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The response of hamsters to primary and secondary infection with *Trichinella spiralis* and to vaccination with parasite antigens

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Abstract

The duration of primary infections with *T. spiralis* was dose-dependent with greater proportional loss of worms from heavily infected hamsters and longer persistence of worms in syngeneic DSN hamsters carrying initially low intensity infections. Intestinal worms were lost more rapidly from challenged immunized animals with over 80% loss of established worms by day 6 post infection, but survival of residual worms for a further 2 weeks. Hamsters carrying initially more than 140 intestinal worms began to lose weight during the second week indicating severe pathology at this stage of infection. Mucosal mast cell numbers increased from 50 cells/20 villus crypt units in uninfected animals to a peak in excess of 150 during week 4 pi, although intestinal mastocytosis persisted long after the loss of the majority of adult worms. Serum antibody responses to muscle stage larval antigen were detected in week 3 and increased subsequently. Both mastocytosis and antibody responses were more intense on secondary exposure to infection. Hamsters vaccinated with muscle stage larval antigen showed only a moderately accelerated loss of the intestinal phase but the fecundity of worms was severely suppressed. Overall it was concluded that the hamster host provided a model of trichinellosis that, in many respects was closer than mice and rats to the pattern of infection seen in economically and clinically important host species.

Introduction

Much of our knowledge of host-parasite relationships involving the nematode *Trichinella spiralis* has come from studies of infections in mice and rats. In both of these hosts infection provokes strong immune responses. During initial infections, immunity is expressed most strongly against worm survival and reproduction, and results in the expulsion of adult worms from the small intestine within about two weeks, although this may be longer in slow-responder strains (Wakelin & Denham, 1983). Data available from the pig show that intestinal infections are more prolonged in this host (Murrell, 1985), and that the effects of immunity are seen primarily in terms of reduced female worm fecundity (Marti & Murrell, 1986). Clinical data from humans also suggests prolonged survival of adult worms (Ozeretskovskaya & Tumolskaya, 1974). Rodent models, particularly those involving rapid-responder hosts, may not therefore accurately represent the type of host-parasite relationship that *Trichinella* has with hosts of economic and clinical significance.

In the course of work on responses to concurrent *Trichinella*-hookworm infections in hamsters, we observed that loss of *Trichinella* in single infections was markedly slower than in other rodents. Only limited data is available in the literature on the kinetics of worm expulsion and associated immune responses in this host (Boyd & Huston, 1954; Ritterson, 1959; Concannon & Ritterson, 1965) and for this reason we have examined the course of infection in detail to determine whether the host-parasite relationship resembles more closely that seen in non-rodent hosts.

Materials and methods

Parasites and hosts

The London isolate of *T. spiralis* (ISS 25) was used throughout. The methods used for the infection of animals, recovery of worms and assays of female worm fecundity were essentially as described previously (Wakelin & Lloyd, 1976; Wakelin & Wilson, 1977). Because of the larger size of the hamster intestine relative to mice, the incubation period for adult worm recovery was extended to 6 h. Worm fecundity was assessed by overnight incubation, the numbers of larvae released representing the maximum output possible under these conditions.

The hamsters used were the syngeneic DSN strain, originally purchased from Intersimian Ltd., Oxford, UK, but now obtained from a closed breeding colony in the Department of Life Science at Nottingham University. Animals were normally 2 to 4 months old at infection. Experimental groups were set up in separate cages 1–2 weeks before infection and were maintained under conventional conditions with access to food and water *ad libitum*.

Preparation of antigens

Outbred CFLP mice were infected with *T. spiralis* and L1 antigen obtained from muscle larvae as described by Goyal & Wakelin (1993). 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 *et al.* (1951), aliquoted and stored at −40°C. Hamsters were vaccinated subcutaneously on day 14 with 100 μg of antigen (1:1 in Freund's complete adjuvant), boosted with 100 μg of antigen in phosphate buffered saline on day 7 and infected on day 0.

