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RESISTANCE OF THE HOOKWORMS *ANCYLOSTOMA CÉYLANICUM* AND *NECATOR AMERICANUS* TO INTESTINAL INFLAMMATORY RESPONSES INDUCED BY HETEROLOGOUS INFECTION

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Abstract—BEHNKE J. M., ROSE R. and LITTLE J. 1994. Resistance of the hookworms *Ancylostoma ceylanicum* and *Necator americanus* to intestinal inflammatory responses induced by heterologous infection. *International Journal for Parasitology* 24: 91–101. Experiments were carried out to ascertain whether the acute inflammatory phase of the intestinal response of hamsters to infection with *Trichinella spiralis* would adversely affect hookworms in concurrently infected animals. The survival and growth of hookworms were unaffected. However, the presence of hookworms reduced the establishment of *T. spiralis*, the initial growth of female worms and their fecundity. The expulsion of *T. spiralis* was also significantly slower in concurrently infected animals and there was significant depression of the serum IgG antibody response to muscle stage and adult worm antigens of *T. spiralis* in concurrently infected animals. These results are discussed in relation to the chronicity of human hookworm infections.

INDEX KEY WORDS: *Ancylostoma ceylanicum*, *Necator americanus*, *Trichinella spiralis*, nematoda; mouse; intestinal inflammation; immunity; evasion of immunity; resistance to inflammation; antibody response; growth.

INTRODUCTION

Human hookworms cause persistent chronic infections both after experimentally administered single pulse infections (Beaver, 1988) and during natural exposure in the field (Bundy, 1990). Moreover, there is little evidence from field studies to support a major role for acquired immunity in regulating human hookworms in communities continuously exposed to infection (Behnke, 1987a, 1991a), although there is good evidence that related hookworm species affecting canine hosts are controlled by host immune responses (Miller, 1971). The reasons for the chronicity of human hookworm infections are not understood but the possibility exists that human hookworms are resilient in the face of host effectors and cannot be eliminated by the intestinal responses generated in the human host.

Following specific activation of CD4+ lymphocytes by antigens liberated from nematodes resident in the intestine, the secretion of cytokines associated with Th-2 cells results in local infiltration of the mucosa by inflammatory cells and eventually in the generation of an environment in which worms cannot survive. The final effector mechanism is still not understood (Moqbel & MacDonald, 1990), but the inflammatory phase of the intestinal response is known to act non-specifically since unrelated parasites, concurrently resident in the intestine are also rejected (Kennedy 1980; Behnke, Cabaj & Wakelin, 1992; Behnke, Bland & Wakelin, 1977; Christie, Wakelin & Wilson, 1979; Ferretti, Gabriele, Palmas & Wakelin, 1984) or have their growth severely impaired (Howard, Christie, Wakelin, Wilson & Behnke, 1978).

In this paper we report on experiments in which we investigated the ability of the hookworms *Necator americanus* and *Ancylostoma ceylanicum* both of which cause patent infections in man (Behnke, 1990), to withstand the consequences of intestinal inflammation heterologously induced by *Trichinella spiralis* (see Rothwell, 1989). Our experiments were carried out in hamsters, which are susceptible to both species of hookworms and to *T. spiralis*, in order to determine whether hookworms would be expelled during the acute inflammatory phase of the response to *T. spiralis* or would survive a response which has severe consequences for other intestinal parasites.
MATERIALS AND METHODS

