Isolates of *Trichuris muris* vary in their ability to elicit protective immune responses to infection in mice

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SUMMARY

Much of what is currently known of the host–parasite interaction between mice and the parasitic nematode *Trichuris muris* has come from experiments using a single parasite isolate (E/N). This isolate has been compared with 2 others which, on morphological criteria, belong to the same species. In 3 inbred strains of mouse that show distinct, genetically determined response phenotypes, there was a consistent pattern in terms of parasite survival time regardless of host strain, E/K worms being expelled early, E/N expelled later and S worms very late or not at all. High-responder CBA mice expelled E/K and E/N worms earlier than low-responder C57 Bl/10 mice. B10.BR mice were permissive to S isolate infection, mounted a very late response to E/N worms but expelled E/K worms effectively by day 25. The differential response of mice to these isolates provides an experimental system for identifying the basis of variation in this host–parasite relationship.

Key words: *Trichuris muris*, nematode, parasite isolate, mouse strain, response phenotype, host genetics.

INTRODUCTION

Trichuris muris, the mouse whipworm, elicits a protective immune response in the majority of inbred strains of mice which results in expulsion of the infection before patency is reached on day 32 post-infection (p.i.). Strong genetic influences govern host strain responsiveness (Else & Wakelin, 1988; Else, Wakelin & Roach, 1989). Mouse strains of high responder (HR) phenotype expel the parasite by the third week of a primary infection and remain resistant to a subsequent challenge infection. Low responder (LR) strains show delayed worm expulsion but still prevent patency; strains which fail to expel the parasite prior to patency are here defined as permissive, although they have been termed non-responders (Else & Grencis, 1991). Response phenotype is determined by both MHC-linked and background genes (Else & Wakelin, 1988) and reflects genetically determined influences on T helper lymphocyte activity (Else & Grencis, 1991). The E/N *T. muris* isolate used in all previous studies has been passaged routinely in immunosuppressed outbred mice since 1964 (Wakelin, 1967). Recently, 2 other isolates have become available. In this study we compare the 3 isolates morphologically and in terms of released proteins. The course of infection with each isolate was followed in HR, LR and NR strains of mice to determine strain–isolate interactions and the effect of isolate adaptation to a laboratory host was also observed. The results show major differences between the isolates in the responses elicited in the host, at the most extreme (in B10.BR mice) varying between early expulsion to complete absence of protective immunity.

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MATERIALS AND METHODS

Parasite

The maintenance of *Trichuris muris* isolates and the methods used for infection and examination of the experimental animals were as described by Wakelin (1967). The E/N isolate was obtained from wild mice by Dr R. C. Rayski at Edinburgh Zoo in 1954 and was supplied to D.W. by Dr J. E. D. Keeling (Wellcome Research Laboratories, London) in 1964. The isolate designated E/K, obtained from Professor Y. Ito (Kitasato University School of Medicine, Kitasato, Japan), was derived from the same stock as the E/N isolate but has been passaged in immunosuppressed mice in Japan since 1969 (Ito, 1991). The third isolate, designated S, was obtained from wild mice in Portugal in 1992 by Dr J. M. Behnke and, like the E/N isolate, has been maintained since that time in immunosuppressed mice. The E/K isolate shows a strikingly different pattern of host–parasite relationship from the other two (Ito, personal communication), offering the possibility of defining parasite-dependent determinants of host responsiveness.

Mice

Outbred female CFLP mice were bred in the Department of Life Science and used at 6–8 weeks of

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age. SPF 6–8-week-old inbred C57Bl/10, B10.BR and CBA mice were obtained from Harlan Olac Ltd (Bicester, Oxon). For passage CFLP mice were immunosuppressed by gamma irradiation (450 Rad.) using a ^{137}caesium source on the day of infection (day 0) followed by s.c. injection with 1·25 mg hydrocortisone-2,1-acetate (Sigma, Poole, Dorset) on days 7 and 13 post-infection (p.i).

Antigen

Adult worms were cultured at 37 °C in RPMI medium to obtain excretory/secretory (E/S) antigen as described by Else *et al.* (1989).

Egg counts

Eggs were recovered from the faeces of infected immunosuppressed CFLP mice using a modification of the zinc sulphate flotation method of Faust *et al.* (1938). Eggs were counted using a McMaster chamber and numbers expressed as eggs per gram of faeces.

