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Non-specific immunodepression by larval and adult *Nematospiroides dubius*

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SUMMARY

Mice infected with *Nematospiroides dubius* generate weaker immune responses to concurrently administered sheep red blood cells (SRBC), than non-infected controls. The experiments described in this paper demonstrate that both adult and larval stages of *N. dubius* cause non-specific immunodepression of the response to SRBC. Mice which had been infected with larvae exposed to 25 krad. of irradiation, which prevents development to the adult luminal stages, produced as weak haemagglutination responses to SRBC as mice infected with normal worms even when SRBC were administered 6 weeks after infection. The removal of adult *N. dubius* by treatment with pyrantel 9, 11 and 15 days after infection with normal larvae did not restore the host's ability to respond to SRBC given on day 14. It was only when the mice had been without worms for 17 days that their capacity to respond normally to SRBC was restored. Mice infected with 60 or 400 transplanted adult worms produced depressed haemagglutination and plaque-forming responses to concurrently injected SRBC when compared with normal or sham-operated controls. The significance of these results is discussed in relation to the possible role of non-specific immunodepression in facilitating the survival of *N. dubius* in the host.

INTRODUCTION

The parasitic nematode *Nematospiroides dubius* survives for a relatively long period of time during a primary infection in its host (Williams, 1982; Williams, Pascoe & Behnke, 1982) and there is growing evidence that the parasite facilitates its own survival by interfering with the host's ability to express an effective response at the intestinal level (Behnke, Wakelin & Wilson, 1978; Hagan & Wakelin, 1982; Dobson & Cayzer, 1982; Behnke, Hannah & Pritchard, 1983). Recent reports have proposed a role for adult worms in suppressing the expression of homologous immunity by the mouse (Behnke *et al.* 1983). Indeed, Dobson & Cayzer (1982) have suggested that this effect may be mediated through circulating immunodepressive factors in the serum of mice harbouring adult worms, a conclusion which is not supported by our demonstration that primary infection serum from CFLP strain mice does not impair immunity adoptively transferred

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by concurrently administered immune mesenteric lymph node cells (Williams & Behnke, 1983).

Non-specific immunodepression during infection with *N. dubius* has also been demonstrated with respect to concurrently administered viruses (Chowaniec, Wescott & Congdon, 1972), sheep red blood cells (Shimp, Crandall & Crandall, 1975; Ali & Behnke, 1983), LPS and oxazalone (Ali, 1983). Non-specific immunodepression of the response to SRBC is dependent on the number of *N. dubius* larvae administered and on the relative timing of infection and injection of cells. Thus non-specific immunodepression is maximal when SRBC are given 2 weeks after infection with the parasite, suggesting that stage-specific immunodepression may be involved (Ali & Behnke, 1983).

However, other reports in the literature indicate that non-specific immunodepression may be caused by both larval and adult stages of the parasite. Thus Jenkins & Behnke (1977), studying the interactions between *N. dubius* and *Trichuris muris* could only explain their results in terms of a rapid immunodepressive effect by the larval stages of *N. dubius*, impairing the effector mechanism of the immune response against *T. muris*. However, the above authors and Behnke *et al.* (1978) proposed an additional role for adult worms, since long-standing infections with *N. dubius* in which only adult worms were present still exerted some immunodepressive effect against the heterologous species. The latter authors also found that elimination of adult *N. dubius* by treatment with pyrantel shortly before infection with *T. spiralis* enabled the mice to respond normally to *T. spiralis*.

In view of the current interest in the mechanisms by which parasites evade host immunity and the growing evidence that in the case of *N. dubius* non-specific immunodepression has a significant role to play in this respect, it was clearly important to determine the relative contribution of larval versus adult worms. In the present paper we extend our previous work by examining non-specific immunodepression of the response to SRBC in mice infected by irradiated larvae of *N. dubius*, by truncated infections in which adult worms were removed before maturity and by transplanted adult worms.

