mice was proportionally greater (day 20, NIH = 79.5%, C57BL/10 = 21.3%). The difference in the onset and intensity of intestinal inflammation between the strains was also reflected in the effects it had on the fecundity of *H. polygyrus* in concurrently infected mice. Figure 7 shows that faecal egg counts declined in concurrently infected NIH mice as early as day 5 after challenge with *T. spiralis* whereas in C57BL/10 mice no appreciable reduction was evident before day 14 post-challenge.

The effect of H. polygyrus *on the expulsion of* T. spiralis *from concurrently infected mice*

Although the primary purpose of this study was to examine the effect of the intestinal inflammation elicited by *T. spiralis* on the survival of *H. polygyrus* in concurrent infections, control groups were included in all experiments to monitor the infectivity of *T. spiralis* and to confirm that expulsion took place at the expected times. In concurrently infected mice, both *H. polygyrus* and *T. spiralis* were counted at autopsy, enabling a comparison of *T. spiralis MWRs* between single and concurrent infection groups. In general the experiments were designed so as to minimize the influence of the known immunomodulatory effects of

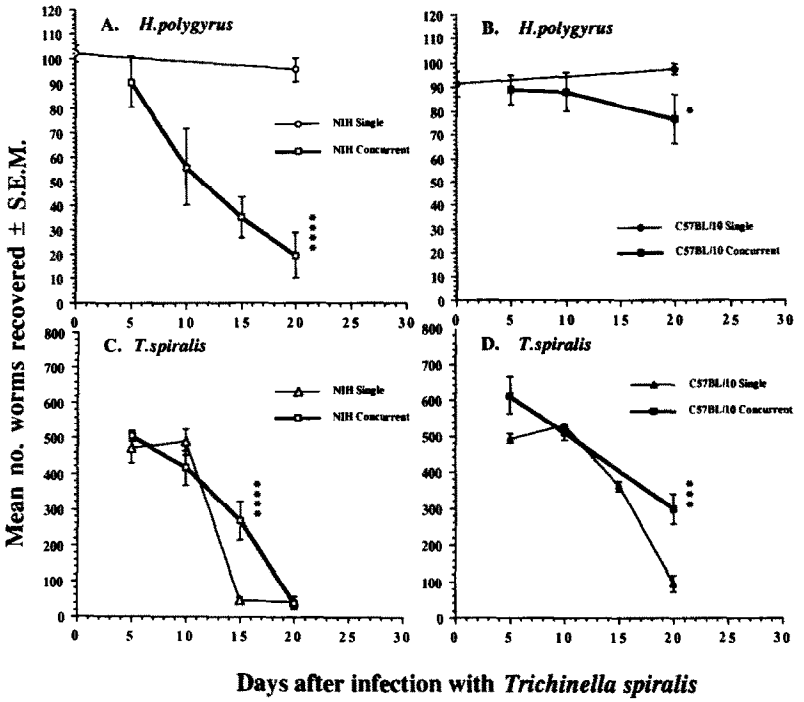

FIG. 6. Comparison of the effect on *H. polygyrus* of primary responses to *T. spiralis* in fast and slow responder mouse strains (Expt 17). A,B. Groups of male NIH or C57BL/10 mice were infected with 100 L3 of *H. polygyrus* on day −14, challenged with 600 muscle larvae of *T. spiralis* on day 0 and were killed on the days shown (n = 6–7) for worm counts. C,D. Additional control groups from both strains monitored the course of primary infection with *T. spiralis* in the presence (n = 6–7 on each occasion) and absence (n = 3 on each occasion) of *H. polygyrus*. Statistical analysis of results: Comparisons of *MWR*s between groups within strains killed on the same day were made using the Mann–Whitney U test and for the groups marked: * $P = 0.05$; *** $0.02 P \geqslant 0.01$; **** $0.01 > P \geqslant 0.001$.

