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Observations on the gross changes in the secondary lymphoid organs of mice infected with *Nematospiroides dubius*

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ABSTRACT

Gross changes in the size of the secondary lymphoid organs were studied during infection with the nematode parasite *Nematospiroides dubius*. In the strong responder NIH strain, the wet weight of the mesenteric lymph nodes (MLN) increased rapidly following infection with 400 larvae to peak on day 28 at approximately three times the resting weight. Enlargement of the spleens was also marked but regression to normal size took place when the MLN had achieved maximum size. In contrast in $C_{57}BL/10$ mice, a slow responder strain, the enlargement of the MLN following infection was relatively slow, and there was no evidence of the regression of the spleen, once maximum enlargement had been achieved.

When adult worms were removed by anthelmintic, the enlarged MLN and spleens returned rapidly to normal size. However, in mice infected with irradiated larvae (25 krad) the MLN stayed enlarged, despite the absence of adult worms but the spleens of these mice returned to normal size fairly rapidly. It was suggested that irradiated worms survive, perhaps as arrested larvae in the intestinal tissue, for a fairly long time, thereby providing a continual stimulus for the MLN.

INTRODUCTION

The nematode parasite *Nematospiroides dubius* has been used as a laboratory model of chronic gastrointestinal infection because in most commonly available mouse strains primary infections last for 9 to 10 months (EHRENFORD, 1954; WILLIAMS, 1982). Despite the host's inability to remove adult worms during this period, their presence in the intestine does not go unrecognized. Thus the initial stages of a primary infection are associated with pronounced haematological changes (BAKER, 1962; CYPESS, 1972; ALI *et al.*, 1985) and with increased immunological activity as reflected in elevated levels of circulating parasite specific antibody (DOBSON, 1982; DOBSON & CAYZER, 1982; PRITCHARD, *et al.* 1983) and in cellular proliferation within the secondary lymphoid organs (LIU, 1965; ALI, 1983). The mesenteric lymph nodes and the spleen increase rapidly in size following a primary infection. Enlargement is detectable on day three (LIU, 1965) and is known to last for at least two to four weeks (PRICE & TURNER, 1983). Longer term studies of gross and histopathological changes in the secondary lymphoid organs have not been reported to date. However, preliminary observations in our laboratory suggested that during a primary infection with *N. dubius*, strong responder (NIH) and weak responder ($C_{57}BL/10$) strains of mice, behave differently with respect to the gross changes in their secondary lymphoid organs. It was felt that this aspect of the host-parasite relationship during a primary infection warranted further investigation. The results of this work are reported here, together with a comparison of the gross response of the secondary lymphoid organs of mice subjected to different doses of infective larvae, and to highly immunogenic primary infection regimes, (e.g., following infection by irradiated larvae (HAGAN *et al.*, 1981) or following an anthelmintic-abbreviated infection in which the worms were prevented from developing to the lumen-dwelling adult stage).

MATERIALS AND METHODS

Animals

Randomly bred CFLP and inbred NIH and $C_{57}BL/10$ mice were bred and maintained under conventional animal house conditions. The animals were used for experiments when 6 to 10 weeks old.

Nematospiroides dubius

The strain of *N. dubius* used was obtained in 1975 from the Wellcome Research Laboratories (Beckenham, Kent) and has been maintained since in CFLP mice. The 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 third-stage larvae of *N. dubius* were exposed to gamma radiation from a Cobalt 60 source in the Chemistry Department of Nottingham University. The methods used have been described previously (BEHNKE *et al.*, 1980).

Anthelmintic

Pyrantel embonate (Strong-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 of lymphoid organs

The animals used in all the experiments reported here were part of a larger study in which the haematological changes were also studied. The results of this work have been published elsewhere (ALI *et al.*, 1985). The mice were killed with chloroform, weighed and were bled out by cardiac puncture. Spleens and mesenteric lymph nodes (MLN) were carefully removed, stripped of fat and placed on a piece of tissue paper moistened with saline to avoid the organs drying before weighing. The wet weights of spleens and MLN were determined by using a sensitive balance (Sartorius 2001 MP). The organs were transferred into small sample bottles and placed in an oven at 60 to 70°C for 48 hours. The dry weights were then determined, but because dry and wet weight data were essentially similar, we have chosen to present only the latter in the present paper.

