STRONGYLOIDES STERCORALIS INFECTIONS IN THE MUSCLES OF MICE: A MODEL FOR INVESTIGATING THE SYSTEMIC PHASE OF STRONGYLOIDIASIS

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Summary

The sequence of events following infection of mice with the intestinal nematode, Strongyloides stercoralis, has been observed. Most infective larvae passed to the muscles where they did not develop further. In mice given primary infections, larvae were found in muscles for the first 9 d or so, then disappeared spontaneously. This was associated with an inflammatory reaction, predominantly eosinophilic and histiocytic in nature, around dying larvae. In mice exposed to the worms previously, both inflammation and worm destruction were hastened indicating the acquisition of resistance. A number of immunological parameters were measured in both primary and challenge infections. Specific antibodies of the IgM and IgG classes appeared, a marked immediate hypersensitivity reaction to injected antigen developed, and a transient blood eosinophilia occurred. No effects on phytohemagglutinin-induced spleen cell transformation were discerned, nor was transformation induced by specific antigen. It is concluded that this system provides a potentially useful model for investigating the systemic phase of strongyloidiasis, particularly with respect to assessing anthelmintic efficacy and the functions of fractionated antigens.

Key words: Strongyloides stercoralis, mice, animal model

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INTRODUCTION

Strongyloidiasis is one of the major human intestinal nematode infections. It is distributed widely throughout the tropics and subtropics, including parts of northern Australia. Infective larvae of the causative organism, Strongyloides stercoralis, penetrate the skin, pass via the bloodstream and possibly the connective tissues to the lungs where they ascend the airways, are swallowed and reach the gut where they mature. This parasite has unusual biological behaviour for a worm, however, 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, and is the basis for the massive infection which may result in death 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 suitable animal models. Patent infections develop in dogs infected with S. stercoralis and auto-infection occurs in some immunocompetent animals, while disseminated infection supervenes in immunosuppressed dogs. Although this model, therefore, resembles strongly the human infection, dogs are large animals that are difficult to work with and expensive to house. Consequently, many investigators have studied S. ratti infection in rodents, especially in rats, but this parasite has the inherent disadvantage of not having the capacity to autoinfect. Since rodents are much easier to manipulate and cheaper to maintain, we investigated several years ago the ability of S. stercoralis to produce patent infections in various inbred strains of mice and in immunodeficient mice. We found that patent infections did not develop but noted in preliminary observations that many infective larvae migrated to the tissues, particularly the muscles. Since this phenomenon offers the possibility of providing a means for studying the systemic phase of Strongyloides infection, we have examined these events in more detail.

MATERIALS AND METHODS

Animals Female Swiss albino mice 18-20 g in weight and 6-8 wk in age were supplied by the Animal Resource Centre, Murdoch, Western Australia.

Parasite The strain of parasite used was derived originally from a human who had acquired the infection in Southeast Asia many years previously. Since that time, the worm has been maintained in dogs. The methods used for the preparation of larvae and infection of mice and dogs have been described elsewhere. Mice were anesthetized, then larvae in 0.2 ml phosphate buffered saline were injected subcutaneously in the midline of the back in the region of the thoraco-lumbar junction.

Muscle worm burdens Mice were killed at intervals and muscles removed. The technique used for the quantification of larvae in muscles has been described elsewhere. Briefly, the hamstring muscles from one leg were removed, weighed, homogenized at high speed for 15 sec in phosphate buffered saline in a Waring blender, then the worms were counted and expressed as the number of larvae per gram of muscle. The methods used for the quantification of larvae in other tissues were the same as have been described for S. ratti infections of mice.

Histopathology The hamstring muscles from the other leg were removed and fixed in formalin for histological examination. Multiple histological sections 5 μm thick were prepared and stained with hematoxylin and eosin.
Immunological studies Soluble antigen was prepared from S. stercoralis filariform larvae as described earlier and then was sterilized by passage through a 0.45-μm filter (Millipore Corporation, Bedford, Massachusetts, USA). The methods used for measurement of footpad reactions, spleen cell stimulation and blood eosinophils were as described previously, except that S. stercoralis antigen was substituted for S. ratti and the following modifications were made: Spleen cells were incubated routinely with 3, 10 or 30 μg of phytohemagglutinin (PHA) or 0.75, 4.0 or 12 μg of S. stercoralis antigen for 3 d then pulsed with 3H thymidine for a further 24 h. Antibodies of the IgM and IgG classes specific for Strongyloides were measured by using an immunofluorescent assay as described earlier, except that S. stercoralis larvae and fluoresceinated sheep anti-mouse IgC and goat anti-mouse IgM reagents (Cappell, Cooper Biomedical, Malvern, Pennsylvania, USA) were used.