Measurement of antibody responses

Parasite-specific IgG responses were measured by a standard ELISA, microtitre plates being coated with 50 μl of worm antigen (5 μg ml^{-1}). Test sera were assayed individually, in triplicate, at a dilution of 1 in 100. Alkaline phosphatase-conjugated Protein A (Sigma-diluted 1:500) was used to detect bound IgG antibody and developed using the substrate p-nitrophenylphosphate (1 mg ml^{-1}). Colour changes were read at 410 nm on a Dynatech MR700 Microplate Reader. In Experiment 2 the results are expressed as optical density values (OD). In experiment 4, where large numbers of sera were assayed (58) in triplicate and several ELISA plates were used, sera from immunized challenged animals bled 10 days after challenge infection and sera from naive hamsters were included on all plates to compensate for inter-plate variation. The data were standardized in relation to these reference sera and are expressed as a relative response, i.e. proportion of the response detected in the immunized challenged group bled on day 10 after challenge.

Measurement of worms

Worms recovered at autopsy were fixed in formol 70% ethanol at 37°C. They were drawn to scale using a camera lucida and the drawings then measured using a digitizer pad and an IBM computer with a programme for conversion of lengths traced into discrete units (courtesy of Dr R. Ramsey). Ten worms of both sexes were measured from each hamster and the mean values for worms from individual hamsters 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 in 20 villus crypt units (VCU) were counted. The mean values/20 VCUs were then calculated for each individual and for the experimental group.

Statistical analysis of results

Data are presented as group mean values ± standard error (S.E.M.). Non-parametric statistical analyses were used because normal distribution of data could not be assumed. When a difference was expected in a specific direction, the procedures described by Meddis (1984) were used and the test statistic z given as appropriate. Where this was not the case, groups were compared by a general one-way ANOVA and the statistic H is given. A specific two-way ANOVA (Meddis, 1984) was employed to assess the effects of the three experimental treatments in experiment 5 on worm recovery and worm fecundity, with time and treatment as the two factors. A general two-way ANOVA was employed to examine differences in mastocytosis. Relationships over time within experimental groups were examined by the Spearman Rank Order Correlation Test and the statistic r_s is given. Probabilities (P) of 0.05 or less were considered significant; exact probabilities are given whenever possible. In certain cases, multiple analyses could not be avoided, and, where these were undertaken, the cut-off value for significance was lowered to $P=0.025$ or 0.01 to avoid type I errors.

Experimental design and results

Time course of a primary infection

Three experiments were carried out, the results of which are summarized in fig. 1.

Experiment 1

Three groups of 7 female hamsters were infected with 500 larvae of *T. spiralis* and were killed on days 7, 14 and

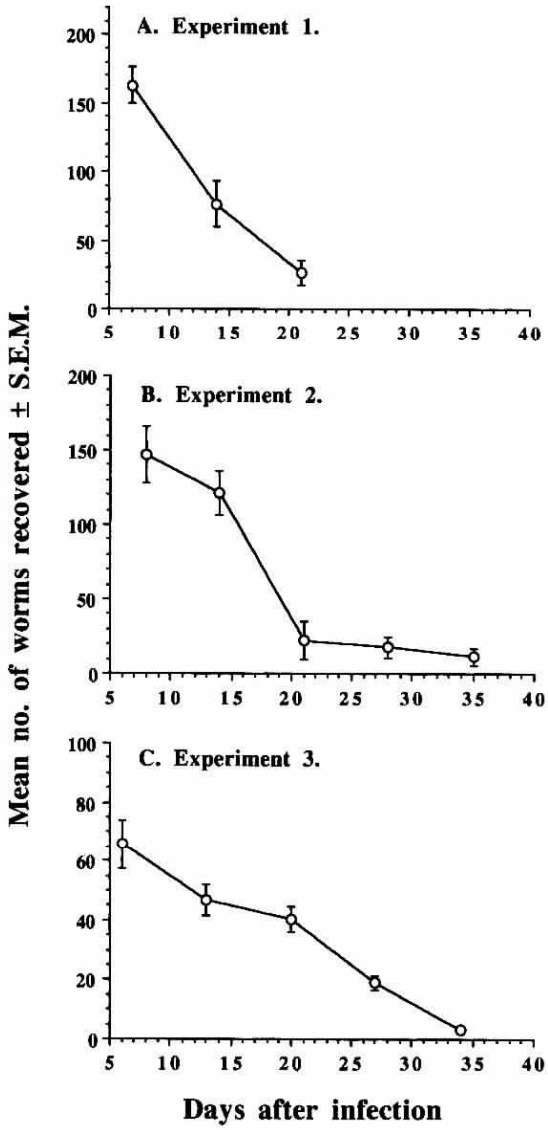

Fig. 1. Survival of *Trichinella spiralis* in hamsters exposed to primary infection. For experimental details and statistical analysis see text.