Statistical analysis of results

The wet weights of the organs are expressed as the mean wet weight (% of body weight) ± S.E. for each experimental group. The presentation of data in this format minimizes the variation between animals arising from differences in body-weight. Where S.E.s on the figures 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

Comparison of the response in different strains of mice

In a pilot experiment male CFLP mice were infected with 400 larvae of *N. dubius* and groups of four animals were killed at 14-day intervals for 98 days. The wet weights of the mesenteric lymph nodes (MLN) were determined and the results are shown in
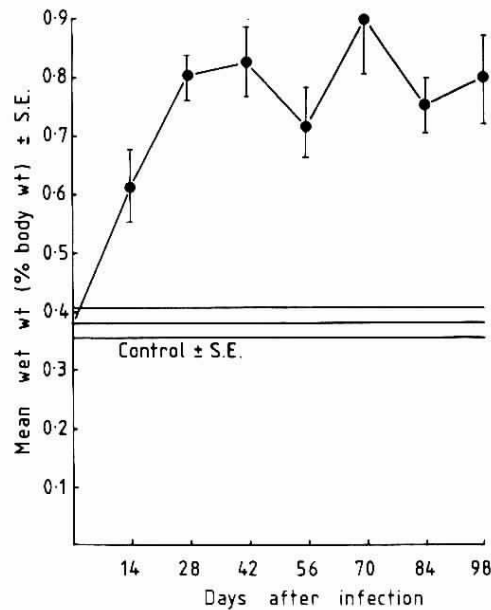
FIG. 1. Increase in the wet weight of the mesenteric lymph nodes of CFLP mice given a single infection with 400 larvae.

Fig. 1. Mesenteric lymph nodes increased from a resting value of 0·38% body-weight to 0·8% by day 28 and remained enlarged, with little further change in size for the remainder of the experiment.

These observations were extended in three separate experiments carried out using NIH, $C_{57}B/10$ or CFLP mice. For logistic reasons it was not possible to compare all strains at the same time. Each experiment comprised 65 mice of which 30 were infected and 35 remained as uninfected controls. The infection levels used were 400 larvae for NIH and CFLP mice and 250 for $C_{57}BL/10$ mice. Five mice from each group were killed at weekly intervals and the MLN and spleens were removed for weighing. The values for the organs in control animals were averaged over the entire six-week period and are presented in Figs. 2 and 3 as a line representing the mean with S.E. bars either side.

It is apparent from the results that the most rapid increase in the MLN occurred in NIH mice. Thus in Fig. 2 it can be seen that by day 28 there was a threefold increase in the weight of the MLNs in NIH mice. In contrast $C_{57}BL/10$ mice were the slowest to respond. The maximum weight of the MLN in this strain was not observed until day 35. CFLP mice had heavier resting values for MLN but their response following infection was as rapid as in NIH mice, although the maximum weight on day 21 represented only a two-fold increase.

The spleens in all three strains, underwent a considerable increase in size within the first two weeks of infection (Fig. 3). Enlargement of spleens was most rapid in NIH and CFLP mice and in both strains the spleens gradually regressed in size after the maximum weight had been achieved on day 14. Only in NIH mice, however, did the value approach control levels within the experimental period. In $C_{57}BL/10$ mice the spleens took longer to achieve maximum weight and remained enlarged until day 42.

