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Stimulation of immunity to *Nematospiroides dubius* in mice using larvae attenuated by cobalt 60 irradiation

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Summary Infective larvae of *Nematospiroides dubius* attenuated by cobalt 60 irradiation are extremely effective at stimulating immunity. Previously, such levels of protection could only be obtained with multiple immunizations of normal larvae. The critical factor underlying this protective response appears to be the dose of irradiation given to the immunizing infection. Various doses of irradiation have been tested and the most effective of these range between 10 and 30 krad. The experiments show that provided this level of irradiation is used, the number of immunizing infections is relatively unimportant. Such use of irradiated larvae will be of value in attempting to analyse the immune mechanisms which operate against *N. dubius*. The possible mechanisms of immunity to *N. dubius* are discussed.

Key words: *Nematospiroides dubius*, nematode, immunity, irradiated larvae

Introduction

The nematode, *Nematospiroides dubius* (*Heligmosomoides polygyrus*) in mice provides a convenient laboratory model of a chronic parasitic infection. After an 8-day tissue phase in the muscular wall of the gut, the parasites emerge to the lumen of the small intestine, where, as adults, they can survive for up to 8 months (Ehrenford 1954).

Coupled with this long survival is the failure of a primary infection to stimulate protective immunity to a subsequent challenge. Most attempts to stimulate immunity have used multiple immunization schedules involving a series of infections and drug treatments (Behnke & Parish 1979a). As shown by Prowse, Ey & Jenkin (1978), two infections would appear to be the minimum effective immunizing dose. They found one dose to be ineffective, but obtained greater than 95% protection with two doses.

The use of irradiation-attenuated parasites to stimulate immunity has never been attempted in this system, although the effects of cobalt 60 irradiation on the survival of the

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parasite have been reported (Behnke, Parish & Hagan 1980). If irradiation-attenuated parasites proved to be effective they would provide an extremely useful tool with which to evaluate the immune mechanisms that operate, often ineffectively, against *N. dubius*.

This paper describes the results of experiments designed to determine, firstly, whether irradiated parasites do stimulate immunity and, secondly, the combination of dose of irradiation and number of immunizing doses that is most effective in stimulating this immunity.

Materials and methods

Experiments were carried out in both Nottingham and Glasgow Universities.

Animals

Inbred male NIH mice were used in all experiments. These were purchased from Hacking and Churchill Limited (Huntingdon) or bred under conventional animal house conditions in the Zoology Department of Nottingham University. Mice, 6–8 weeks old at the start of each experiment, were killed in groups of six or seven.

Nematospiroides dubius

The strain of *N. dubius* used in the present study was obtained in 1975 from the Wellcome Research Laboratories, Beckenham, and has since been maintained in outbred CFLP mice. The maintenance of the parasite, and the methods used for infection and recovery of worms have already been described (Behnke & Wakelin 1977, Jenkins & Behnke 1977).

Irradiation of infective larvae

Larvae were irradiated using a cobalt 60 source as described by Behnke *et al.* (1980).

Anthelmintic

Pyrantel embonate (strongid-P paste, Pfizer) was used to remove adult *N. dubius* from infected mice. A dose of 100 mg/kg was administered orally as an aqueous suspension. This dose level is known to be adequate for the removal of all adult worms from the intestinal lumen (Behnke & Wakelin 1977).

Faecal egg counts

Half a gram to one gram of fresh faeces collected each morning from the pooled faeces of all the mice in each group was dispersed in 30 ml of 50% saturated saline. This suspension was washed through a sieve (aperture size 300 μm) with 100% saturated saline and the eggs were counted after flotation in standard McMaster counting slides as described by Gordon & Whitlock (1939).

The counts were expressed as the number of eggs per gram of faeces.

Statistical analysis of results

The results were analysed by the non-parametric Wilcoxon test. A value of $P < 0{\cdot}05$ was considered to be significant.

Results

EFFECT OF IMMUNIZATION WITH NORMAL OR IRRADIATED LARVAE

In the first experiment, two groups of mice were infected with 200 normal or 200, 10 krad irradiated larvae on day 0 and both these groups, plus a group of age-matched controls, were treated with pyrantel on days 34 and 35. All the groups were infected with 100 normal larvae on day 42 and mice were killed on 14 and 35 days post-challenge. The results are shown in Figure 1.

Table 1. Experiment 2. Comparison of normal and irradiated larvae in stimulation of immunity

	Immunization	Worm recoveries (mean ± s.d.) Day + 14 post-challenge (100 *N. dubius*)
A	1 × 100 (25 krad) larvae	51 ± 14·5
B	1 × 100 (normal) larvae	72 ± 10·5
C	No immunization	92 ± 10·1

A vs C, B vs C, $P < 0{\cdot}05$ both combinations.

