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Attempts to establish infections with *Strongyloides stercoralis* in mice and other laboratory animals

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ABSTRACT

Infection of a dog with *Strongyloides stercoralis* filariform larvae resulted in a persistent infection. Patent infections were not seen in rabbits, guinea-pigs, rats and 11 inbred strains and one outbred strain of mice. Manipulation of factors known to influence *S. ratti* infections in mice, such as age and sex of the host and the route of larval presentation, did not facilitate the appearance of rhabditiform larvae in the stools. Administration of immunosuppressive doses of corticosterioids to rabbits, guinea-pigs and C57B1/6 mice did not permit complete development. Similarly, the course of infection was not altered in T cell-deficient hypothymic (nu/nu) mice. The fate of filariform larvae applied to the skin of mice was ascertained; filariform larvae were observed to migrate from the skin via the lungs to the muscles within several days of infection. Although *S. stercoralis* does not develop to maturity in the small intestine of mice, this system does allow *in vivo* studies of the actions of anthelmintics against filariform larvae as well as a number of aspects of the immune response to this parasite.

INTRODUCTION

Strongyloidiasis is one of the major intestinal nematode infections. The causative organism, *Strongyloides stercoralis*, has peculiar biological behaviour for a worm since it has the capacity to replicate within the human host. It is this ability which accounts for the long duration of infection which may sometimes be seen. Similarly, it is the basis for the massive infection which may result in death, particularly in immunosuppressed persons.

Despite the importance of this disease, very little is known about the factors which influence the host-parasite relationship. One of the major reasons for this deficiency is the lack of a suitable animal model. Some strains of *S. stercoralis* have been shown to infect primates, dogs and cats but as yet there have been no reports of patent infections in small laboratory animals. Infection of such animals would provide considerable benefits as they are easier to manipulate and more economical to house.

The factors determining innate resistance of a particular host to infection are ill-understood, but the genetic factors are of undoubted importance (WAKELIN, 1978). Consequently, we have assessed the infectivity of *S. stercoralis* for several different host species and for a number of inbred strains of mice. Furthermore, since immune suppression may facilitate infection, we have investigated the effects of immunosuppression and immunodeficiency on the susceptibility to infection of some of these animals.

MATERIALS AND METHODS

Animals

Ten inbred strains of mice, A/J, AKR/J, AQR/SF, BALB/c, C57B1/6, C3H/He, C3H.SW, DBA/2, NZW and SJL/J mice were generously supplied by Dr. G. F. Mitchell (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Vic.). In addition, CBA inbred mice and Canberra multicoloured outbred mice were supplied by the Animal Breeding Unit, University of Western Australia (Perth, W.A.). In later studies, C57B1/6 mice were also obtained from this latter source. Unless otherwise stated, female C57B1/6 mice 18–22 g in weight were used. Congenitally athymic nude

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mice (nu/nu) backcrossed with BALB/c mice were supplied by Dr. G. F. Mitchell.

Female Sprague-Dawley rats, female New Zealand half-lop rabbits and male and female random bred guinea-pigs were supplied by the Animal Breeding Unit (University of W.A.). A mongrel dog, seven weeks old, was purchased from a pet shop; *S. stercoralis* larvae were not recovered from the stools before infection.

Strongyloides stercoralis

S. stercoralis was obtained from the faeces of a patient who had acquired the infection in Southeast Asia during the Second World War. Moistened faeces were kept for two weeks at room temperature on a watchglass in a petri dish containing water. Filariform larvae were removed from the water in the petri dish, washed twice in phosphate buffered saline (PBS) and adjusted to the desired concentration. Percutaneous infection (p.c.) was performed by applying filariform larvae to a shaved section of the anterior abdominal wall as described previously (DAWKINS *et al.* 1980). When larvae were injected subcutaneously (s.c.) 400 i.u./ml benzyl penicillin (Commonwealth Serum Laboratories, Melbourne, Vic.) and 400 μg/ml streptomycin sulphate (Glaxo Australia Pty. Ltd., Boronia, Vic.) were added.

Larvae were sought in the faeces of all animals. Faeces were collected, weighed, broken up with a glass rod in a small volume of water then suspended in 10 ml of water. Multiple 0·5 ml samples were examined for larvae as described previously (DAWKINS *et al.*, 1980).

Larvae were recovered from the tissues of mice as described elsewhere (DAWKINS & GROVE, 1981). In brief, tissues and organs from individual mice were homogenized at high speed for 15 sec in a Waring blender, suspended in 50 ml of PBS, centrifuged at 400 g for 10 min and then the number of larvae in the pellet counted.

Corticosteroids

Animals were treated with prednisolone (Predsol retention enema, Glaxo Australia Pty. Ltd., Boronia, Vic.) at an immunosuppressive dose of 1 mg/kg/day. This was administered in the drinking water in the case of rabbits and guinea-pigs, and by intra-oesophageal intubation of mice.

RESULTS

Patent infection in a dog

A puppy was infected p.c. with 2,000 filariform larvae. Rhabditiform larvae were first seen in the faeces three weeks later. Infection has persisted since that time and this animal has served as a source of *S. stercoralis* for many of the experiments described in this paper.