Statistics All tests of significance were done using Student's “t” test.

RESULTS

Worm burdens
Forty mice were injected subcutaneously with 3,000 larvae, then the muscle worm burdens were quantified in groups of 4 mice after 2 h and 1, 3, 5, 7, 9, 12 and 15 d. The numbers of larvae per gram of muscle at these times were 0, 15 ± 12 (S.D.), 55 ± 33, 55 ± 46, 52 ± 54, 50 ± 24, 0 and 0, respectively. Complete autopsies were performed on mice on day 15 and no worms were found in any tissues.

A second experiment was undertaken to determine whether prior infection hastened the rate of disappearance of worms after challenge infection. Twenty-four mice were injected subcutaneously with 300 larvae, then were given another 400 larvae 4 mth later. After another 2 mth, these mice together with the same number of age-matched control mice were injected subcutaneously with 8,000 larvae. The muscle worm burdens are shown in Fig. 1. In mice given a primary infection, similar results were obtained to those found in the first experiment, with most larvae having disappeared by day 12. In mice given a challenge infection, however, there was a highly significant accelerated destruction of larvae.

Pathological studies
Twenty-one mice were injected subcutaneously with 10,000 larvae. The pathological appearances in the muscles were then examined in groups of 3 mice at intervals over the next 2 wk. No abnormalities were seen in sections taken 2 h after injection but after 24 h, scattered larvae were seen. These worms were extracellular in location and were not surrounded by an inflammatory reaction (Fig. 2). Similar observations were made 3 and 6 d after infection. On the 8th day, the earliest stage of a generalized interstitial inflammatory infiltrate was observed. The inflammatory reaction at this stage was predominantly eosinophilic in nature and was not focused around worms. By the 10th day, however, a marked inflammatory reaction was seen in the interstitial tissues with accumulations of large numbers of cells around many larvae. Most of the cells were eosinophils but a few histiocytes were present. The cuticle of each worm was poorly defined, and the cells appeared to be invading the surface of the degenerating larvae (Fig. 3). The adjacent muscle fibres appeared normal. A few worms had no surrounding inflammation at this period.

By the 13th day after infection, all the worms were involved, with granulomas comprised of histiocytes, giant cells and eosinophils being found around remnants of necrotic larvae (Fig. 4).

The inflammatory reaction and destruction of larvae were accelerated in mice which had been exposed to the infection previously. The same mice were used for this experiment as were employed for the assessment of muscle worm burdens in challenged mice. Two days after challenge infection, sparse numbers of worms were seen and no inflammation was noted. Four days after challenge, larvae appeared undamaged and the muscle was infiltrated with small numbers of eosinophils. By 6 d after infection, an intense inflammatory infiltrate of eosinophils and macrophages was seen around dying larvae and foci of adjacent muscle fibres were necrotic (Figs 5, 6). Similar observations were made 8 and 12 d after infection.

Immunological responses
In the first experiment, 90 mice were given a primary infection with 1,000 larvae, then various parameters were measured in groups of 6 mice at weekly intervals. In a second experiment designed to assess the effects of prior exposure, 72 mice were infected with 1,000 larvae, then half of them were challenged 6 wk later with 1,000 larvae. Groups of 6 challenged and 6 control mice were assessed at intervals.

(1) Serum antibody levels Specific antibodies of the IgM class were first detected 1 wk after infection, peaked at 2 wk, then declined slowly over the ensuing weeks and were absent 10 wk after infection (Fig. 7). These antibodies were also present in the serum of mice which were given a challenge infection; the titre was augmented after the second exposure. IgG antibody titres followed a similar pattern, except that their appearance was delayed by 1 wk in mice with a primary infection and the rise in titre was accelerated in animals given a challenge infection.

(2) Footpad reactions Immediate hypersensitivity footpad reactions 15 min after injection of antigen are shown in Fig. 8. Significant reactivity was first noted 4 wk after primary infection (P < 0.02). The size of the reaction continued to increase until 10 wk after infection. In mice given a challenge infection, there was an anamnestic response with the increase of footpad thickness occurring earlier and to a greater degree.

No significant footpad reactions were seen 5, 24 or 48 h after injection during the 10 wk of observation of mice with primary infections or in the 6 wk of observation of animals with a challenge infection.