MATERIALS AND METHODS
Animals

Randomly bred CFLP mice were used throughout this work. The mice were originally obtained from commercial suppliers (Hacking and Churchill Ltd, Huntingdon) and were bred and maintained under conventional animal house conditions.

Nematospiroides dubius

The strain of *N. dubius* used in the present study was obtained in 1975 from the Wellcome Research Laboratories (Beckenham, Kent), and has been maintained since in CFLP mice. Maintenance of the parasite and the methods used for infection of animals and recovery of worms have already been described (Behnke & Wakelin, 1977; Jenkins & Behnke, 1977). The infective 3rd-stage larvae of *N. dubius* were exposed to gamma radiation from a ^{60}Cobalt source in the Chemistry

Department of Nottingham University. The methods used for irradiation have previously been published (Behnke, Parish & Hagan, 1980). In some experiments adult worms were introduced directly into the small intestine as described by Behnke *et al.* (1983).

Determination of haemagglutination titres and plaque-forming cells to SRBC

Standard laboratory techniques were used to measure haemagglutination titres and plaque-forming cells to SRBC in infected mice. A detailed description of the methods involved was reported by Ali & Behnke (1983).

Anthelmintic

Pyrantel embonate (Strongid-P paste, Pfizer) was used to remove adult *N. dubius* from the intestines of infected mice. A dose of 100 mg/kg body weight was administered orally as an aqueous suspension. This dose level is known to be more than 95 % effective in removing adult worms from the intestinal lumen. Treatment with pyrantel 24 h before infection with *N. dubius* has no effect on larval establishment.

Statistical analysis of results

Unless otherwise stated groups of 6 mice were used throughout this work. The results are expressed as the group mean $\pm$ s.e. Where s.e.'s on the Figs overlap, only one s.e. is shown or alternatively the s.e. bar of one group is shown slightly to one side of the others. Statistical significance was determined by the non-parametric Wilcoxon test (Sokal & Rohlf, 1969). A value of $P < 0.05$ was considered to be significant.

RESULTS
The immune response to SRBC in mice infected with irradiated larvae

The exposure of the infective 3rd-stage larvae of *N. dubius* to gamma irradiation has a profound effect on the subsequent development of these larvae in the host, especially when they are exposed to a high level of radiation, for example 25 krad. At this level of irradiation, the larvae do not complete their development and hence they are a useful experimental tool since after infection mice experience only the larval stages of this parasite (Behnke *et al.* 1980).

In the first experiment (Table 1), groups of 6 mice were infected either with larvae exposed to different levels of irradiation (Groups B, C, and D) or with normal larvae (Group E) or were left uninfected (control Group A). All the infected groups were given *N. dubius* 14 days before injection with SRBC. The mice were bled every 4 days and the haemagglutination antibody titres were followed for 20 days. All the infected groups irrespective of whether the larvae were normal or irradiated had considerably reduced haemagglutination titres on day 4 and all these groups appeared to be equally immunodepressed. On day 8 the difference between the control group (A) and the infected groups was smaller but nevertheless still significant. The mean worm recovery from the infected groups established that

Table 1. *The immune response to SRBC in mice infected with irradiated larvae of* Nematospiroides dubius

		Haemagglutinating antibody response† to SRBC on different days (mean ± s.e.)				
Days after SRBC injection	No. of mice	Group A (no larvae)	Group B (25 krad.)	Group C (15 krad.)	Group D (5 krad.)	Group E (normal larvae)
4	6	6·5 ± 0·3	4·3 ± 0·4‡	4·0 ± 0·4‡	3·8 ± 0·5‡	3·3 ± 0·3‡
8	6	8·5 ± 0·2	7·3 ± 0·3‡	7·2 ± 0·3‡	7·3 ± 0·4‡	6·6 ± 0·3‡
12	6	9·8 ± 0·3	9·0 ± 0·4‡	8·8 ± 0·5‡	8·5 ± 0·4‡	7·8 ± 0·3‡
16	6	9·0 ± 0·3	8·4 ± 0·2‡	8·6 ± 0·2	8·2 ± 0·4‡	8·6 ± 0·5
20	6	6·5 ± 0·2	6·3 ± 0·3	6·1 ± 0·3	6·0 ± 0·4	6·3 ± 0·3
Mean worm recovery ± s.e.		—	1·5 ± 0·55	121·6 ± 25·5	285·5 ± 18·5	423·2 ± 3·22