Parasites and hosts. Infective larvae of N. americanus were obtained in 1983 from Dr Rajasekariah of CIBA-GEIGY Hindustan Ltd, Bombay, India and the parasite has been maintained since by regular passage through hamsters as described originally by Sen (1972) and Behnke, Paul & Rajasekariah, 1986). It is important to note here that N. americanus will mature to patency only following exposure of neonatal (1–3 day old) hamsters to percutaneous infection (Rajasekariah, Deb, Dhage & Base, 1985). Experiments with N. americanus were therefore limited by the need for synchronized mating of female hamsters and by resulting litter sizes. A. ceylanicum was also obtained from Dr Rajasekariah and was passaged through adult hamsters using techniques which have been described by Garside & Behnke (1989). Larvae were administered orally to mature hamsters throughout this study. The strain of T. spiralis and the methods used for the infection of animals and recovery of worms have been described previously (Wakelin & Lloyd, 1976). Briefly, the small intestines were opened longitudinally and incubated for 6 h at 37°C, suspended in nylon mesh in beakers of Hanks’s saline, in a water bath. After this time the mesh was removed carefully and the contents either examined immediately or fixed by the addition of concen-
trated formaldehyde. Both the tissues contained in the mesh and the mesh were carefully examined for the presence of remaining hookworms and pieces which may have been cut at autopsy but in general the majority of worms migrated into the saline and were counted subsequently in Petri dishes under the low magnification of a binocular microscope. Assays of female worm fecundity were carried out as described by Wakelin & Wilson (1977). All the animals used in this work were syngeneic DSN hamsters originally purchased from Intersimian Ltd. Oxford, U.K., but now maintained, under conventional conditions with access to food and water ad libitum, as a closed breeding colony in the Department of Life Science at Nottingham University. Both sexes were used but, with the exception of experiment 5 involving N. americanus, the animals comprising any single experiment were of the same sex, as stated. Neither the establishment nor the course of infection with N. americanus and A. ceylanicum in hamsters differ between the sexes (Rose & Behnke, 1990; Garside & Behnke, 1989). Experimental groups were set up in separate cages 1–2 weeks before infection.

Preparation of antigens. Outbred CFLP mice were infected orally with T. spiralis and L1 antigen was obtained from muscle digests as described by Wakelin & Lloyd (1976). Adult worm homogenate was prepared from worms collected 7 days p.i. The parasites were washed 10 times in ice-cold sterile PBS and were homogenized in minimal volume of PBS using a glass tissue homogenizer held in an ice bath. The resulting suspension was centrifuged at 10,000 g for 1 h at 4°C to remove coarse particulate matter. The supernatant was filtered (0.22 μm filter, Millipore), analysed for protein concentration using a method modified from Lowry, Rosebrough, Farr & Randall (1951), aliquotted and stored at −40°C.

Measurement of antibody responses. Parasite specific IgG responses were measured by a standard ELISA. Briefly, ELISA microtitre plates were coated with 50 μl of worm antigen (5 mg ml−1). Alkaline phosphatase conjugated Protein A (sigma) was used to measure specific responses. After addition of the substrate (P-nitrophenylphosphatase) colour changes were read at 410 nm on a Dynatech MR700 Microplate Reader. Sera were assayed individually in triplicate at a dilution of 1 in 1000, after storage at −40°C.

Measurement of worms. All the worms recovered at autopsy were collected, fixed in formal 70% ethanol (37°C) and were kept in separate tubes until measured. They were drawn to scale using a camera lucida arrangement attached to a microscope. The drawings were then measured using a digitizer pad and an IBM computer with a program for conversion of lengths traced into discrete units (courtesy of Dr R. Ramsey). Ten worms of both sexes were measured from each hamster and a mean value for the length of male and female worms from individual hamsters was derived. These mean values were then used to calculate the overall group mean.

Mast cell counts. A 2 cm length of small intestine taken 10 cm from the pyloric sphincter was fixed in Carnoy’s fixative and processed using standard histological techniques. Sections cut at 5 μm were stained with Alcian Blue, counter-stained with Safranin O and mounted in DPX using the method of Alizadeh & Wakelin (1982) with the following modifications. Sections were stained for 25–30 minutes in Mayer’s haematoxylin, then for 20–25 minutes in phosphate buffered Safranin O before processing and mounting in DPX. Five non-adjacent sections were examined and in each the mast cells evident in 20 villus crypt units (VCU) were counted. Totals counted on each section were averaged to yield the number of mast cells/20VCUs for each animal and these values were used to calculate a mean value/20VCUs for pertinent experimental groups.

Statistical analysis of results. For clarity data are presented as group mean values ± s.e.m. Non-parametric statistical procedures were used to analyse the data sets, because normal distribution of data could not be assumed. When a difference was expected in a specific direction, a priori hypotheses were tested by the procedures described by Meddis (1984) and the test statistic z is given as appropriate. In the event of a hypothesis being rejected [P= Not significant (NS)], groups were compared by a general one-
way ANOVA in order to determine whether there were any significant differences between the groups and the statistic H as given. A two-way ANOVA (Meddis, 1984) was employed to assess the effects on growth of worms, specifying infection (concurrent vs single) and worm sex (male vs female) as the two factors. Relationships over time within experimental groups were examined by the Spearman Rank Order Correlation Test and the statistic r, is given. Probabilities (P) of 0.05 or less were considered significant but throughout exact probabilities are given. In certain cases, multiple analyses could not be avoided, and, where these were undertaken, the cut-off value for significance was lowered to
Establishment and course of infection with Trichinella spiralis in hamsters carrying mature A. ceylanicum (exp 2)

