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With 1 figure in the text

Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*

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SUMMARY

Primary immune expulsion of *Trichuris muris* was markedly delayed by concurrent infection with *Nematospiroides dubius*. Maximum delay of expulsion was dependent on size and timing of *N. dubius* infection relative to *T. muris* infection. In NIH mice infection with 400 *N. dubius* larvae immediately before or after *T. muris* infection was found to be most effective in suppressing expulsion. Infection on day 8 of *T. muris* infection, when mice are sensitized to *T. muris*, also impaired expulsion. From this evidence it is suggested that the larvae of *N. dubius* are immunosuppressive and that the efferent role of the immune response to *T. muris* is inhibited. The results are discussed in terms of non-specific immunosuppression and their relevance to the tropical disease situation is emphasized.

INTRODUCTION

Nematospiroides dubius, a trichostrongyle parasite of the small intestine of mice, gives rise to chronic primary infections, in which the worms persist with little evidence of reduced fecundity for up to 8 months (Ehrenford, 1954; Bartlett & Ball, 1972). There have been several reports indicating that this parasite interferes with the immune response to concurrently administered, non-related antigens. Chowaniec, Westcott & Congdon (1972) demonstrated suppression of the immune response to influenza virus in *N. dubius*-infected mice, and the response to sheep erythrocytes has been found to be markedly depressed during an infection with *N. dubius* (Shimp, Crandall & Crandall, 1975).

Collwell & Wescott (1973) reported that the longevity and the duration of egg production of *Nippostrongylus brasiliensis* were greatly prolonged in mice concurrently infected with *N. dubius*. More recent work, however (Jenkins, 1975), has indicated that this phenomenon may be associated with larval *N. brasiliensis* adapting to an immune response initiated by *N. dubius* since there is evidence of cross-immunity between these closely related parasites. The expulsion of primary *Trichinella spiralis* infections is also delayed in *N. dubius*-infected mice (Behnke, Wakelin & Wilson, in preparation) but in this situation there is no evidence of cross-immunity between the parasites involved.

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T. spiralis, *N. brasiliensis* and *N. dubius* live in the small intestine of the mouse and therefore the distribution of these parasites overlaps, a factor which may contribute to interaction between them. The present study examines the effect of concurrent *N. dubius* infections on the primary expulsion of *Trichuris muris*, a parasite of the caecum and colon, which in contrast does not overlap in distribution with *N. dubius* in the intestine of the host.

MATERIALS AND METHODS

Mice

Male mice of the inbred NIH and Balb/c strains were bred in this laboratory and were used at 6–8 weeks of age. Immune expulsion of primary *T. muris* infections from these strains is complete by approximately 15 and 22 days after infection respectively (Wakelin, 1975).

Parasites

(*a*) *Trichuris muris*. The methods used for maintenance of the parasite and infection of mice were essentially as described by Wakelin (1967). An infective dose of 400 eggs was used throughout this study.

T. muris were measured using a camera lucida and a calibrated map measurer. At least 30 worms were measured from each experimental group.

(*b*) *Nematospiroides dubius*. The strain of *N. dubius* was obtained in 1975 from the Wellcome Research Laboratories, Beckenham, and the parasite was maintained as stock infection in outbred CFLP mice. Infective 3rd-stage larvae were cultured as described by Bryant (1973). Mice were infected orally with the required number of larvae in 0·2 ml of suspension.

The infectivity of the cultures used in the experiments was assessed by worm counts, made not less than 10 days after infection. Mice were killed by an overdose of chloroform and the entire small intestine was removed. The worms were recovered by a modified Baermann technique as described by Wakelin & Lloyd (1976), but incubation was continued for 6–7 h. After the completion of incubation, 1 ml of formalin was added to each flask and these were stored either at room temperature or at 4 °C until examination was possible. The worms were transferred to a Petri dish and counted under a binocular dissecting microscope. In all experiments described here 90–100 % of the administered dose of *N. dubius* was recovered at autopsy. *T. muris* infection had no apparent effect on *N. dubius*, therefore the *N. dubius* counts are omitted from the results.