A second experiment using a shorter immunizing period and 25 krad irradiated larvae was carried out. Two groups of mice were infected with 100 normal or 100 irradiated larvae on day 0 followed by pyrantel treatment on days 14 and 15. Challenge infection was given on day 28 and all groups, including challenge controls, were killed on day 14 post challenge. The results are shown in Table 1.

In experiment 1, larvae irradiated at 10 krad clearly conferred greater protection against a subsequent challenge than did normal larvae, as assessed by worm recoveries on both day 14 and day 35 post-challenge. Although, in terms of numbers of worms recovered, there was no significant difference in immunizing capacity between normal and 25 krad irradiated larvae in experiment 2, unlike experiment 1, the challenge worms recovered were smaller and less mature than those recovered from mice immunized with normal larvae. It would seem, therefore, that mice immunized with irradiated larvae are more capable of arresting the development (see Behnke & Parish 1979b) of a subsequent challenge than are mice immunized with normal larvae.

EFFECT OF DIFFERENT DOSES OF IRRADIATION ON IMMUNOGENICITY OF *N. DUBIUS* LARVAE

The above results showed that irradiated larvae were more effective at stimulating immunity than normal larvae. An investigation of the effect of varying the dose of irradiation on the immunogenicity of larvae was undertaken. A total of five experiments was carried out. In two of these, a protocol similar to that of experiment 1 was used, but with mice killed on day 35 post-challenge only and mice were immunized with 200 normal larvae or with larvae irradiated at 5, 10, 15, 20, 30 and 40 krad. The combined results are shown in Figure 2 (expt 3). In a further experiment groups of mice were infected with 300 normal, 7·5, 15 or 25 krad irradiated larvae, treated with pyrantel on days 14 and 15, and challenged with 100 normal larvae on day 18. All the mice were killed 14 days later. The results are shown in Table 2 (expt 4).

Table 2. Experiment 4. Effect of dose of irradiation on immunity stimulated by *N. dubius* larvae

	Immunization	Worm recoveries (mean ± s.d.) Day +14 post challenge (100 *N. dubius*)
A	1 × 300 (25 krad) larvae	58 ± 9·6
B	1 × 300 (15 krad) larvae	71 ± 10·6
C	1 × 300 (7·5 krad) larvae	77 ± 9·9
D	1 × 300 (normal) larvae	85 ± 10·6
E	No immunization	91 ± 11·0

A vs C, A vs D, A vs E, B vs D, B vs E, $P < 0{\cdot}05$ all combinations.

In both these experiments, the protective effect of immunizing with irradiated larvae increased with increasing dose of irradiation. Although this is evident from the mean worm recoveries shown in Figure 2, the variation within the groups was large. However, as the figures shown above the barlines indicate, the numbers of mice in each group which were >90% protected against a challenge infection increased with increasing dose of irradiation.

EFFECT OF VARYING THE NUMBER OF IMMUNIZING DOSES ON THE RESPONSE TO CHALLENGE INFECTION

In this experiment (expt 5) all immunizing doses were of 300 normal or 300, 25 krad irradiated larvae. Four groups of mice were immunized at 16-day intervals with 4 ×, 3 ×, 2 × and 1 × 300, 25 krad irradiated larvae and two other groups were immunized with 4 × and 1 × normal larvae. Each immunizing dose was terminated using pyrantel after 14 days. All groups, including age matched controls were challenged with 100 normal larvae and killed on day 35 post-challenge. Faecal egg counts from these groups are shown in Figure 3 and day 35 post-challenge worm recoveries in Figure 4. This experiment again