The second experiment was carried out to confirm the results of exp 1 and to determine whether the presence of A. ceylanicum would alter the subsequent course of infection with T. spiralis in hamsters. Twenty-five female hamsters, arranged in 5 groups \([n = 7 \text{ (A)}, 6 \text{ (B)}, 6 \text{ (C)}, 3 \text{ (G)} \text{ and } 3 \text{ (I)})\] were infected with 50 L3 of A. ceylanicum on day -15. On day 0 groups A, B and C were challenged with 500 muscle larvae of T. spiralis. A further 21 sex- and age-matched hamsters, arranged in three groups of 7 each (D, E and F), were also infected with the same dose of T. spiralis on day 0. Two additional groups (H and J, \(n = 3\) in each case) remained uninfected with either parasite throughout in order to provide sera from naive animals for antibody assays. The results are summarized in Figs. 1 and 2 and Table 2.

Following the results of exp 1, the a priori hypothesis that the presence of hookworms would impair the establishment of T. spiralis was examined in a specific test. The establishment of T. spiralis in hamsters carrying a patent A. ceylanicum infection (group A), as assessed by worm counts on day 7 p.i., was significantly reduced (20.6% reduction relative to control group (D), \(z = 1.86, n_1, n_2 = 7.7, P = 0.0314\)). Two weeks p.i., T. spiralis worm burdens were similar in single and concurrently infected animals (general test groups B vs E, \(H = 1.31, n_1, n_2 = 6.7, P = NS\)) but by day 21, the concurrently infected hamsters (group C) had more T. spiralis than those given T. spiralis alone (group F; \(H = 5.9, n_1, n_2 = 6.7, P = 0.015\)). As in exp 1 there was no overall significant effect of concurrent vs single infection on the size of worms on day 7 (specific prediction by two-way ANOVA, \(z = 1.61, P = NS\)) but comparison of mean worm lengths showed that male and female worms from the concurrently infected animals (group A) were 90.1 and 71.6% respectively of the length of those from the control group (D).

Analysis by a general test again indicated that male but not male worms from concurrently infected animals were smaller (general test \(H = 9.843, n_1, n_2 = 7.7, P = 0.002\; H = 1.894, n_1, n_2 = 7.7, P = NS\) relative to those of the control group (D). By day 14 the mean length of female worms from concurrently infected hamsters (group B) was 96.3% of that from controls (group E). However, male worms from group B were smaller than those from group E (92.9% of the length of group E males) and the difference was just significant (general test \(H = 4.067, n_1, n_2 = 6.7, P = 0.044\)).

In contrast A. ceylanicum were not affected by the host response to T. spiralis. There was no significant loss of worms from concurrently infected hamsters during the course of the experiment \(r_1 = -0.089, n = 19, P = NS\) and no difference in worm burdens...
| Group                | No. of hamsters | Mean no worms recovered ± S.E.M. | T. spiralis* Mean length ± S.E.M. (mm) | Fecundity† Mean No. worms recovered L 1/F/h ± S.E.M. | A. ceylanicum† Mean No. worms recovered ± S.E.M. |
|---------------------|----------------|----------------------------------|----------------------------------------|---------------------------------------------------|-----------------------------------------------|
| (A) A. ceylanicum + T. spiralis | 8              | 228.5 ± 11.8§                    | 2.88 ± 0.1                             | 3.76 ± 0.44                                       | 22.4 ± 4.7                                    |
| (B) T. spiralis alone | 10             | 281.1 ± 12.7§                    | 3.18 ± 0.29                             | 4.15 ± 0.08                                       | ---                                           |
| (C) A. ceylanicum alone | 5              |                                  |                                       |                                                   | 29.6 ± 6.6                                    |

* 500 larvae of T. spiralis were given on day 0 and all the animals were killed for worm counts on day 7.
† 60 larvae of A. ceylanicum were administered on day – 14, i.e. 2 weeks before challenge with T. spiralis.
‡ Fecundity is expressed as mean no. of larvae/female worm/h of incubation.
Statistical analysis of results: § H = 6.2 P = 0.013. For analysis of other groups see text.
Resistance of hookworms to inflammation

between concurrently infected animals and hamsters carrying A. ceylanicum alone on either day 7 or day 21 p.i.