Worm and egg measurements

Male and female adult *T. muris* worms of each isolate were recovered from immunosuppressed CFLP mice and their outlines drawn under an appropriate magnification using a dissecting microscope fitted with a camera lucida. The drawings were then converted into units of length by computer using a bit-pad. Eggs were measured under a compound microscope using an eye-piece graticule. Intestinal worm burdens, worm and egg measurements were subject to statistical analysis by the Mann–Whitney U test. A value of $P > 0.05$ was considered non-significant.

Infection time-course experimental procedure

Groups of HR CBA, LR C57Bl/10 and permissive B10.BR mice were infected with 400 embryonated *T. muris* eggs of each isolate on day 0. The mice were killed in groups of 5 at time-points after infection for worm burden determination. The fact that all strains were infected with eggs of the same batch of each isolate on the same day provided an internal control for initial worm establishment at day 14.

Electrophoretic separation of E/S material

SDS–PAGE gels (12% (w/v) acrylamide, 0·27% (w/v) *bis*acrylamide) were prepared as described by Laemmli (1970). For electrophoresis, samples were mixed with an equal volume of 0·5 M Tris (pH 6·8) containing 4% (w/v) SDS, 20% (v/v) glycerol and 0·042% (w/v) bromophenol blue. Gels were electrophoresed for 2–4 h at 300 V, fixed for 60 min at room temperature in methanol–acetic acid, and stained as directed using a silver stain kit (Sigma).

RESULTS

Egg production in immunosuppressed CFLP mice

Infections with each isolate became patent by day 32/35 (Fig. 1). The number of eggs released rose to a peak at day 50 p.i. and then declined rapidly. The patterns of egg production were similar for each isolate. The results also show that the most recently derived S isolate was equally capable of sustaining an infection in a laboratory-bred host.

Worm and egg measurements

Table 1 shows the mean lengths for 10 male and 10 female worms of each isolate recovered on day 35 p.i. from immunosuppressed CFLP mice. No significant difference was found, for either sex, between the isolates. The mean lengths and widths of fertilized eggs recovered from faeces on day 35 also showed no significant difference between the isolates, except that the S isolate eggs were significantly ($P = 0.04$) shorter than those of the E/K isolate.

Infection kinetics

A minimum of 3 replicate experiments was carried out to determine the time-course of infection with each isolate in the 3 strains of mice used. The data shown refer to representative experiments in each case.

Infection kinetics in high-responder CBA mice

The worm burdens on days 14, 18, 24 and 30 p.i. for each isolate of *T. muris* are shown in Fig. 2A. Worms of the E/N-isolate were expelled completely by day 24 p.i. Expulsion of the S isolate was significantly delayed compared with the E/N-isolate, about 30% of the initial worm burden still remaining on day 30 p.i. The E/K isolate was completely expelled by day 18 p.i. The number of E/K worms recovered on day 14 was significantly lower ($P = 0.004$) than that in permissive mice infected at the same time (Fig. 2C), indicating that expulsion had been initiated prior to this time.

Infection kinetics in low-responder C57Bl/10 mice

Worm recoveries on days 14, 21 and 28 p.i. are shown for each isolate in Fig. 2B. Expulsion of the E/N isolate occurred later than in CBA mice, more than 60% of the day 14 burden remaining at day 21. Similarly, a large proportion of the S isolate infection was recovered at day 28, when no E/N worms were

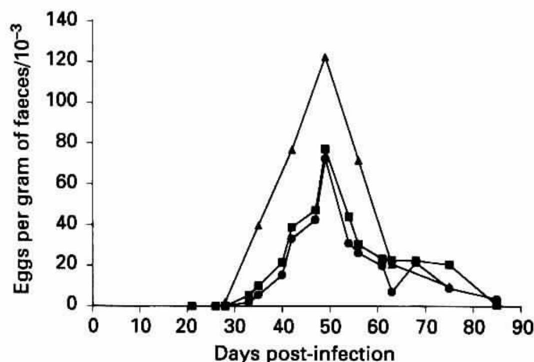

Fig. 1. A comparison of parasite egg release between 3 isolates of *Trichuris muris* during infection in immunosuppressed CFLP mice. The mice were given 400 embryonated eggs of the E/N (■), E/K (▲) and S (●) isolates and immunosuppressed by a combination of irradiation and hydrocortisone treatment.