21 post infection (pi). A mean of 162.9 worms was recovered on day 7 pi but over the following 2 weeks worm counts declined by 83.7% (fig. 1A). The reduction of worm burden with time was significant ($r_s = -0.857$, $n = 21$, $P < 0.001$). However, 21 days after infection, all 7 hamsters still harboured adult worms, the mean worm burden being 26.6 ± 9.0 (16.3% of the initial establishment).

Experiment 2

Twenty male hamsters were given 500 muscle larvae and then killed in batches of four on days 8, 14, 21, 28 and 35 pi. A mean of 147.3 worms was recovered on day 8 and there was no significant change in worm burden before day 14 pi (fig. 1B). By day 21 the worm burden was reduced by 84.7% relative to day 8 pi, however, only one of the four hamsters killed on day 21 was entirely without adult worms and all 8 animals from the two groups killed on days 28 and 35 harboured adult worms. The mean burden on day 35 was 11.3 worms, 7.6% of the originally established parasite population. There was a highly significant negative relationship between worm burden and time pi ($r_s = -0.733$, $n = 20$, $P < 0.001$).

Experiment 3

Fifteen male hamsters were infected with 300 muscle larvae and killed in groups of 3 at intervals from 6 to 34 days pi (fig. 1C). A mean of 65.7 worms was recovered on day 6 and by day 34 pi 95.4% of these had been lost. The negative correlation between worm burden and time pi was highly significant ($r_s = -0.95$, $n = 15$, $P < 0.001$). Muscle larval burdens were measured in hamsters killed 27 and 34 days pi. The yield of larvae was 5856.7 ± 907 ($n = 3$) and 9950.8 ± 1082.1 gm^{-1} of muscle ($n = 3$) respectively.

Fecundity of female worms during primary infection

Fecundity was measured in experiment 3 on days when sufficient worms were available to enable the assay to be carried out. The mean fecundity/female worm was 12.3 ± 1.6 (day 6), 11.1 ± 1.23 (day 13), 7.7 ± 1.35 (day 20) and 10.1 ± 0.51 (day 27). On day 34 insufficient numbers of female worms were recovered. Over the period 6 to 27 days pi there was no significant change in the fecundity of female worms.

Time course of a secondary infection (experiment 4)

Sixty-two male hamsters were arranged into 11 groups of 5 each and a further two groups comprising 3 and 4 animals. Thirty-two hamsters were infected with 266 muscle larvae on day 0 and four were killed 7 days later to determine the level of worm establishment. The mean worm

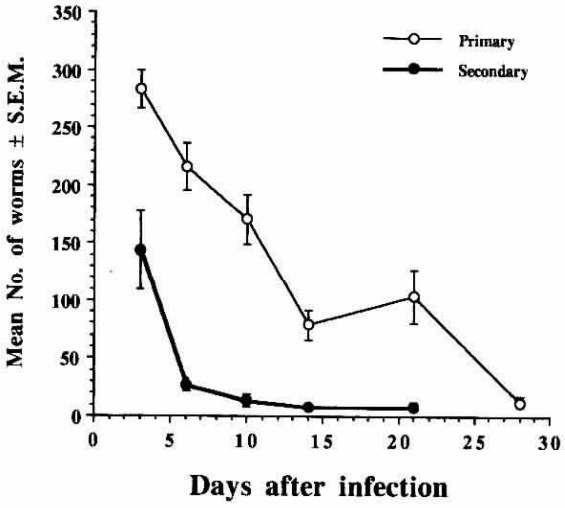

Fig. 2. Experiment 4. Survival of *Trichinella spiralis* in hamsters exposed to primary and secondary infections. For experimental details and statistical analysis see text.