Antibody responses to L1 and adult T. spiralis antigens were measured on day 21 p.i. with T. spiralis and the results are presented in Table 2. Sera taken earlier than day 21 did not show reactivity against these antigens. On day 21 p.i. all the hamsters infected with T. spiralis (groups C and F) responded against both antigens relative to uninfected controls. The response against adult worm antigens was significantly greater in hamsters with T. spiralis alone (specific test for group C vs F, \( z = 1.71, P = 0.044 \)) but there was no significant difference in the response to larval antigen (\( z = 0.43, P = \text{NS} \)).

Survival of an established infection with N. americanus during the acute phase of the host response to Trichinella spiralis (exp 5)

The experimental design employed in expts 3 and 4 was used to determine whether N. americanus was equally resistant to the non-specific effects of the inflammatory response elicited by T. spiralis. Litters of neonatal hamsters, including both sexes, were exposed percutaneously to 140 infective larvae of N. americanus when 2 days old and were challenged with T. spiralis 5 weeks later. One group of hamsters (\( n = 4 \)) was killed on the day of challenge, i.e. 5 weeks after exposure to N. americanus, to confirm establishment
| Group*                                                                 | Exp 2                                                                 | Mean optical density ± S.E.M. | Exp 6                                                                 |
|-----------------------------------------------------------------------|----------------------------------------------------------------------|-------------------------------|---------------------------------------------------------------------|
|                                                                       | A. ceylanicum                                                        |                               |                                                                     |
|                                                                       | given day - 15.                                                      |                               |                                                                     |
|                                                                       | Sera from day 21 p.i.                                               |                               |                                                                     |
|                                                                       | No. of hamsters | Adult worm antigen | L1 antigen             | No. of hamsters | Adult worm antigen | L1 antigen             | No. of hamsters | Adult worm antigen | L1 antigen             |
| A. ceylanicum                                                         | 6 | 0.238 ± 0.010†        | 0.266 ± 0.033            | 9 | 0.369 ± 0.021‡        | 0.472 ± 0.045§         | 9 | 0.422 ± 0.036||     | 0.411 ± 0.061**       |
| + T. spiralis                                                        | 7 | 0.278 ± 0.015†        | 0.264 ± 0.012            | 4 | 0.866 ± 0.380‡        | 0.763 ± 0.109§         | 4 | 0.587 ± 0.018||     | 0.687 ± 0.069**       |
| T. spiralis alone                                                    | 3 | 0.184 ± 0.006   | Nd                      | 7 | 0.020 ± 0.006   | 0.115 ± 0.002         | 7 | 0.050 ± 0.004   | 0.144 ± 0.007         |
| A. ceylanicum alone                                                  | 3 | 0.171 ± 0.006   | 0.145 ± 0.002         | – | Nd                     | Nd                      | – | Nd                     | Nd                      |
| Uninfected                                                            | 3 | 0.171 ± 0.006   | 0.145 ± 0.002         | – | Nd                     | Nd                      | – | Nd                     | Nd                      |

* For details see text. Nd = Not measured.

Statistical analysis: Groups with the same symbols have the following statistical values.

† z = 1.71, P = 0.0436.
‡ z = 2.47, P = 0.0068.
§ H = 4.67, P = 0.031.
|| z = 2.62, P = 0.004.
** H = 5.36, P = 0.021.
Resistance of hookworms to inflammation

Table 3—Survival of *A. ceylanicum* during the acute phase of the response to *T. spiralis*