Table 1. Measurements of adult worms and eggs of 3 isolates of *Trichuris muris* recovered from immunosuppressed mice infected with 400 eggs

	Length of adult worms (mm) (Mean ± S.D.)		Size of eggs (μm) (Mean ± S.D.)	
Isolate	Males	Females	Length	Breadth
E/N	23·4 ± 3·0	31·7 ± 4·3	131 ± 6	64 ± 3
E/K	22·3 ± 3·0	31·1 ± 2·8	133 ± 5	65 ± 2
S	21·8 ± 2·9	30·1 ± 2·9	128 ± 4*	65 ± 5

* Eggs of S isolate significantly shorter than those of the E/K isolate ($P = 0·04$).

found. As in CBA mice, the E/K isolate was expelled rapidly, but significantly more worms were recovered from C57Bl/10 mice at day 14.

Infection kinetics in permissive B10.BR mice

The worm burdens recovered from B10.BR mice infected with the 3 isolates on days 14, 20, 25 and 32 p.i. are shown in Fig. 2C. The S isolate showed no significant ($P > 0·05$) decrease in the number of worms during this time. Expulsion of the E/N isolate was slower than in the CBA and C57Bl/10 mice, but there was a significant ($P = 0·001$) decrease in the worm burden on day 32 p.i., suggesting that B10.BR mice did respond to the E/N isolate. The E/K isolate was expelled rapidly, expulsion being effectively complete on day 25 p.i.

SDS–PAGE separation of ES proteins

Fig. 3 shows a 12% acrylamide gel of 10 μg/lane of ES proteins from the 3 isolates of *T. muris*. The E/N and E/K isolates showed a similar distribution of

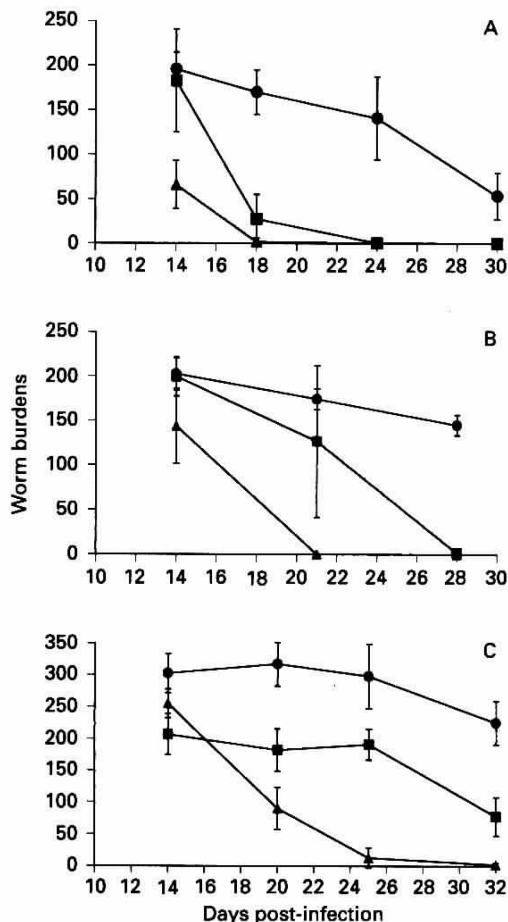

Fig. 2. (A) The expulsion kinetics of inbred CBA mice infected with 3 isolates of *Trichuris muris*. The CBA mice were infected with 400 embryonated eggs of the E/N (■), E/K (▲) and S (●) isolates and killed for worm burden recovery on days 14, 18, 24 and 30 post-infection (p.i.). (B) The expulsion kinetics of inbred C57Bl/10 mice infected with 3 isolates of *T. muris*. The mice were infected with 400 embryonated eggs of the E/N (■), E/K (▲) and S (●) isolates and killed for worm burden recovery on days 14, 21 and 28 p.i. (C) The expulsion kinetics of inbred B10.BR mice infected with 3 isolates of *T. muris*. The B10.BR mice were infected with 400 embryonated eggs of the E/N (■), E/K (▲) and S (●) isolates and killed for worm burden recovery on days 14, 20, 25 and 32 p.i.

protein bands and both were different from that of the S isolate at bands 80, 59, 26, 23, 21, 19 and 14 kDa.