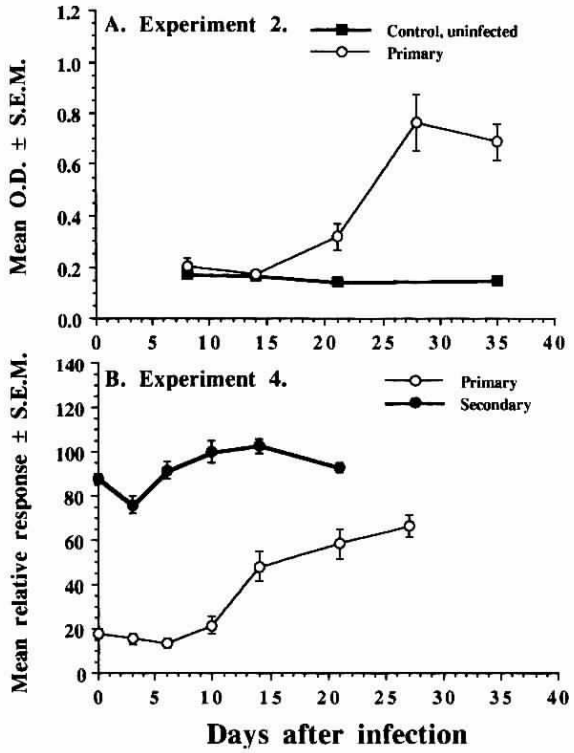

Fig. 3. Serum antibody responses to muscle larvae homogenate antigens.

recovery was 224.8 (84.2% establishment), a level which would have led to severe weight loss, accordingly all the remaining animals were treated with fenbendazole at 250 mg/kg body weight on days 10 and 14 pi. Forty-four days after the immunizing infection 25 of the originally infected animals together with a further 30 hamsters were challenged with 500 larvae. Three of the originally infected animals were not challenged and provided background data for serum immunoglobulin and mucosal mast cell levels.

In naive hamsters receiving only the challenge infection, the number of worms recovered on day 3 post challenge was 283.5 (fig. 2). In animals which had been previously infected worm burdens were nearly 50% lower. Worm loss ensued in both immunized and previously uninfected animals and the negative relationship between worm recovery and time pi was highly significant in both cases ($r_s = -0.908$, $n = 30$, $P < 0.001$; $r_s = -0.753$, $n = 23$, $P < 0.001$, respectively). There was no overlap in worm burdens from immunized and primary infection animals autopsied on any single day. However, the immunized animals rejected worms faster than the challenge control group with 80.7% loss of established worms by the former between days 3 and 6 pi compared with only 23.9% by the latter groups. Despite the accelerated response to challenge infection in immunized animals, none of the hamsters completely cleared the intestinal phase of the infection. On day 21 after challenge, the mean worm recovery was 8.8 ± 3.73 but one animal still had 20 adult worms. Likewise, even on day 27 pi the worm burden of the challenge infection control group was 12.6 ± 4.58, representing the persistence of 4.4% of the originally established worms.

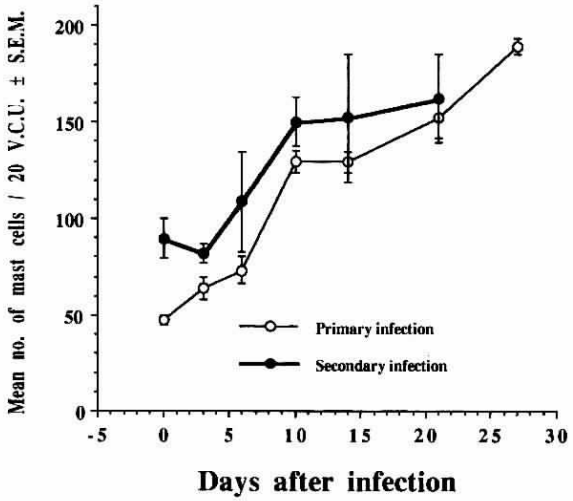

Fig. 4. Experiment 4. Mucosal mast cell response following primary and secondary exposure to *Trichinella spiralis*. For experimental details and statistical analysis see text.