| Group                              | Mean no. of worms recovered ± S.E.M. on days after infection with *T. spiralis* *n* | *A. ceylanicum* | *T. spiralis* |
|------------------------------------|---------------------------------------------------------------------------------------|-----------------|---------------|
| Experiment 3                       |                                                                                       |                 |               |
| *A. ceylanicum* only               | 0                                      | 28.3 ± 6.1      | 5             |
|                                    | 5                                      | 34.5 ± 2.8      | 21            |
|                                    | 21                                     | 23.2 ± 1.6      | 5             |
|                                    |                                         | 21              |               |
| *A. ceylanicum + T. spiralis*      | Nd                                     | 25.7 ± 4.9      | 28.5 ± 3.0    |
|                                    |                                         | 96.2 ± 15.6     |               |
|                                    | [6]                                    | [6]             | [3]           |
| *T. spiralis* only                 | –                                      | –               | 270.5         |
|                                    |                                         | 122.8 ± 13.8    | [4]           |
| **Experiment 4**                   |                                                                                       |                 |               |
| *A. ceylanicum* only               | 0                                      | 35.8 ± 9.4      | 7             |
|                                    | 7                                      | 22.8 ± 12.0     | 28            |
|                                    |                                         | 28              | 7             |
|                                    | [5]                                    | [5]             | [4]           |
| *A. ceylanicum + T. spiralis*      | Nd                                     | 37.5 ± 6.6      | 37.0 ± 9.5    |
|                                    |                                         | 104 ± 2.0       | 9.5 ± 4.3     |
|                                    | [4]                                    | [4]             | [4]           |
| *T. spiralis* only                 | –                                      | –               | 87.8 ± 10.4   |
|                                    |                                         | 43.4 ± 13.9     | [6]           |
|                                    | [5]                                    |                 |               |

In Exp 3 hamsters were given 47 L3 of *A. ceylanicum* on day −21 and were challenged 3 weeks later with 480 muscle larvae of *T. spiralis*.

In Exp 4 hamsters were given 95 L3 of *A. ceylanicum* on day −28 and were challenged 4 weeks later with 400 muscle larvae of *T. spiralis*.

of worms and 23.8 ± 6.1 worms were recovered. A second group (*n* = 5), not challenged with *T. spiralis*, was killed 21 days later to determine the survival of *N. americanus* in the absence of *T. spiralis*, and 38.4 ± 11.3 worms were recovered. The difference in recovery of worms was not statistically significant. Seven hamsters carrying *N. americanus* were challenged with *T. spiralis* (490 muscle larvae) and were killed for worm counts either 5 (*n* = 4) or 21 (*n* = 3) days later. Eight uninfected age-matched hamsters were infected only with *T. spiralis* and were killed on the same days [Day 5 (*n* = 4) and 21 (*n* = 4)]. Figure 3 summarizes the results. There was no impairment of the establishment of *T. spiralis*, nor was the duration of infection with *T. spiralis* prolonged significantly by the presence of *N. americanus* in concurrently infected animals. Despite some variation in the hookworm burdens, the survival of *N. americanus* to day 21 after challenge with *T. spiralis* was not impaired in concurrently infected animals, despite the loss of 92.5% of *T. spiralis* over this interval.

Establishment of *A. ceylanicum* during the acute phase of the host response to *T. spiralis* (Experiment 6)

Finally an experiment was carried out to determine whether the larval stages of *A. ceylanicum* are more susceptible to heterologously elicited intestinal inflammation. It was therefore necessary to infect animals first with *T. spiralis* and then to administer challenge inocula with *A. ceylanicum* at times adjudged to coincide with the onset of the acute expulsion phase of...
The experiment comprised 57 male hamsters which were divided into 10 groups (A–J). One group (J) of 4 hamsters was not infected, and provided sera from naive animals to control for antibody responses. One animal from this group was killed on each of days 8, 14, 21 and 35 relative to infection with *T. spiralis* in the other groups. Five groups of 4 hamsters each were infected with *T. spiralis* and were killed for worm counts 8 (group E), 14 (group F), 21 (group G), 28 (group H) and 35 (group I) days p.i. Samples of intestinal tissue were also processed for mast cell counts from these animals. Two of the remaining groups (B, *n* = 9 and D, *n* = 9) were infected with *T. spiralis* but subsequently challenged orally with 61 L3 of *A. ceylanicum* on days 8 (group B) or 14 (group D). Groups A (*n* = 7) and C (*n* = 8) were infected with *A. ceylanicum* on the same days on which groups B and D were given hookworms and acted as controls for establishment and survival of hookworms. Groups A and B were killed 28 days after infection with *T. spiralis* (day 20 following *A. ceylanicum*) and groups C and D on day 35 (day 21 following *A. ceylanicum*). Worm burdens are presented in Fig. 4, the effect on the growth of *A. ceylanicum* is shown in Table 4 and antibody responses are recorded in Table 2.