† Log$_2$ titre: groups infected with 400 larvae exposed to different levels of radiation.
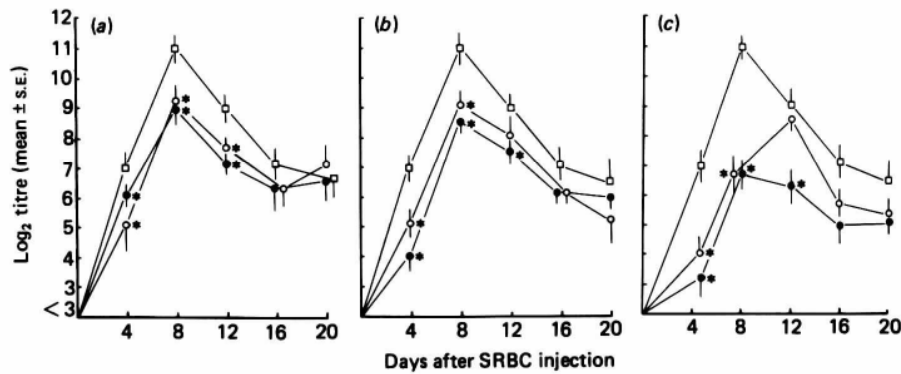‡ Significantly lower than control Group A.

Fig. 1. Haemagglutination response to SRBC in CFLP mice infected with 400 normal larvae or 400 irradiated larvae of *Nematospiroides dubius* given 6 (*a*), 4 (*b*) or 2 (*c*) weeks prior to challenge with SRBC. (●), Group infected with 400 normal larvae; (○), group infected with 400 irradiated larvae (25 krad.); (□), control group – no infection. The mean worm recovery from the groups infected with normal or irradiated larvae were as follows: 6-week interval (*a*), 402 ± 15·3 and 7·3 ± 3·4, respectively; 4-week interval (*b*), 386·6 ± 9·7 and 4·0 ± 3·5, respectively; 2-week interval (*c*), 431·5 ± 4·5 and 8·8 ± 4·7, respectively. Statistical analysis of results; * – significantly lower than the corresponding control group.

mice infected with larvae exposed to high levels of irradiation (25 krad.) harboured very few adult worms (1·5 ± 0·5) when compared to Groups C, D or E. In all subsequent experiments, therefore, larvae were irradiated with 25 krad.

Fig. 1 illustrates the results of an experiment in which groups of 6 CFLP mice were infected either with normal or irradiated larvae (25 krad.) at different times in relation to the challenge with SRBC. Three time intervals were considered, 6, 4 or 2 weeks. It can be seen from the results that the groups of mice infected with normal or irradiated larvae behaved identically with respect to the response to

Table 2. *Direct plaque-forming cell response to SRBC in CFLP mice infected with normal or irradiated larvae of Nematospiroides dubius*

Group	No. of mice	Infection with N. dubius†	SRBC	Mean PFC/spleen ±s.e.‡	Percentage reduction	Mean PFC/10^6 spleen cells ±s.e.	Percentage reduction	Mean worm recovery ±s.e.
A	6	Irradiated larvae	+	27333±1488·6	42·3	30·02±1·96*	39	7·0±1·68
B	6	Normal larvae	+	22300±558·1	52·9	20·85±1·76**	57·7	420·0±8·29
C	6	No infection	+	47333±2296·9	—	49·2±3·12	—	—
D	4	No infection	−	1700±173·2	—	1·50±0·19	—	—

Statistical analysis: groups were compared to group C. *$P = 0.013$; **$P = 0.008$.

† Mice were infected with 400 normal or irradiated larvae 14 days before injection of SRBC.