Growth of worms during primary and secondary infections

Worms were measured during the course of experiment 1. Male worms measured 1.31 ± 0.06 ($n = 7$), 1.69 ± 0.03 ($n = 7$) and 1.67 ± 0.06 mm ($n = 3$) on days 7, 14 and 21 pi

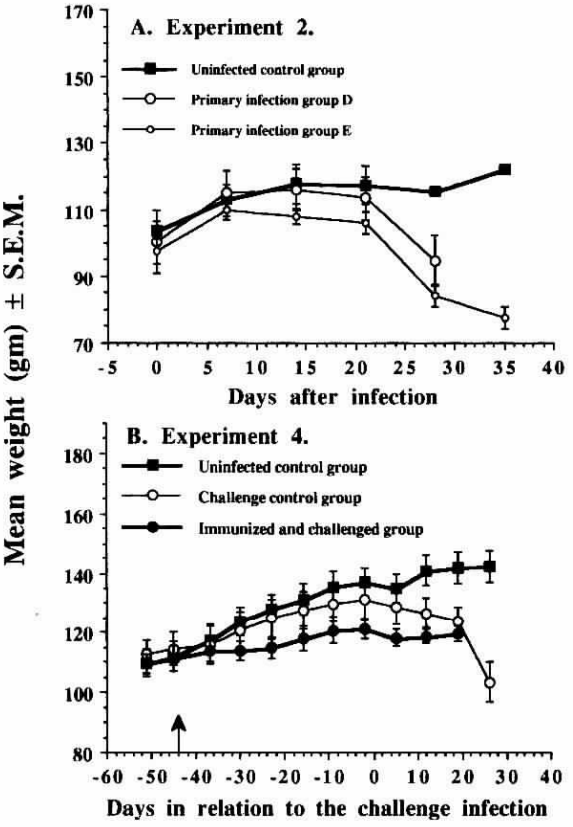

Fig. 5. Changes in the weight of hamsters following primary and secondary exposure to *Trichinella spiralis*. In B, the arrow indicated day of primary infection. The challenge infection was given on day 0.

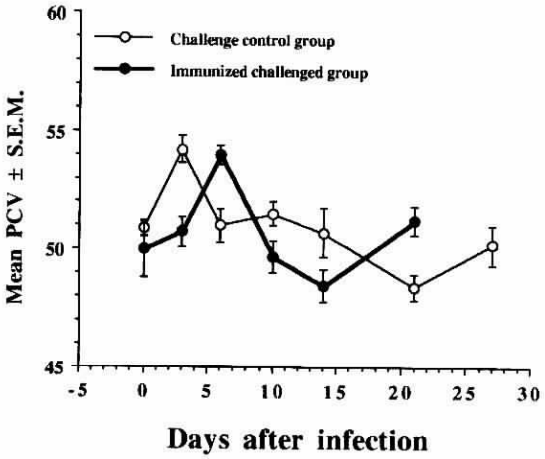

Fig. 6. Experiment 4. Changes in PCV during the course of primary and secondary infections with *Trichinella spiralis*.

respectively. Female worms measured 2.92 ± 0.06, 3.23 ± 0.04 and 3.43 ± 0.07 mm over the same period. Analysis of these data by a specific one-way ANOVA predicting increase in length across the three time points gave highly significant results ($z = 2.97$, $P = 0.0015$ and $z = 3.39$, $P = 0.00005$, respectively).

In experiment 4, female worms from the two groups killed on day 6 after challenge were measured. Those from the challenge control group measured 2.75 ± 0.04 mm, whereas those from the immunized, challenged hamsters were significantly smaller at 2.34 ± 0.08 mm ($z = 2.45$, $P = 0.0071$).

Serum antibody response to muscle larval and adult worm antigens

The serum antibody response to muscle larval antigen is shown in fig. 3. In experiment 2 there was little variation in the OD reading for sera from the naive control animals killed on days 8, 14, 21 and 35 pi, but hamsters infected with *T. spiralis* showed a significant increase over background levels by day 21 pi (fig. 3A). Antibody titres for days 21 and 35 pi were increased further and there was a significant positive correlation between OD and days pi ($r_s = 0.834$, $n = 20$, $P < 0.001$).