Eight days after infection with *T. spiralis* a mean of 147 worms were recovered representing 29.4% establishment (Fig. 4A). By day 21, 85% of the worms had been rejected with the greater loss occurring 14–21 days p.i. Loss of worms was accompanied by mastocytosis with mast cell counts increasing from 58.8 ± 13.9/20 VCU on day 8 p.i. to 143.5 ± 13.44 on day 21 and 154.0 ± 2.8/20 VCU on day 35. There was a highly significant positive correlation between mast cell density and time after infection (*r* = 0.803, *n* = 12, *P* = 0.002). Concurrently infected animals did not harbour more worms than hamsters infected only with *T. spiralis* on day 28 (group B, 8.2 ± 1.2, specific test *z* = -1.55, *P* = NS) nor on day 35 (group D, 14.4 ± 3.9, specific test *z* = 0.62, *P* = NS).

The establishment of *A. ceylanicum* was marginally different on the two occasions when larvae were given with 54% (group A) and 70% (group C) as assessed by worm recoveries from control hamsters on days 28 and 35 p.i. with *T. spiralis*, respectively. However, *A. ceylanicum* established and survived in the concurrently infected animals (groups B and D) without significant loss of worms relative to the control groups (groups A and C, specific test in both cases, *z* = 1.43 and 0.48, respectively, *P* = NS). Moreover, there was little effect on the growth of *A. ceylanicum*: analysis by two-way ANOVA yielded *z* = 1.31 and *z* = -0.25 respectively, *P* = NS.

Antibody responses of hamsters infected with *T. spiralis* and killed on days 28 (groups B and H) and 35 (groups D and I) were compared using *T. spiralis* adult worm and muscle stage (L1) antigens (Table 2). Following the results of Experiment 2 the specific test was applied to ELISA data derived employing adult worm antigen (group B vs H, *z* = 2.47, *n* = 9, *n*2 = 9.4, *P* = 0.0068; group D vs I, *z* = 2.62, *n* = 9.4, *P* = 0.0044) indicating significant differences between concurrently and singly infected animals. The res-
Table 4—The growth of Ancylostoma ceylanicum following establishment during the acute phase of the host response to Trichinella spiralis (Exp 6)

| Group | No. of hamsters | Mean length (mm) ± S.E.M. |
|-------|-----------------|--------------------------|
|       | Male worms      | Female worms             |
| A. ceylanicum given 8 days after T. spiralis* |
| (A) A. ceylanicum alone | 7 | 5.06 ± 0.11 | 5.65 ± 0.11 |
| (B) A. ceylanicum + T. spiralis | 9 | 4.76 ± 0.10 | 5.49 ± 0.10 |
| A. ceylanicum given 14 days after T. spiralis† |
| (C) A. ceylanicum alone | 8 | 5.08 ± 0.11 | 5.67 ± 0.09 |
| (D) A ceylanicum + T. spiralis | 8 | 5.14 ± 0.07 | 5.77 ± 0.11 |

* 500 larvae of T. spiralis were given to group B on day 0 and 61 larvae of A. ceylanicum to both groups on day 8. All the animals were killed for worm counts and measurements 20 days later on day 28.

† 500 larvae of T. spiralis were given to group D on day 0 and 61 larvae of A. ceylanicum to both groups on day 14. All the animals were killed for worm counts and measurements 21 days later on day 35.

See text for statistical analysis.

Discussion

The experiments described in this paper have shown conclusively that the hookworms N. americanus and A. ceylanicum have the capacity to survive the changes which occur in the intestinal environment during the acute phase of the host response to T. spiralis and, in this respect, they contrast with other species of GI nematodes and tapeworms which are severely affected when present during such responses (Behnke et al., 1992). Hamsters infected with T. spiralis mounted a primary response the kinetics of which were similar to those described for mice and rats, with worm expulsion commencing after the first week of infection and >90% loss of worms by week 4, accompanied by mastocytosis as in other rodents (Wakelin & Lloyd, 1976; Alizadeh & Wakelin, 1982). Our results indicate that hookworms can withstand a temporary phase of heterologously-induced inflammation in the intestine of their hosts and suggest that they are resilient in the face of host-immune effectors, possibly explaining why human hookworms are chronic under field conditions.

One explanation for our data may be that the worms held on to the mucosal tissues with sufficient firmness to ensure their survival at the height of the response. Hookworms are known to bite deep into the mucosa, penetrating the submucosa on occasion (Bonne, 1942). However, we were aware that a proportion of the worms which we recovered from concurrently infected animals were unattached (data not shown) and we do not consider that firm attachment to the mucosa is the sole explanation for our findings.