Comparison of different infection levels

An experiment was carried out in CFLP mice, in which three groups of 30 animals were infected with 400, 275 or 65 larvae of *N. dubius*. A further group of 20 uninfected
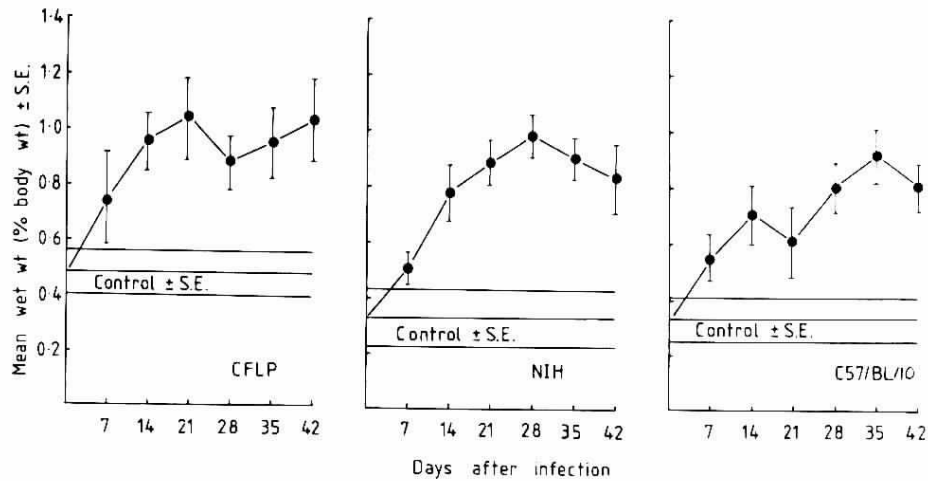
FIG. 2. Increase in the wet weight of the mesenteric lymph nodes of three different strains of mice given a single infection of *N. dubius*. The pooled mean worm recovery from CFLP, NIH and $C_{57}BL/10$ mice was as follows: $370·4 \pm 17·6$; $422·6 \pm 13·8$; $233·9 \pm 11·4$ respectively.
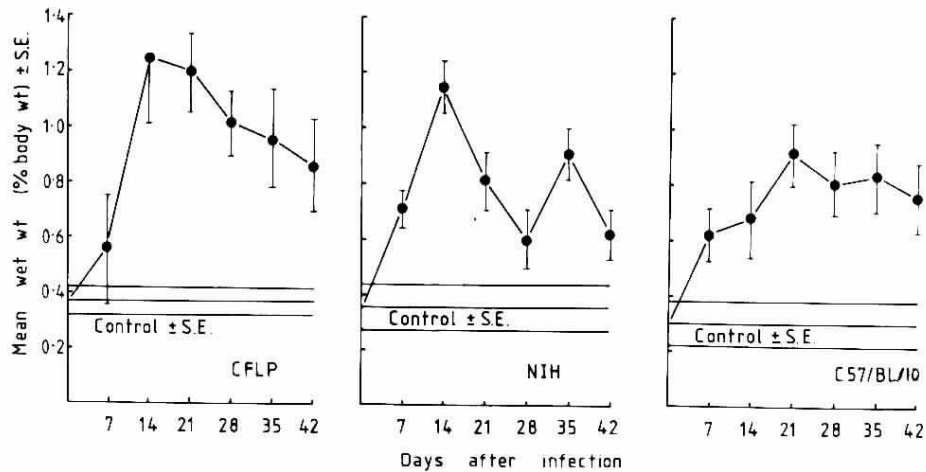
FIG. 3. Increase in the wet weight of the spleens of three different strains of mice infected with *N. dubius*.

mice was included as the control group. Five mice from each group were killed and autopsied at weekly intervals and the results are presented in Fig. 4.

The increase in the wet weight (% of body-weight) of both MLN and the spleens was dependent on the number of larvae given. Mice infected with 275 and 400 larvae showed a rapid increase in the weight of the MLN and spleen but there was little to distinguish between these infection levels. Although in both cases the former group had numerically smaller mean weights throughout the experiment, this was never a statistically significant difference. Interestingly, in mice infected with 65 larvae an increase in the weight of the MLN was not observed until day 14 and then only a transient increase was recorded, the MLN in this group returning to control levels by day 28 despite the continuing presence of adult worms. The spleens of these mice hardly increased above control levels.
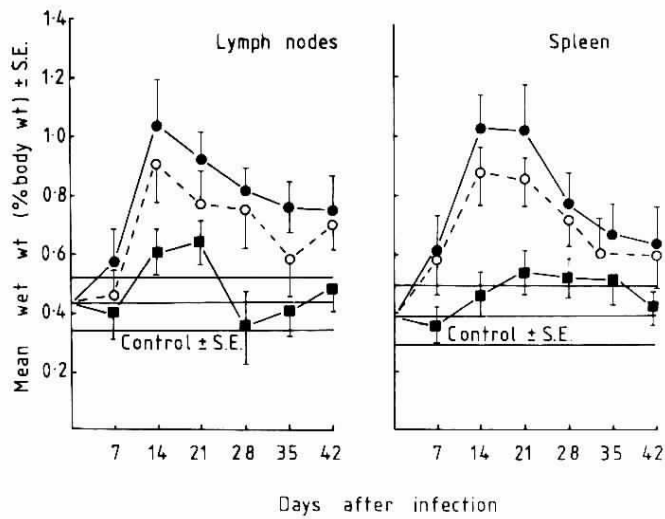
FIG. 4. Increase in the wet weight of the mesenteric lymph nodes and spleen in CFLP mice given a single infection with 400 (●), 275 (○) or 65 (■) larvae of *N. dubius*. The mean no. of worms recovered from these groups was $368 \pm 12\cdot8$, $272\cdot3 \pm 9\cdot8$, $63\cdot6 \pm 5\cdot9$ respectively.