In experiment 4 the primary response detected in the challenge control group was somewhat faster with a rise in titre above that of naive animals by day 14 pi (fig. 3B). The immunized group had higher antibody titres before challenge but showed little further change after challenge.

Mucosal mast response

Mucosal mast cells were quantified in experiment 2, 3 and 4; the results for the latter are summarized in fig. 4. All three experiments revealed a significant rise in mast cell counts from approximately 50 cells/20 VCU in uninfected hamsters to a peak count in excess of 150 by weeks 4–5 pi (experiment 2, $r_s = 0.803$, $n = 12$, $P = 0.002$; experiment 3, $r_s = 0.92$, $n = 19$, $P < 0.001$). Elevated counts persisted for at least 44 days after a primary infection and counts were boosted on challenge ($r_s = 0.721$, $n = 20$, $P < 0.001$ and $r_s = 0.954$, $n = 24$, $P < 0.001$ respectively for the immunized challenged and challenge control hamsters in experiment 4).

Changes in weight and PCV during infection

Figure 5A shows changes in weight of two of the infected groups and uninfected controls during the course of infection in experiment 2. Initially all three groups gained weight, but whereas the naive uninfected animals continued to gain weight slowly both infected groups lost weight, with a mean loss of 17.5% by group A by day 28 and a loss of 21.5% by group B by day 35.

Selected data from experiment 4 is illustrated in fig. 5B. This shows the uninfected control group A ($n = 5$) gaining weight slowly throughout the period of the experiment (29.5% gain in weight between day 0 and day 77 of the experiment: days −51 and 26 respectively in fig. 5B, where changes are expressed in relation to the day on which the challenge infection was administered). The challenge control group (N, $n = 5$), which was eventually killed for worm counts 27 days after the challenge infection, initially, during the period when they were not yet infected, also gained weight in parallel with group A. However, within 2 weeks of the infection administered on day 44 of the experiment (day 0 in fig. 5B), the mean weight began to decline and by day 27 after challenge these animals had lost 21.0% of their weight relative to 8 days before challenge (i.e. day −8 in fig. 5B). The immunized animals (group H, $n = 5$) did not gain weight as rapidly as the other groups in the period before challenge but in contrast to group N, their weight remained stable following challenge infection.

Changes in PCV following primary and secondary infection

Figure 6 illustrates the changes in PCV recorded in experiment 4. Despite the variation from day to day all the mean values are well within the normal range for the PCV value of healthy uninfected hamsters. However, whilst there was no significant change in PCV of the immunized, challenged group with time ($r_s = -0.135$, $n = 26$, $P = $ NS), that of the challenge control groups (i.e. animals carrying a primary infection) declined significantly ($r_s = -0.443$, $n = 35$, $P = 0.008$).

The response of hamsters to vaccination with muscle larvae antigens

Finally an experiment was carried out to determine whether hamsters would respond to vaccination with a standard antigen preparation known to induce strong immunity to infection in mice. The experiment comprised seven groups of male hamsters ($n = 5$ in each case). Two groups were infected with 300 *T. spiralis* (groups A and B), two were vaccinated (groups C and D) and three left untreated (E, F and G) at the start of the experiment. A further 3 hamsters were given only the immunizing infection given to groups A and B, and were killed for worm counts on day 6 pi; 66 ± 8 worms were recovered. On day 42 groups A–F were challenged with 300 larvae while group G was left uninfected to provide baseline data for relevant parameters. One group from each of the experimental treatments was killed on day 6 after challenge (A, C and E) and the other groups (B, D and F), together with the naive animals (group G) on day 11 after challenge.