It was more surprising, but consistent with the above view, that the infective larvae of A. ceylanicum established without loss and grew normally when they were administered to hamsters, 8 and 14 days after infection with T. spiralis at the height of the response to the latter species, when mast cell counts were rising and T. spiralis were about to be rejected. It is again possible that these stages escaped from the direct influence of the abnormal environment in the gut mucosa and lumen by penetrating the mucosa or by entering the crypts of Lieberkühn temporarily, as other species have been shown to do (Behnke et al., 1992). However, the experimental design (involving administration of challenge inocula of hookworms either on day 8 or 14) would have ensured that in one of these combinations L4 stages, which are non-nomigratory, would have been in the lumen during the acute inflammatory phase. Any prolonged residence in the tissues as a means of avoiding the hostile environment on the mucosal surface and in the lumen would have been reflected in reduced growth relative to controls.

Whilst hookworms were unaffected by the presence of and the host response to T. spiralis, there were a number of indications that the latter species was affected by the presence of hookworms. Thus in experiments where T. spiralis followed an earlier infection with A. ceylanicum, there was a significant
reduction in the recovery of T. spiralis, female worms were smaller and their fecundity was depressed. Despite the statistical significance of these observations in some experiments, the magnitude of the effect on T. spiralis was not marked to such an extent that it would have influenced the course of intestinal inflammation. Considerably smaller worm burdens than those recorded from concurrently infected animals in this study are known to be sufficient to elicit a primary response in other rodents (Wakelin & Lloyd, 1976). On balance we conclude that the interference with the establishment of T. spiralis stemmed most likely from the pathological changes in the intestinal mucosa which followed infection with hookworms (Behnke, 1991b), and we do not consider these to have been a major impediment to the sequence of immunological events which ultimately culminated in the expulsion of T. spiralis.

A possible explanation for the chronicity of hookworm infections is that these worms, like H. polygyrus, are immunomodulatory and depress immunological activity in their local mucosal environment (reviewed by Behnke, 1987b). In this context, IgG antibody responses to adult T. spiralis antigens were depressed in concurrently infected hamsters relative to controls (Table 2) and lower serum antibody concentrations were also evident to larval antigens in animals which had harboured A. ceylanicum for 3–4 weeks before challenge with T. spiralis. However, all concurrently infected hamsters expelled T. spiralis, albeit marginally slower than controls in some instances, indicating that despite immunodepression the mucosal response was of sufficient intensity to ensure worm expulsion. Even in Exp 6, in which marked depression of serum IgG antibody responses was observed, the expulsion of T. spiralis was not significantly delayed (Fig. 4). In comparison mice concurrently infected with H. polygyrus show a marked depression of serum antibody responses to heterologous antigens (Ali & Behnke, 1983) and delayed expulsion of T. spiralis, with adult worms of the latter species persisting for some 2–4 weeks after total loss from control groups infected with T. spiralis alone (Behnke, Wakelin & Wilson, 1978).

In conclusion, the results reported in this paper suggest that hookworms have some immunomodulatory properties and indicate that, unlike other parasitic nematodes and tapeworms, hookworms can survive the non-specific components of host-protective intestinal immune responses elicited by concurrent infections. However, the explanation for their greater resilience vis à vis other gastrointestinal nematodes remains to be elucidated.

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REFERENCES

Ali N. M. H. & Behnke J. M. 1983. Nematospiooides dubius: factors affecting the primary response to SRBC in infected mice. Journal of Helminthology 57: 343–353.

Ali Zadeh H. & Wakelin D. 1982. Comparison of rapid expulsion of Trichinella spiralis in mice and rats. International Journal for Parasitology 12: 65–73.

Beaver P. C. 1988. Light, long-lasting Necator infection in a volunteer. American Journal of Tropical Medicine and Hygiene 39: 369–372.

Behnke J. M., Bland P. W. & Wakelin D. 1977. The effect of the expulsion phase of Trichinella spiralis on Hymenolepis diminuta infection in mice. Parasitology 75: 79–88.

Behnke J. M., Wakelin D. & Wilson M. M. 1978. Trichinella spiralis: delayed rejection in mice concurrently infected with Nematospiooides dubius. Experimental Parasitology 46: 121–130.