RESULTS

Concurrent infection with N. dubius *and* T. muris

Several preliminary experiments were carried out in which NIH mice were infected with 400 larvae of *N. dubius* and 400 eggs of *T. muris* on day 0. The mice were killed 15 days later and the worm burdens were assessed. The results of one

Table 1. *Effect of concurrent infection with* Nematospiroides dubius *on the expulsion of a primary infection of* Trichuris muris *from NIH mice*

Infection	No. of *T. muris* recovered (mean ± S.D.) Day of (*T. muris*) infection 9	15
T. muris	91·8 ± 24·3	2·5 ± 2·1
T. muris + *N. dubius*	Not done	107·5 ± 13·5

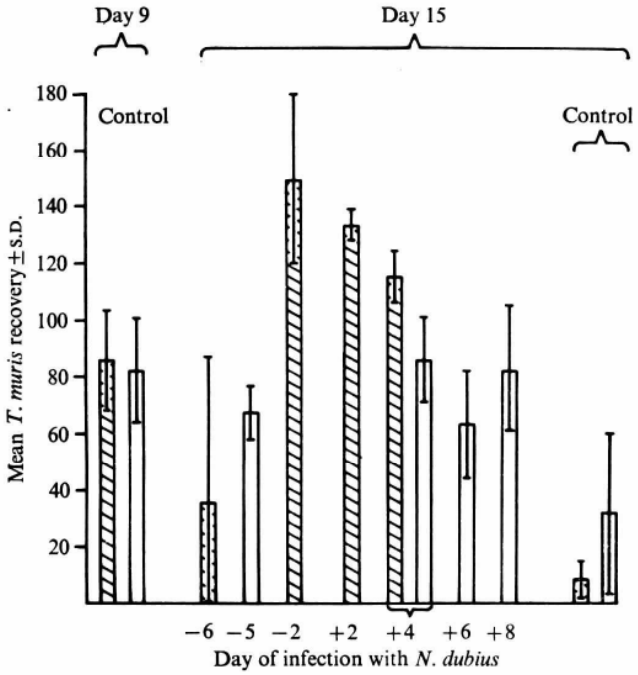

Fig. 1. Effect of *Nematospiroides dubius* infection at various times on survival of *Trichuris muris* in NIH mice (*T. muris* infection day 0, control mice were infected with *T. muris* only.) Shaded bars, Expt. 1; open bars, Expt. 2.

such experiment are presented in Table 1 and it can be seen that mice concurrently infected with *N. dubius* had not expelled *T. muris* by day 15 whereas control animals had lost most of their worms.

Determination of time of infection with N. dubius *for maximum suppression relative to* T. muris *expulsion*

Two experiments were carried out to determine the infection time with *N. dubius* required to achieve maximum suppression of the effective host response to *T. muris*. The results are given in Fig. 1.

In the first experiment (shaded bars) groups of NIH mice were given 400 larvae of *N. dubius* on either days −6, −2, +2 or +4 relative to *T. muris* infection

Table 2. *Duration of the prolonged survival of* Trichuris muris *in Balb/c mice concurrently infected with* Nematospiroides dubius

Infection	No. of larval *T. muris* recovered (mean ± S.D.) Day of *T. muris* infection 10	15	22	29
Expt. 1				
T. muris only	107·3 ± 9·9	102·0 ± 3·6	0·5 ± 0·9	Not done
T. muris + *N. dubius* (400)	102·6 ± 17·8	118·6 ± 14·8	129·3 ± 41·2	Not done
Expt. 2				
T. muris only	80·8 ± 7·5	Not done	0 ± 0	0 ± 0
T. muris + *N. dubius* (300)	85·0 ± 10·2	Not done	115·0 ± 11·8	10·6 ± 15·8

(day 0). In the second experiment (open bars) the respective intervals were days −5, +4, +6 or +8. Groups of control mice, infected with *T. muris* only were killed on days 9 and 15, whereas the doubly-infected mice were killed on day 15.