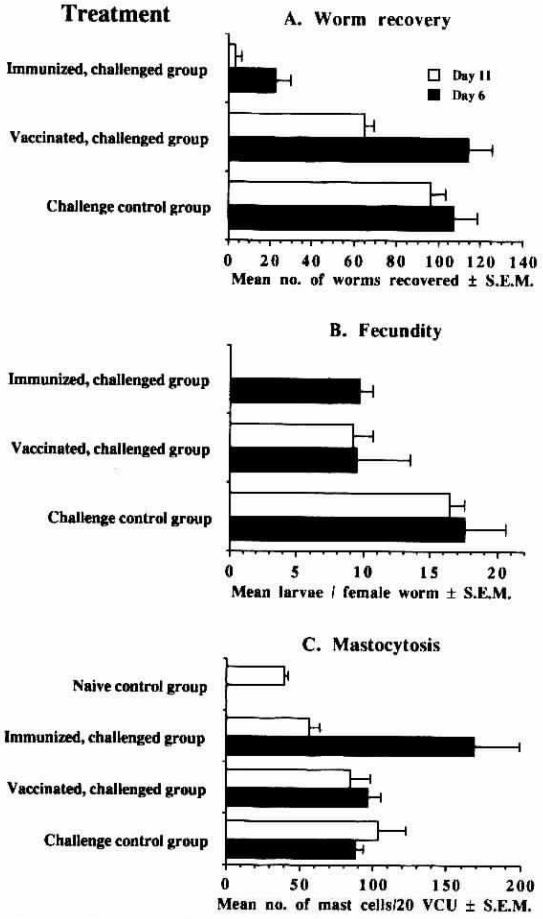

Fig. 7. Experiment 5. The response of hamsters to challenge with *Trichinella spiralis*, following vaccination with muscle larval antigen or immunization with a primary infection.

Figure 7A summarizes the worm burdens recovered on both days after challenge infection. The results were analysed by a two-way ANOVA and the specific prediction that worm burdens in groups A & B < C & D < E & F was found to be significant ($z=4.19$, $P=0.0003$). It is apparent from the data that the immunized group (A) already lost 78.9% of the worm burden relative to the challenge control group E. In comparison, the vaccinated animals did not show accelerated loss of worms on day 6 but their worm burdens were 32.4% smaller than those of the control group F on day 11 pi.

The fecundity of female worms was highest in the challenge control group (fig. 7B) and a test of the specific hypothesis A & B < C & D < E & F gave $z=3.77$, $P=0.00011$. However, it is evident from the data that counts for worms from both vaccinated and immunized groups differed little from each other.

Analysis of the mast cell response (fig. 7C) by a general two-way ANOVA, with time pi and treatment as the factors, gave $H=0.45$, $P=$NS for effect of treatment. There was, however, a significant time effect ($H=5.99$, $P < 0.05$) and a significant interaction between time and treatment ($H=6.71$, $P < 0.05$). This arose mainly through the mast cell response of immunized challenged hamsters, which was most intense on day 6 but subsided to background levels by day 11 (in contrast to earlier experiments). The vaccinated and challenge control groups had more cells than the naive control group but there was little to distinguish between them and little change from day 6 to day 11 pi.

Discussion

A wide variety of mammals is susceptible to infection with *T. spiralis*, the Chinese hamster being one of the few species known to have a substantial degree of natural resistance to the muscle but not intestinal phase of the parasite (Concannon & Ritterson, 1965; Takada & Tada, 1987). In the most commonly used rodent hosts (mice and rats), primary infections establish successfully, but then elicit a strong acquired immunity that operates rapidly enough to reduce both the reproduction and the survival of adult worms (Wakelin & Denham, 1983). The duration of the intestinal phase of infection in these hosts shows considerable variation, depending upon host genotype and number of larvae given (Wakelin, 1980; Bell *et al.*, 1983). It is usually between 2 and 3 weeks, although small numbers of adult worms may persist for much longer. Female worms liberate larvae for a relatively short period during the intestinal phase, the majority of new-born larvae being released between days 5 and 12 pi (Kennedy, 1980). Data derived from experimental infections in pigs and from clinical observations of human cases show that, in primary infections, the survival and reproduction of adult worms is considerably more prolonged than that seen in rodents (Murrell, 1985). There are, accordingly, significant differences in terms of the parasitological and pathological consequences of infection. In this respect, therefore, existing rodent models of trichinellosis have some limitations. Hamsters are known to be highly susceptible to a number of parasitic infections, and the experiments reported here were designed to determine whether the course of infection, and the accompanying immune and inflammatory responses, in the DSN strain would be similar to those seen in other rodents or resemble more closely those patterns recorded in non-rodent hosts.