DISCUSSION

The recently derived S isolate of *T. muris* established infections in immunosuppressed outbred mice comparable in intensity and duration with the long-term laboratory-adapted E/N and E/K isolates. All 3 isolates were morphologically similar, and only minimal differences were seen in the sizes of the

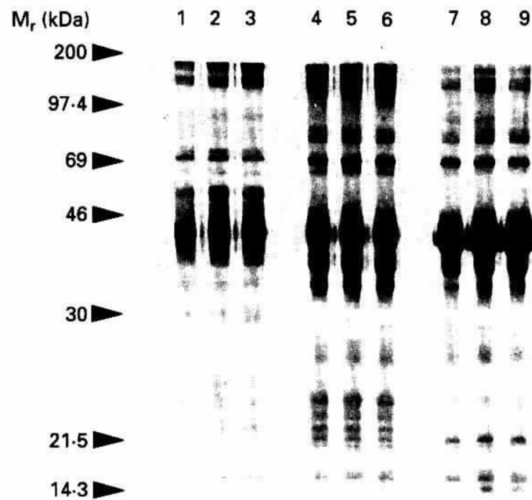

Fig. 3. SDS–PAGE electrophoretic separation of ES proteins from 3 isolates of *Trichuris muris*; 10 μg/lane of ES protein was run on a 12% acrylamide gel. The gel was stained using a silver stain kit (Sigma). Lanes 1–3 ES protein from the S isolate; Lanes 4–6 from the E/K isolate; Lanes 7–9 from the E/N isolate.

adult male and female worms or in fertilized eggs. When ES material released from adult worms maintained *in vitro* was separated on SDS–PAGE, similar protein banding patterns were obtained, although that of the S isolate showed some differences from the other two. These similarities suggest that the isolates belong to the same species, a conclusion supported by the fact that *T. muris* is apparently the only species recorded from *Mus musculus* (Skrjabin, Shikhobalova & Orlov, 1957). Despite these similarities, the 3 isolates showed some striking differences in the patterns of infection established in inbred strains of mice.

Three strains were used, whose response phenotypes have been defined previously in relation to infections with the E/N isolate. The rank order of these strains in terms of worm expulsion of this isolate, as determined by Else & Wakelin (1988), was CBA (expulsion in 3 weeks), C57Bl/10 (expulsion in 4 weeks) and B10.BR (no expulsion). The present data confirm this rank order, but show that B10.BR mice are capable of a late response to the E/N isolate. No strain appeared capable of expelling the S isolate completely in the period studied, only CBA mice achieving a significant reduction, whereas all expelled the E/K isolate within 25 days. The relative kinetics of infection with the 3 isolates (i.e. expulsion time: E/K < E/N < S) were similar in each strain.

The most interesting patterns of infection were seen in B10.BR mice, originally designated non-responsive to the E/N isolate because of its inability to bring about worm expulsion, this phenotype reflecting the additive influences of B10 background genes and of MHC-linked genes associated with the *k* haplotype (Else & Wakelin, 1988; Else *et al.* 1990). Recent studies have shown that non-responsiveness is associated with a switch in T helper (TH) lymphocyte activity from the TH2 to the TH1 subset, and with a predominant IgG_{2a} anti-worm antibody response (Else, Hultner & Grencis, 1992; Else, Entwistle & Grencis, 1993). It has been suggested that the TH switch is brought about in response to immunomodulatory antigens released by the later larval stages (Else *et al.* 1989, 1993).

It is clear that the B10.BR mice are fully capable of expelling the E/K isolate (as has also been shown by Ito – personal communication), and indeed do so comparatively rapidly. In contrast, they are quite unable to affect survival of the S isolate before patency, and show a delayed expulsion of E/N worms.

These differential patterns of host–parasite interaction between an inbred mouse strain and 3 isolates of a single species of nematode raise some important questions about the underlying immune phenomena. It can be predicted from the present observations and existing data on TH subset responses that the response of B10.BR mice to S isolate *T. muris* should be TH1 dominated, whereas that to the E/K isolate should be TH2 dominated. Equally, anti-S isolate IgG isotype responses should be predominantly IgG_{2a} and anti-E/K isolate responses predominantly IgG_1.

The greatest variation in host response was seen in mice infected with the S and E/K isolates. However, differences in host response to the E/N and E/K isolates were almost as marked, particularly in B10.BR mice, and this is surprising, given that both isolates originate from the same stock. This difference must have arisen during the 30 or so years that they have been maintained in separate laboratories. It is likely that less than 100 passage generations have been completed in that time, making the divergence quite remarkable. The availability of these isolates and the clear-cut difference in host–parasite relationships that they establish in mice provides an excellent model for addressing the molecular bases of this rapidly established phenotypic separation and of the distinct differences elicited in host immune response.

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