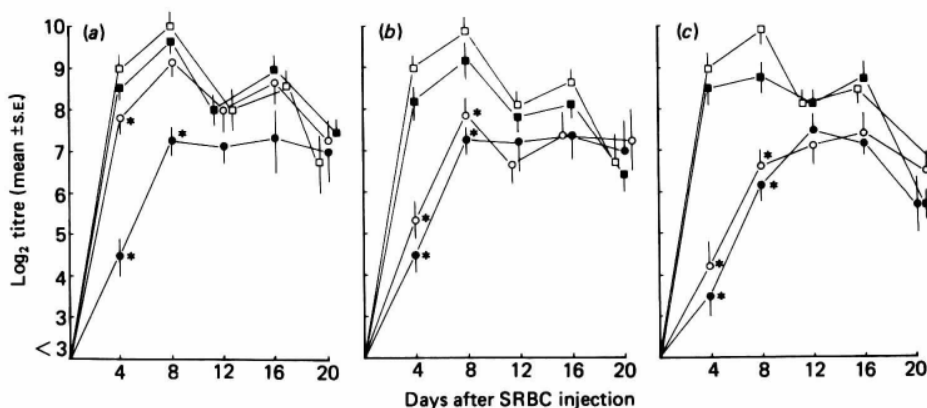‡ PFC were determined 4 days after SRBC injection.

6

Fig. 2. Haemagglutination response to SRBC in CFLP mice in which *Nematospiroides dubius* were removed by treatment with pyrantel, at various times prior to the administration of SRBC. (●) Group infected with 400 larvae; (○) group infected but given anthelmintic; (■) control group given anthelmintic; (□) control group – no infection. The mean worm recovery from the infected groups and infected groups treated with pyrantel were as follows: (a) $416·0 \pm 12·2$ and $2·3 \pm 1·0$ respectively; (b) $370·0 \pm 7·4$ and $1·5 \pm 0·8$ respectively; (c) $406·0 \pm 11·3$ and $5·0 \pm 1·4$ respectively. Statistical analysis of results: * – significantly lower than the corresponding control group. The interval between removal of *N. dubius* and challenge with SRBC was (a) 17 days; (b) 10 days; (c) 3 days.

SRBC at any particular interval. Depression of the response (day + 4) was maximal when a 2-week interval was used but decreased with a 4-week interval and was least in mice infected for 6 weeks.

In a final experiment (Table 2) mice were infected with normal or irradiated larvae of *N. dubius*, challenged with SRBC 14 days later and the direct plaque-forming cell responses to SRBC were determined on day 4 after SRBC administration. It was found that normal and irradiated larvae depressed the response by 52·9 and 42·3 % respectively.

The immune response to SRBC in mice exposed to abbreviated infections with N. dubius

An alternative approach by which infection can be limited to only the larval stages of *N. dubius*, consists of treating the host with anthelmintic drugs immediately after the larval parasites have completed their development to juveniles and have returned to the intestinal lumen.

Accordingly, several experiments were carried out along these lines and representative experiments are presented here. Fig. 2 summarizes the results of one such experiment in which groups of mice were infected with *N. dubius* at various intervals before the administration of SRBC (4, 3 and 2 weeks). One group of infected mice was given pyrantel 9, 11 and 15 days after the infection. Treatment with pyrantel on day 9 should remove 50–80 % of the worm burden, and the dose on day 11 should have reduced the worm burden to less than 5 % of that in untreated mice. Therefore, taking day 11 as the day on which > 95 % of the worms

would have been eliminated, the experiment comprised 3 groups of mice which experienced larval worms only and were without infection 3 days, 10 days and 17 days respectively on the day SRBC were given. The results show that the haemagglutination response to SRBC was not significantly altered in control mice treated with pyrantel. In mice infected with *N. dubius* on day −14 (Fig. 2c) and day −21 (Fig. 2b) and subsequently treated with pyrantel, the response was as depressed as in the groups which retained the worm burden. Only the group which was without worms for 17 days prior to the administration of SRBC recovered its ability to respond normally to SRBC (Fig. 2a).