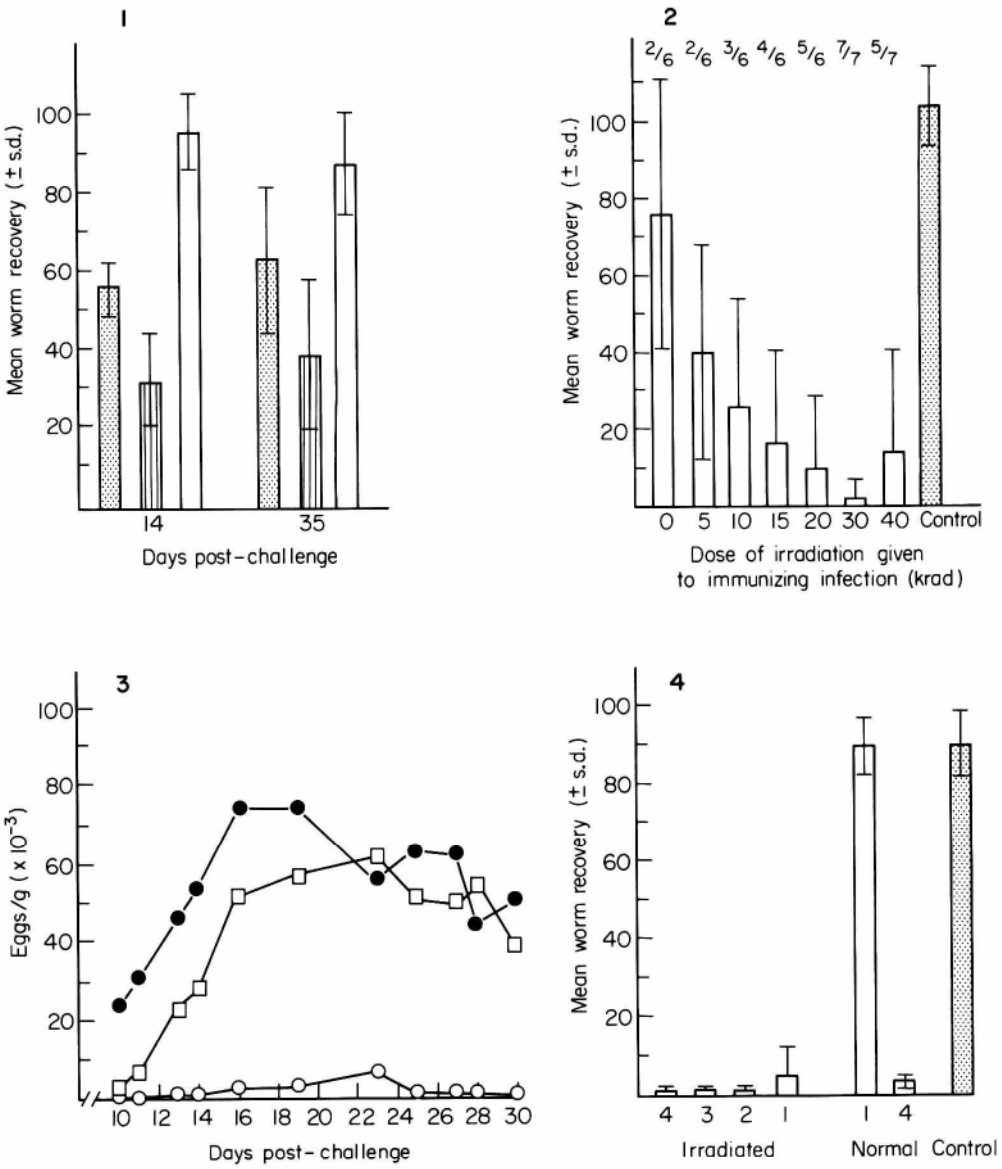

Figure 1. Experiment 1. Mean worm recoveries, days 14 and 35 post-challenge. Mice immunized with 200 ⊠ normal larvae, or ▥ (10 krad) irradiated larvae. Control group, □ no previous infection. Challenge infection 100 normal *N. dubius* larvae.

Figure 2. Experiment 3. Mean worm recoveries, day 35 post-challenge from mice immunized with 300 larvae irradiated at 0, 5, 10, 15, 20, 30 and 40 krad. Control group, no previous infection ▦. Challenge infection 100 normal *N. dubius* larvae. Figures above bar-lines indicate the number of mice in each group, >90% protected, against challenge.

Figure 3. Experiment 5. Pattern of egg production in groups of mice after challenge infection with 100 normal *N. dubius* larvae: □ immunized 1 × 300 normal *N. dubius* and ○ immunized 1 × 300 (25 krad) *N. dubius*, prior to challenge. Control group no previous infection. Counts expressed as eggs per gram of faeces.

Figure 4. Experiment 5. Mean worm recoveries, day 35 post challenge with 100 normal *N. dubius* larvae. Groups of mice immunized 1×, 2×, 3× and 4 × 300 (25 krad) irradiated *N. dubius* or 1× and 4× normal *N. dubius* prior to challenge. Control group ▦ no previous infection.

partial immunity resulting from a single immunizing infection; macrophages from these mice have been shown to adhere to infective third stage larvae *in vivo*. However, only after two immunizing infections with normal larvae did they obtain more than 95% protection. Coinciding with this protection was a blood eosinophilia and the appearance of eosinophils in peritoneal exudates. They concluded that eosinophils may be involved in a second immune mechanism which gives rise to this high level of protection. As yet we have not determined whether eosinophils appear after a single immunization with cobalt 60 irradiated larvae.

By attenuating larvae with cobalt 60 irradiation, the characteristics of infection with *N. dubius* have been dramatically altered. Single infections with normal larvae produce only poor immunity which is manifested by a delay in the onset of patency, possibly due to a slight delay in larval development. Irradiated larvae, on the other hand, produce a highly protective immunity which greatly reduces the number of parasites reaching maturity.

The results of this work indicate that once activated, the immune mechanisms of NIH mice, which normally fail to work against a single *N. dubius* infection, are extremely effective. Irradiated larvae will provide a means by which the underlying mechanisms of immune responsiveness and non-responsiveness in various strains of mice can be studied.

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