H. polygyrus (Behnke *et al.*, 1978) and therefore, *H. polygyrus* infections were kept low (100–150 larvae in most experiments, although some utilized higher doses, e.g. Expt 1). Only some of the data are shown. Thus Fig. 1 shows that during Expt 1, there was no major effect on the expulsion of *T. spiralis*, although a few larvae did persist longer in the concurrent infection group. However, Figs. 5 and 6 show quite clearly that *T. spiralis* did persist longer in concurrently infected animals despite the low doses of *H. polygyrus* which were used on those occasions. In most of the remaining experiments for which we do not present quantitative data, *T. spiralis* and *T. pseudospiralis* persisted longer in the concurrent infection groups compared with mice infected only with *Trichinella* spp.

DISCUSSION

Despite the inability of most mouse strains to expel primary worm burdens of *H. polygyrus* as rapidly as *T. spiralis*, the experiments reported in this paper clearly establish that two such strains (NIH and C57BL/10) have the capacity to mount an intestinal inflammatory response of sufficient intensity to cause the expulsion of many, in some experiments the majority, of *H. polygyrus*. Thus the important conclusion from our experiments is that these mouse strains have the components necessary to mount an intestinal inflammatory response capable of driving the adult worms out but normally fail to express the response and in consequence tolerate chronic primary infections with *H. polygyrus*. This conclusion again focuses attention on the importance of the immunomodulatory strategy utilized by adult *H. polygyrus* in avoiding host immunity and it emphasizes that this strategy is essential to the parasite because *H. polygyrus* is susceptible to the non-specifically acting effectors of inflammatory responses which it has failed to prevent.

Our experiments defined the conditions under which expulsion of *H. polygyrus* could occur. The intensity of the concurrent *T. spiralis* infection was an important factor, because although some loss occurred when a low intensity *T. spiralis* infection was used, this was not always predictable and consistent effects

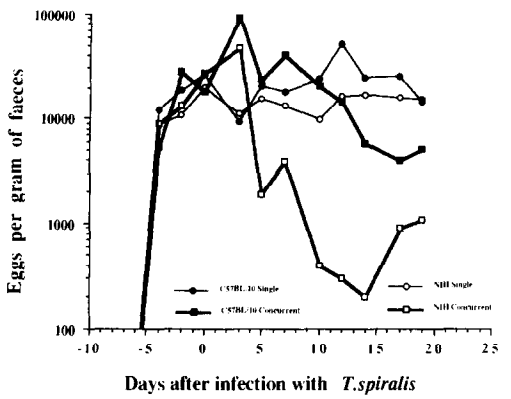

FIG. 7. Faecal egg counts (*H. polygyrus*) during concurrent primary infections with *T. spiralis* in fast and slow responder mouse strains (Expt 17). For details of this experiment and explanation of the key denoting groups see legend to Fig. 6.

were observed only when the higher intensity infections were employed. There was a significant negative correlation between the proportion of *H. polygyrus* surviving the inflammatory phase of the *T. spiralis* challenge and the dose of *T. spiralis* administered, although this relationship was not linear as seen in Fig. 2. We explored also the effect of *T. pseudospiralis* which is reputed to be less pathogenic than *T. spiralis* (Przyjalkowski *et al.*, 1981; Przyjalkowski & Pykalo, 1988) and to persist for longer after primary infection. However, NIH mice expel both species at about the same time and our experiments suggest that the inflammatory effect generated by both *Trichinella* spp. is comparably detrimental to the survival of *H. polygyrus* in concurrently infected animals.

One of the interesting observations was the variation in the proportion of *H. polygyrus* eliminated when the relative timing of infection with the two species was varied. The proportion of *H. polygyrus* expelled declined as larvae were given nearer to the time of infection with *T. spiralis*. This reduction can be explained by the position of its larval stages. When mice were infected with *H. polygyrus* on days 0–5, L3 would have penetrated deeply into their normal site of development in the *muscularis mucosa*, and would have been relatively secure from expulsion at the time of peak inflammation, i.e. days 5–10, emerging from this site only after their normal development had been completed, some 8–10 days later (Bryant, 1973; Sukhdeo, O'Grady & Hsu, 1984). By this time conditions in the gut lumen would have returned almost to normal. The subsequent decline in survival when *H. polygyrus* larvae were given after *T. spiralis* on days 5–10 is explained by a failure to establish, since in these animals *H. polygyrus* L3 larvae would have been attempting to exsheath and penetrate the intestinal mucosa at the peak of the inflammatory phase. Finally the second decline in the proportion of *H. polygyrus* being eliminated, occurring when larvae were given on days 11–15, is explained by the less inflamed environment following the expulsion of *T. spiralis*.