It can be seen from the results that all the groups infected with *N. dubius* showed a delay in immune expulsion of *T. muris* compared with mice infected with *T. muris* only, irrespective of the interval between administration of the two species of parasite. In the first experiment the most marked suppression was produced by *N. dubius* given on either days −2 or +2. A consistent degree of suppression was achieved in the second experiment where *N. dubius* was given on four occasions in relation to infection with *T. muris*.

Time course of T. muris *infection in Balb/c mice concurrently infected with* N. dubius

Two experiments were carried out in order to determine the duration of the survival of *T. muris* in Balb/c mice concurrently infected with *N. dubius*. In the first experiment a group of mice was infected with 400 larvae of *N. dubius* and infected 2 days later with *T. muris*, whilst a control group received *T. muris* only. Six mice from each group were killed 10, 15 or 22 days after the *T. muris* infection.

In the second experiment the dose of *N. dubius* was reduced to 300 larvae because the previous infection (400) was found to be lethal for about $\frac{1}{3}$ of Balb/c mice. Groups of doubly-infected and control mice were killed on days 29 and 35 as well as days 10 and 22 in order to determine whether the prolonged survival of *T. muris* extended to patency. The results of both experiments are presented in Table 2.

It is clear from these results that *N. dubius* exerted a considerable suppressive effect on the immune expulsion of *T. muris*. Although control mice had fully expelled the *T. muris* infection by day 22, in concurrently-infected mice no loss was observed at this time. In the second experiment the majority of *T. muris* had

Table 3. *Larval recoveries of* Trichuris muris *from NIH and Balb/c mice concurrently infected with different numbers of* Nematospiroides dubius *larvae*

No. of *N. dubius* larvae administered	Mean no. of *T. muris* recovered ± S.D.	
	Balb/c (killed day 22)	NIH (killed day 16)
400	Not done	82·5 ± 35·0
300	139·7 ± 62·1	34·0 ± 26·4
200	84·8 ± 65·5	71·5 ± 38·2
100	25·6 ± 45·8	30·2 ± 13·2
Control	15·3 ± 1·4	4·6 ± 5·2

The mean worm recovery from infectivity control groups was as follows: Balb/c (killed day 10) – 95·0 ± 10·1; NIH (killed day 9) – 93·0 ± 9·8.

been lost by day 29 from the experimental group, and by day 35 no *T. muris* were present in either control or doubly-infected groups.

The suppressive effect of *N. dubius* on the host response to *T. muris* was also evident from a comparison of the growth of *T. muris* in *N. dubius*-infected and control mice. On day 10, worms from both groups were similar in length, whereas on day 15 the *T. muris* recovered from the doubly-infected group were considerably longer (*T. muris* + *N. dubius* 2186 μm ± 462, $P < 0{\cdot}001$) than those from the group infected with *T. muris* alone (day 15 *T. muris* only 1629 μm ± 327).

Dose of N. dubius *required to delay expulsion of* T. muris

Two experiments were carried out in Balb/c and NIH mice respectively to establish the relationship between the level of infection with *N. dubius* and the degree of suppression of the host response to *T. muris*.

Groups of Balb/c mice were infected with 100, 200 or 300 larvae of *N. dubius* and 2 days later they were challenged with *T. muris*. Two groups of control mice were given *T. muris* alone and one such group was then killed on day 10, to determine the infectivity of the *T. muris* inoculum. The remaining control group and the experimental groups were killed on day 22. The results of this experiment (Table 3) show that all three levels of infection with *N. dubius* resulted in delayed expulsion of *T. muris* and that the degree of suppression was positively related to the dose of larvae administered.

NIH mice were used in the second experiment and because the expulsion of *T. muris* occurs earlier in this strain of mice, the experimental protocol was modified accordingly. Thus, the infectivity of the *T. muris* inoculum was assessed by the worms recovered from a control group killed on day 9, whereas the remaining groups were killed on day 16. An additional group given 400 larvae of *N. dubius* was included in this experiment since, unlike Balb/c mice, NIH mice tolerate this level of infection. The results of this experiment are in general agreement with those of the previous experiment although the degree of suppression was much more variable in relation to the level of infection with *N. dubius*. The group given 300 larvae of *N. dubius* was inconsistent compared to the mice given 200 or 400

larvae. Overall it can be seen that mice infected with *N. dubius* had more *T. muris* larvae than the control group and the rejection of the latter parasite was delayed in all of the experimental groups. The most consistent suppression was achieved in the group given 400 larvae of *N. dubius*.