In a replicate experiment, 30 CFLP mice were infected with 400 larvae of *N. dubius* on day −14. Six infected and 6 control mice were challenged with SRBC on day 0 and plaque-forming cells (PFC)/spleen were determined 4 days later. The infected mice had 62·4 % fewer plaques than the control group (41 000 ± 2150 PFC/spleen). The remaining 24 infected mice together with 24 control mice were treated with anthelmintic on days 0, +1 and +2 and groups of 6 infected and 6 control mice were challenged with SRBC on days +2, +7, +14 and +21 respectively. The results confirmed that there was some recovery of the hosts' ability to respond to SRBC when *N. dubius* was removed before injection with antigen but this recovery was slow, and even mice which had been without infection for 14 days (31·2 % reduction in PFC/spleen) and 21 days (42 % reduction in PFC/spleen) were still markedly depressed.

The immune response to SRBC in mice infected by transplanted adult worms

The role of adult worms was examined by transplanting adult worms from donor mice into recipients which had not experienced larval infection. By way of comparison, groups were also included which had been given a larval infection and others (sham controls) which were subjected to laparotomy but which received saline without worms.

Fig. 3 summarizes the results from 2 experiments, one in which approximately 400 worms were transplanted (Fig. 3a) and the other in which 60 worms were given (Fig. 3b). The first experiment (Fig. 3a) comprised 4 groups of mice, namely; an untreated control group, a sham-operated control group, a group given adult worms by laparotomy on day −14 and a group infected with 400 larvae of *N. dubius* on day −14. All the mice were given SRBC on day 0. The results show that there was a marked depression in haemagglutination titre of both infected groups on day 4 and day 8 and a significantly reduced peak titre on day 12. Both control groups (normal and sham operated) responded almost identically throughout the course of the experiment. As part of this experiment some mice were killed 4 days after the administration of SRBC for the determination of the plaque-forming response. The control group had 36 200 ± 3460 PFC/spleen and the sham-operated group had 11·05 % fewer PFC/spleen. In contrast, both infected groups were significantly depressed (group given adult worms − 60·07 % reduction and group given larvae − 64·5 % reduction in PFC/spleen).

In the second experiment (Fig. 3b) a group of mice received approximately 60 adult worms by laparotomy. It is clear that even at this level of infection adult worms were able to exert a marked immunodepressive effect on the host.
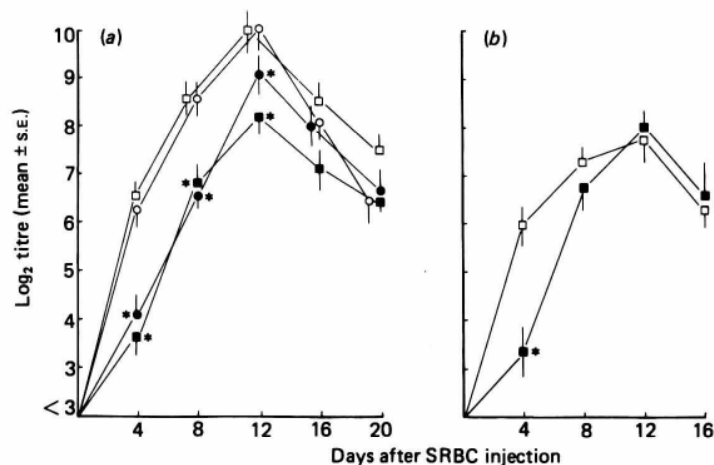
Fig. 3. Haemagglutination response to SRBC in CFLP mice infected by transplanted adult worms. (●), Group infected with 400 larvae; (○), sham-operated control group; (■), group infected with 400 (*a*) or 60 (*b*) transplanted adult worms; (□), control group – no infection. The mean worm recovery was as follows: (*a*) group infected with larvae – 425.0 ± 14.6, group infected with adult worms – 335.0 ± 25.3; (*b*) group infected with 60 adult worms – 54.5 ± 9.8. Statistical analysis of results; * – significantly lower than the corresponding control group.