Rabbits, guinea-pigs and rats

Two rabbits, four guinea-pigs and three rats were injected s.c. with 1,000 filariform larvae. Patent infections failed to develop during the following six weeks.

Two rabbits and two guinea-pigs were treated with prednisolone beginning one day before the s.c. injection with 5,000 and 2,500 filariform larvae respectively. Patent infections did not develop.

Mice: attempts to achieve patent infection

(a) *Infection in inbred and outbred strains of mice.* Mice were injected s.c. with 400 filariform larvae derived from a patient. Four female animals were in each group. The faeces were collected from individual mice one, two, three, and four weeks after injection; no larvae were seen.

(b) *Influence of age*. Ten weanling female C57B1/6 mice and 10 female C57B1/6 mice three months old were injected s.c. with 200 filariform larvae and their faeces examined one, two, and three weeks after injection; no larvae were found.

(c) *Influence of sex*. Ten weanling male C57B1/6 mice were infected with 1,000 larvae derived from the dog. Faeces were examined one, two, three, and four weeks after infection; no larvae were seen.

(d) *Influence of route of infection*. Ten animals were infected percutaneously with 1,000 filariform larvae; a patent infection did not develop. One mouse was infected by intra-oesophageal intubation of 1,000 filariform larvae; no rhabditiform larvae were seen in the faeces.

(e) *Influence of corticosteroid administration*. Five mice were treated with prednisolone beginning one day before p.c. infection with 600 filariform larvae derived from the dog. Faeces were examined weekly for the next four weeks; no larvae were seen.

(f) *Infection in hypothymic mice*. Three BALB/c nu/nu mice were injected s.c. with 600 filariform larvae derived from the dog. Faeces were examined weekly for the next four weeks; no larvae were seen.

Mice: route of larval migration

In order to follow the fate of larvae after application to the skin, C57B1/6 mice were infected p.c. with larvae derived from the dog and were then killed by cervical dislocation one hour and one and four days after infection. The skin, lungs, small intestine, striated muscles, brain, liver, spleen and kidneys were all examined for filariform larvae.

Filariform larvae were found in the skin; 40%, 10% and 0·5% of larvae applied were recovered at one hour, one day and four days after infection, respectively. Larvae were found in small numbers in the lungs when examined one and four days after infection. By the fourth day after infection 7% of the larvae applied were found in the striated muscles and 0·5% were seen in the small intestine; all larvae were motile. No larvae were ever recovered from the other organs.

DISCUSSION

Rats and, more recently, mice infected with *S. ratti* have been the systems most frequently used for investigating factors affecting the host-parasite relationship in strongyloidiasis. Unfortunately, this model has a major disadvantage since *S. ratti* does not appear to replicate in these animals (Grove & Dawkins, in press), thus autoinfection does not occur as in human strongyloidiasis.

Considerable differences have been demonstrated in the susceptibility of inbred strains of mice to other intestinal nematodes including *Nematospiroides dubius*, *Trichuris muris* and *Trichinella spiralis* (reviewed by Wakelin, 1978). We have recently observed the same phenomenon in mice infected with *S. ratti* (Dawkins *et al.*, 1980). We therefore infected several species of animals, as well as a number of strains of mice, with *S. stercoralis*. Although the dog developed a chronic infection, other animals failed to develop patent infections.

Since young mice and male animals are more susceptible to infection with *S. ratti* (Dawkins *et al.*, 1980), we examined the effects of these parameters on infection with *S. stercoralis* in C57B1/6 mice. Again, no patent infections were seen. Similarly, manipulation of the route of infection failed to influence the course of infection.

We have shown in a strain of mouse innately resistant to infection with *S. ratti*, that administration of corticosteroids partly abrogated this immunity (Grove & Dawkins,

in press). Consequently we assessed the effects of immunosuppression on innate resistance to infection with *S. stercoralis*. Patent infections did not develop, however, in mice given pharmacological doses of corticosteroids or in T-cell-deficient hypothymic mice.

In order to ascertain the fate of *S. stercoralis* filariform larvae placed on the skin, various tissues were examined at several intervals after infection. FÜLLEBORN (1927) demonstrated that in unsuitable hosts, such as the rabbit, development was not completed but that *S. stercoralis* larvae persisted in the skin. In contrast to those observations, we found that in mice, larvae penetrated the skin and passed to the lungs in a fashion similar to that observed with *S. ratti* (DAWKINS & GROVE, in press). Instead of continuing the usual migration to and maturation in the gastro-intestinal tract however, many larvae localized in the muscles. A similar phenomenon of arrested development in muscles has been observed with ancylostomes in paratenic hosts such as rodents (NORRIS, 1971) and swine (STONE *et al.*, 1979).

Even though mice infected with *S. stercoralis* do not constitute an ideal model of human strongyloidiasis with maturation of worms and auto-infection, this system may still provide a useful investigatory tool. It allows *in vivo* studies of the efficacies and modes of action of anthelmintics against *S. stercoralis* larvae, rather than the less relevant *S. ratti*, as well as permitting quantification of a number of aspects of the immune responses to this worm.

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