Behnke J. M., Paul V. & Rajasekariah G. R. 1986. The growth and migration of Necator americanus following infection of neonatal hamsters. Transactions of the Royal Society of Tropical Medicine and Hygiene 80: 146–149.

Behnke J. M. 1987a. Do hookworms elicit protective immunity in man? Parasitology Today 3: 200–206.

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

Behnke J. M. 1990. Laboratory animal models. In: Hookworm Disease. Current Status and New Directions (Edited by Schad G. A. & Warren K. S.) pp. 105–128. Taylor and Francis, London.

Behnke J. M. 1991a. Immunology. In: Human Parasitic Diseases, Vol. 4, Hookworm Infections (Edited by Gilles H. J. & Ball P. A. J.), pp. 93–155. Elsevier, Amsterdam.

Behnke J. M. 1991b. Pathology. In: Human Parasitic Diseases, Vol. 4, Hookworm Infections (Edited by Gilles H. M. & Ball P. A. J.), pp. 51–91. Elsevier, Amsterdam.

Behnke J. M., Cabaj W. & Wakelin D. 1992. Susceptibility of adult Hymenolepsis polygyrus to intestinal inflammatory responses induced by heterologous infection. International Journal for Parasitology 22: 75–86.

Bonne C. 1942. Invasion of the wall of the human intestine by ancylostomes. American Journal of Medicine 22: 507–509.

Bundy D. A. P. (1990) Is the hookworm just another geohelminth? In: Hookworm Disease. Current Status and New Directions (Edited by Schad G. A. & Warren K. S.), pp. 147–164. Taylor and Francis, London.

Christie P. R., Wakelin D. & Wilson M. M. 1979. The
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effect of the expulsion phase of *Trichinella spiralis* on *Hymenolepis diminuta* infection in rats. *Parasitology* 78: 323–330.

Ferreti G., Gabriele F., Palmas C. & Wakelin D. 1984. Interactions between *Trichinella spiralis* and *Hymenolepis nana* in the intestine of the mouse. *International Journal for Parasitology* 14: 29–33.

Garside P. & Behnke J. M. 1989. *Ancylostoma ceylanicum*: observations on host-parasite relationship during primary infection. *Parasitology* 98: 283–289.

Howard R. J., Christie D., Wakelin D., Wilson M. M. & Behnke J. M. 1978. The effect of concurrent infection with *Trichinella spiralis* on *Hymenolepis microstoma* in mice. *Parasitology* 77: 273–279.

Kennedy M. W. 1980. Immunologically mediated, non-specific interactions between the intestinal phases of *Trichinella spiralis* and *Nippostrongylus brasiliensis* in the mouse. *Parasitology* 80: 61–72.

Lowry O., Rosebrough N., Farr A. & Randall R. 1951. Protein measurement with folin–phenol reagent. *Journal of Biological Chemistry* 193: 265–275.

Meddis R. 1984. *Statistics Using Ranks. A Unified Approach*. Basil Blackwell Publishers, New York.

Miller T. A. 1971. Vaccination against the canine hookworm diseases. *Advances in Parasitology* 9: 153–183.

Moqbel R. & MacDonald A. J. 1990. Immunological and inflammatory responses in the small intestine associated with helminthic infections. In: *Parasites: Immunity and Pathology. The Consequences of Parasitic Infection in Mammals* (Edited by Behnke J. M.), pp. 249–282. Taylor and Francis, London.

Rajasekari G. R., Deb B. N., Dhage K. R., Bose S. 1985. Site of resistance to *Necator americanus* in hamsters. *Acta Tropica* 42: 333–340.

Rose R. A. & Behnke J. M. 1990. *Necator americanus* in the DSN hamster: density-dependent expulsion of adult worms during primary infection. *Parasitology* 100: 469–478.

Rothwell T. L. W. 1989. Immune expulsion of parasitic nematodes from the alimentary tract. *International Journal for Parasitology* 19: 139–168.

Sen H. G. 1972. *Necator americanus*: behaviour in hamsters. *Experimental Parasitology* 32: 26–32.

Wakelin D. & Lloyd M. 1976. Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology* 72: 173–182.

Wakelin D. & Wilson M. M. 1977. Transfer of immunity to *Trichinella spiralis* in the mouse with mesenteric lymph node cells: time of appearance of effective cells in donors and expression of immunity in recipients. *Parasitology* 74: 215–224.