DISCUSSION

The present paper examines the relationship between non-specific immunodepression and the developmental stage of the parasite involved. Three experimental approaches were used, namely irradiated larvae, truncated and transplanted infections. Each resulted in the host having only limited experience of either adult or larval parasites.

In a previous publication (Ali & Behnke, 1983) we demonstrated that maximum levels of non-specific immunodepression were observed when mice were infected 14 days before administration of SRBC and that the degree of immunodepression was less after infections of longer duration (i.e. 6 weeks). This implied that whilst adult worms were capable of inducing some degree of immunodepression, there was an additional component, maximal at 14 days but waning with time, possibly caused by the trauma of emerging larval parasites. The present demonstration that adult worms do cause non-specific immunodepression raises the possibility that this phenomenon and the depression of homologous immunity (Behnke *et al.* 1983) are related events and may have a common cause, i.e. a worm-derived mechanism which prevents the host from expelling worms during a primary infection. A further possibility is that in heavy longstanding infections the major physiological priority for the host is the maintenance of homeostasis in order to counter the pathological consequences of infection. The presence of numerous secretory/excretory proteins produced in large quantities by the parasite (Day, Howard, Prowse, Chapman & Mitchell, 1979) may pose a formidable assault on the host's mechanisms of defence. Under these circumstances the host may simply be unable to give priority to unrelated immune responses. Furthermore, there is evidence

of a recovery in the host's ability to respond to SRBC in longer infections (Fig. 1), a feature which is not compatible with the fact that homologous immunity is depressed for a considerably longer period of time, since primary infections last for some 8 months or more (Williams *et al.* 1982).

The experimental approaches involving irradiated and truncated infections have established that larval parasites can also induce strong non-specific immuno-depression. Indeed, in mice infected by irradiated larvae in which very few adults mature, this effect was so long-lasting that depressed SRBC responses were still demonstrable when SRBC were given 6 weeks after infection (Fig. 1). There was some recovery here but it was not significantly different from that of mice which had been infected concurrently with normal worms and had retained the full worm burden on the day SRBC were given. Additional evidence for recovery from immunodepression induced by larvae was provided in the experiment illustrated in Fig. 2, where mice which had been without infection for 17 days were observed to respond normally. On the basis of these observations it can be concluded that larval *N. dubius* cause non-specific immunodepression and that this effect is long-lasting carrying over into the period of adult worms. The considerably longer lasting effect of the irradiated worms may be explained by their survival as stunted individuals within the tissues of the intestinal tract. It is not known how long irradiated parasites may survive as larval forms within the walls of the intestine.

The observation that the non-specific immunodepression caused by larval parasites is long-lasting suggests that the mechanisms involved may be different from those concerned in the evasion of host immunity. Three lines of evidence support this conclusion. Firstly, mice infected with irradiated larvae (25 krad.) develop solid immunity to challenge infection with *N. dubius* even when given 14–21 days after the irradiated immunizing infection (Hagan, Behnke & Parish, 1981; Robinson & Behnke, 1983), this despite the non-specific immunodepression which has been described in the present paper. Secondly, mice treated with pyrantel 9 and 10 days after a single primary infection with *N. dubius* express very strong immunity to re-infection when challenged on day 21. In the absence of adult worms, these mice show 90 % protection (Robinson & Behnke, 1983) and hence the long-lasting immunodepression induced by larval parasites cannot extend to challenge with the homologous infection. Finally, Behnke *et al.* (1978) found that when mice infected with *N. dubius* were treated with pyrantel 4 days prior to infection with *T. spiralis*, the host's ability to expel the latter parasite within the normal period was completely restored.

In conclusion, our experiments have demonstrated that both larval and adult stages of *N. dubius* cause non-specific immunodepression of the response to SRBC. However, the precise significance of this phenomenon to the mechanism by which the parasite evades host immunity is still not clear. Nevertheless, the factors involved are of considerable interest and may explain why only limited success has been achieved in vaccinating with the excretory/secretory products of this parasite (Hurley, Day & Mitchell, 1980).

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