DISCUSSION

It is evident from the results that mice concurrently infected with *N. dubius* have their ability to expel *T. muris* greatly impaired. This finding was consistent for the two mouse strains used which show different rates of expulsion for *T. muris* (see Materials and Methods). The degree of interference with the immune response to *T. muris* was, however, variable between experiments, within experiments and in relation to a variety of experimental conditions. Some of this variation is thought to be due to differences between batches of mice. The size of *N. dubius* infection was important, only the heaviest infection with *N. dubius* produced a consistent delay in the immune response to *T. muris* (Table 3). The relative timing of infection was also critical, suppression being maximal when the larval phase of *N. dubius* overlapped the *T. muris* infection for some time (Fig. 1).

Mice produce a strong immune response to the nematode *T. muris* which is expelled before the 4th week of infection (Wakelin, 1975). *T. muris* is a parasite of the caecum and therefore does not overlap in distribution with *N. dubius* and there is little possibility of direct interaction between these two species of parasite. *N. dubius* stimulates inflammation in the small intestine which may affect other organisms in the gut. The inflammation produced by the infection levels used in our experiments did not extend to the caecum as assessed by gross visual observation, however, this does not rule out the possibility of physiological changes in the large intestine.

Although there is much information concerning the immune response of the mouse to *N. dubius*, there is relatively little information relating to the long-term survival of the mature parasite in primary infections. There is some information indicating that *N. dubius* has a potent non-specific immunosuppressive effect on concurrent non-related responses in the host (Chowaniec *et al.* 1972; Shimp *et al.* 1975) and this immunosuppressive effect may be a mechanism by which the parasite maintains itself in the host for long periods. If this is so then *N. dubius* may also suppress responses to other helminths during concurrent infections, as our experiments suggest.

Antigenic competition may be an important factor in delaying the response to *T. muris* when *N. dubius* is given immediately before or after *T. muris* infection; however, this cannot be the only explanation since mice given *N. dubius* 8 days after infection with *T. muris* also showed delayed rejection of the latter parasite (Fig. 1). Since it is known that mice become sensitized to *T. muris* before the 8th day of infection (Jenkins, in preparation), the immunosuppression must in this case have been exerted by the larval stages of *N. dubius* and must have acted against the effector mechanism of the immune response against *T. muris*. The rapidity with which larval *N. dubius* produced immunosuppression raises the

possibility that when given earlier in the *T. muris* infection the suppression may have acted against the afferent role of the immune response.

The evidence that immunosuppression can be caused by adult *N. dubius* is less clear-cut, but the fact that *T. muris* is retained in Balb/c mice until at least day 22 of infection when the concurrent *N. dubius* infection had been adult for approximately 15 days is strongly suggestive (Table 2).

Brown, Crandall & Crandall (1976) showed that *N. dubius* caused an increased IgG catabolism in infected mice and suggested that this is a contributing factor to immunosuppression, but the cause of the breakdown of IgG is not known. There is evidence that the immunosuppressive effect of *T. spiralis* new-born larvae on responses to non-related antigens is attributable to the release of agents toxic to lymphoid cells (Faubert, 1976). The results of our experiments show that a strong immunosuppressive effect is generated by *N. dubius*, particularly during the larval phase in the intestine wall. The rapidity of the suppression induced by infection on day 8 of *T. muris* infection suggests that a similar mechanism may be operating in *N. dubius*-infected mice.

The results of this study are pertinent to the natural situation. Human endemic hookworm and whipworm infections overlap over large areas of the world and if immunosuppression is a general phenomenon then hookworm infections may well prolong the survival of *Trichuris trichiura* in human hosts. The model system described here is therefore relevant to the study of pathogenesis associated with concurrent tropical diseases.

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