(3) Spleen cell stimulation a. Phytohemagglutinin stimulation. The stimulation indices when spleen cells from the control and infected groups of mice were incubated with 10 μg of PHA and compared with non-stimulated cells were 15 ± 9 (S.D.) and 20 ± 15, respectively, at week 0 of primary infection. No significant differences between the 2 groups of mice were seen 0, 1, 2, 3, 4 and 6 wk after
Fig. 1 Worm burdens (mean ± SEM) expressed as larvae per gram of muscle at various times after primary and challenge infections. Statistically significant differences between the two groups of mice are indicated.

Fig. 2 Day 1 after primary infection. An infective larva lying between striated muscle fibres 1 d after infection. No inflammatory reaction is present. H & E × 640 (Original magnifications are given in Figs 2-6).

Fig. 3 Day 10 after primary infection. A degenerating larva between muscle fibres and surrounded by eosinophils, macrophages and giant cells. H & E × 640

Fig. 4 Day 13 after primary infection. Remnants of a necrotic larva illustrating fragmented cuticle surrounded by a dense inflammatory infiltrate. H & E × 640

Fig. 5 Day 6 after challenge infection. Necrotic larva surrounded by a dense inflammatory infiltrate with necrosis of some adjacent muscle fibres. H & E × 160

Fig. 6 High power view of Fig. 5 showing a necrotic larva and inflammatory infiltrate composed predominantly of eosinophils and macrophages. H & E × 400
(4) Blood eosinophil levels A marked eosinophilia was seen 2 wk after primary infection (Fig. 9). This subsided over the next several weeks, there being no significant difference in blood eosinophil levels between infected and control mice by 4 wk after infection. In mice given a challenge infection, there was an accelerated response with the peak blood eosinophil level occurring one week after challenge; this level was not significantly higher than the peak found in mice with a primary infection.
DISCUSSION

We have investigated the course of infection in mice infected with S. stercoralis and have assessed the host response to the parasites. Since previous observations had indicated that the majority of worms were located in the muscles, we concentrated on this tissue in measuring worm burdens. In mice with primary infections, larvae reached the muscles quickly, then resided there for the first 9 d or so. They then disappeared rapidly, not only from these tissues, but also from all the organs of the host animal. Histopathological examination disclosed that this event was associated with an inflammatory reaction round the larvae in the muscles and destruction of the parasites 10 d or more after infection. This time sequence suggested that the mice may have acquired immunity to the worm, resulting in killing of the parasites. This belief was supported when the course of a challenge infection was determined in animals that had been exposed previously. As would be expected if immunity had been acquired, the inflammatory reaction was mounted earlier and larvae were eradicated from the tissues more quickly. The inflammatory infiltrate was composed primarily of eosinophils and histiocytes, and these cells appeared to be invading the cuticular surface of the dying worms.

We measured a number of immunological concomitants of these events. Specific antibodies of both the IgM and IgG classes appeared in the serum, and the titres of these antibodies were augmented after challenge infection. Five hours (Arthus) and 24 and 48 h (delayed) hypersensitivity reactions to antigen injected into the footpads were not seen, but a pronounced immediate (15 min) hypersensitivity reaction was found; this was first noted 3 wk after infection, increased progressively for the 10 wk of observation of the primary infection, and was enhanced in mice who had been exposed previously to the parasite. We were unable to detect any effects of the stimulation of spleen cell transformation by PHA, or to demonstrate the appearance of sensitivity to Strongyloides antigen on the part of these cells. Blood eosinophil levels, however, increased markedly following primary infection and appeared more rapidly in mice that received challenge infection. All these events are consistent with an immunological basis for the inflammation observed around larvae in the muscles.

Thus, although this system has some major disadvantages, it does allow dissection of the host-parasite relationship during the systemic phase of infection. Firstly, it permits an assessment of the efficacy of various anthelmintics against migrating larvae. Indeed, in our first attempt at this type of study, we were almost misled because we did not then appreciate the kinetics of infection and examined the muscles after all the larvae had disappeared spontaneously. Secondly, it provides a means by which the functions of various Strongyloides antigens can be examined. Strongyloides antigens can be fractionated and injected into mice, and the animals can be challenged with S. stercoralis infective larvae. Those antigens which are capable of inducing resistance to infection and/or of provoking an inflammatory reaction can be identified by measuring muscle worm burdens and assessing muscle inflammation. Compared with experiments in dogs, this can be done much more simply and economically, both in terms of finance and the quantities of antigen required. When promising anthelmintics or antigens are identified, they can be investigated further in the canine model.

The value of this system would be enhanced if another model could be developed for the investigation of the intestinal phase of strongyloidiasis. We are currently investigating the feasibility of this approach by attempting to recover S. stercoralis adult worms from the bowel of a host animal and transferring them to the intestinal tract of mice.

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References