It is clear from the data in figs 1–4 that hamsters, like mice and rats, make effective immune responses to primary infections. This results in the loss of adult worms, the rate of which appears to be dose-dependent. In experiments where burdens of about 150 worms were established, loss was virtually complete within 4 weeks, but when fewer worms were established, worm loss was slower, some 30% of the initial burden remaining at 4 weeks. Our results contrast with earlier finding by Boyd & Huston (1959) and Ritterson (1959) who observed complete loss of worms by 15 days and 3 weeks pi respectively and even more so with Sadun & Norman (1956) who reported that most worms were rejected by hamsters in the first week following infection. However, our findings are supported by Concannon & Ritterson (1965), who reported that worm burdens declined slowly following the initial establishment of 97 worms (6 dpi) with an average of 24 worms surviving until day 18 pi. Somewhat surprisingly, worms continued to grow despite the development and expression of the host's immune response. When hamsters were challenged after a primary infection, worm expulsion was rapid, burdens being reduced to less than 20% of those present in controls within 6 days (figs 2 & 7).

The prolonged survival of intestinal worms in our study was associated with an extended reproductive period. Female worms continued to release newborn larvae throughout infection, and muscle larval burdens showed a steady increase with time. It is likely that the accumulating muscle burdens provoked intense local inflammation with resultant muscular disfunction and host inappetance leading to the drastic weight loss which we observed. Although we did not quantify feeding nor monitor pathological changes in the host's muscles, Ritterson (1959) observed inflammatory, neutrophil- and eosinophil-rich, infiltration of infected hamster muscles, with associated degenerative changes in muscle fibres, as early as 14 days pi, earlier than would be expected in mice. By 21 days pi basophils were evident in infected muscles. Mice seem to tolerate comparable adult worm burdens without suffering weight loss to the same degree and in part this may be explained by the shorter survival of adult worms and hence lower total output of larvae by females but equally it is conceivable that hamsters sustain greater damage to muscles through their more intense responses to the muscle phase of trichinellosis. In this respect the hamster may be a more relevant model of human infections than other rodents.

These parasitological observations suggest that the immunological response to *T. spiralis* in hamsters may differ qualitatively or quantitatively from that described in detail in rats and in rapid responder strains of mice; it appears to be more similar to that seen in slow responder strains such as B10.BR mice (Wakelin, 1980). Two parameters of this response were followed, mucosal mastocytosis (an index of intestinal inflammation) and parasite-specific IgG antibody levels. Infection stimulated a rise in mucosal mast cell density, initially rapid over the first 10 days and then a steady increase to nearly 4 times baseline levels by day 30 pi (figs 4 & 7). In absolute terms this value represents a lower level of mastocytosis than that seen in mice and rats, and the pattern of response is quite different. In both rats and mice there is a peak of mastocytosis at about the time of worm loss, followed by a decline to baseline values (Alizadeh & Wakelin, 1982). The IgG antibody response, in contrast, followed a pattern that is more consistent with that described elsewhere, levels rising significantly from the second week of infection (Jungery & Ogilvie, 1982; Almond & Parkhouse, 1986).

An interesting difference between the responses of rodents and pigs to *T. spiralis* is seen when hosts are immunized with parasite antigen before infection. In the majority of mouse strains and in rats growth and fecundity of a challenge infection are reduced and worm expulsion is accelerated (Wakelin & Denham, 1983). In pigs, however, vaccine-induced immunity is expressed most strongly against worm reproduction, worm survival being much less affected (Gamble *et al.*, 1986). The data summarized in fig. 7 show that the response of vaccine-immunized hamsters to challenge is more like that of pigs than of mice and rats. The initial expression of immunity (day 6 post challenge) was a significantly reduced female worm fecundity, but there was no effect on worm numbers, even though infection-immunized hamsters had only 20% of the control burden at this time. Even on day 11 post challenge the vaccinated animals retained more than 50% of their initial worm burden.

Collectively, the data described in this paper suggest that the hamster, although less well-defined genetically and immunologically than other rodents, may nevertheless provide a model of trichinellosis that is closer in many respects to the pattern of infection seen in economically and clinically important host species.

Acknowledgements

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