The final two series of experiments exploited situations in which the inflammatory response to *T. spiralis* was known to be altered in time. Thus mice experiencing a secondary *T. spiralis* infection mount a vigorous rapid inflammatory response (Wakelin & Lloyd, 1976; Alizadeh & Wakelin, 1982). In contrast poor responder mice such as C57BL/10 reject *T. spiralis* more slowly, later and with less vigour than fast responders such as NIH (Wakelin, 1980). The observations that adult *H. polygyrus* were lost earlier in NIH mice experiencing a secondary response to *T. spiralis* and considerably later in C57BL/10 mice are in accordance with our hypothesis that it is the non-specific effectors of the inflammatory response which make life untenable for *H. polygyrus*.

One more approach could have been used to confirm the requirement for an active immune system and the expression of an inflammatory response but this was not available to us. Immunodepression of concurrently infected mice could have been exploited to demonstrate that in the absence of an inflammatory response both species can co-exist together. However, the combination of immunodepression and two relatively pathogenic intestinal parasites is more than mice can tolerate at the doses that are required. Small-scale exploratory pilot experiments convinced us that this approach was not one which we wished to pursue further.

The reciprocal interaction, i.e. the effect of *H. polygyrus* on the expulsion of *T. spiralis*, was not an objective of this work but nevertheless our data confirm that there is an immunodepressive influence of *H. polygyrus* which was detectable in some experiments despite out attempts to minimize the reciprocal interaction. The majority of experiments were carried out with low doses of *H. polygyrus*, large enough for a reduction in the worm burden to be detected but low enough not to cause a major impairment of the response to *T. spiralis*. Behnke *et al.* (1978) demonstrated that expulsion of *T. spiralis* was most consistently impaired when the infection intensity of *H. polygyrus* exceeded 200 adult worms and in most of these earlier experiments doses of 200–400 worms were used. However, in spite of our precautions it was quite clear from some of the results we have presented here, as well as from the large amount of additional data arising from necessary control groups which we have not included, that the immunodepressive influence of *H. polygyrus* was still evident in concurrently infected

mice and that there was a degree of impairment of the response to *T. spiralis*. Nevertheless, *T. spiralis* were rejected and the response carried with it a proportion of *H. polygyrus*. However, it was equally interesting to observe that in most experiments some *H. polygyrus* managed to survive. Even when the infection intensity of *T. spiralis* was high and over 90% of *H. polygyrus* were lost, the remainder survived and continued to produce eggs as normal. Clearly, the intestinal effectors were not 100% effective against *H. polygyrus* even at the best of times demonstrating again the resilience and adaptability of this parasite in the face of inflammatory host protective responses.

In summary, our experiments have demonstrated unequivocally that mice can reject *H. polygyrus* through the same combination of intestinal inflammatory effectors which are normally invoked in response to *T. spiralis*. Most worms are susceptible to these effectors and when located in an inflamed intestine cannot escape from their damaging influence. However, a variable proportion of worms always survived even in immune mice responding to homologous challenge with *T. spiralis* in which intestinal inflammation was at its most intense. Chronic primary infections of *H. polygyrus* most likely maintain themselves by preventing host inflammatory effectors from being triggered in the first place and it is conceivable that in some mice aggregations of adult worms exerted sufficient local immunomodulatory influence to minimize the release of inflammatory mediators in the vicinity of the site occupied by the parasites (Behnke, 1978). Alternatively a subpopulation of worms may have been more resistant than the remainder even when immune effectors were triggered. This resilience in the face of host effectors could be attributable to the worm's relatively high free oxygen radical scavenging enzymes (Smith & Bryant, 1986) or to other, as yet undetermined qualities but either way it is likely that the parasite has a second line of defence against host immune responses.

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