The effect of truncated infections and infection with irradiated larvae

Finally two experiments were carried out in which the CFLP mice were prevented from experiencing adult worms. In the first of these, irradiated larvae were used. Thus two groups of 36 mice each, were infected either with normal or irradiated (25 krad) larvae. In the second experiment two groups of 30 mice were infected with normal larvae, but one group was treated with pyrantel on day 9, 11, 13 and 16 after infection. A further group of 20 mice was left without infection but was treated with anthelmintic and a final group of 20 untreated mice served as the control to this second experiment. For ease of interpretation comparable data from both experiments are set alongside one another. The results are presented in Figs. 5 and 6.

Pyrantel by itself had no effect on the size of the MLN and spleen but when adult worms were removed by treatment with pyrantel, the enlarged MLN and spleens returned rapidly to normal size. This result indicated that the continued enlargement of both organs during a primary infection was dependent on the presence of adult worms in the intestine. Interestingly, however, the MLN stayed enlarged in mice given irradiated larvae, which developed very few adult worms ($8\cdot4 \pm 1\cdot5$). In contrast, the spleens of mice infected with irradiated larvae returned to normal size fairly rapidly. By day 28, the spleens of these mice were within the control range whilst those of mice given normal larvae were still grossly enlarged.

DISCUSSION

The enlargement of the secondary lymphoid organs in animals undergoing a primary infection, reflects the immunological activity and especially the proliferation of lymphoid cells within these tissues (HUMPHREY & WHITE, 1971). In the present work we have investigated the gross changes taking place in the mesenteric lymph nodes and the spleens of mice infected with *N. dubius* and subjected to a variety of experimental conditions in order to ascertain the relationship of these changes to the intensity of infection, the continuing presence of adult worms and the responder status of the host.
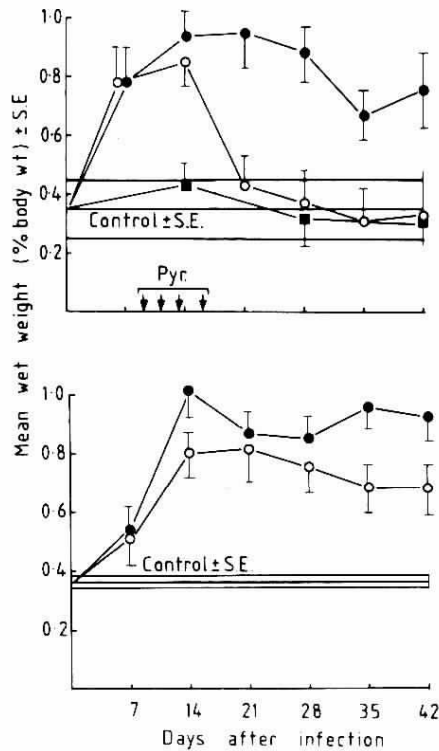
FIG. 5. (a, top) Increase in the wet weight of the mesenteric lymph nodes in CFLP mice given a single infection with 500 larvae of *N. dubius* followed by treatment with anthelmintic drug. Mice infected with 500 larvae (●); mice infected with 500 larvae, followed by treatment with anthelmintic on days 9, 11, 13 and 16 (○) and mice given anthelmintic alone (■). PYR denotes the days on which the anthelmintic pyrantel was administered.

FIG. 5. (b, bottom) Increase in the net weight of the mesenteric lymph nodes in CFLP mice given a single infection with 500 normal larvae (●) or 500 irradiated larvae (○) of *N. dubius*.

The three strains of mice which were studied underwent varying patterns of change in their secondary lymphoid organs. The strong responder NIH mice, showed the greatest increase in the size of the MLN and when compared to the weak responder $C_{57}BL/10$ mice, this response was extremely rapid. It must be noted, however, that $C_{57}BL/10$ mice received fewer infective larvae, an infection level of 250 larvae being used for this strain because higher infection doses would have been lethal. The MLN would be expected to process a significant proportion of material of parasite origin during an intestinal infection and it could be that the efficiency of the reticuloendothelial system in the MLN of NIH mice safeguarded the circulation and the remainder of the body from possible toxins (LIU, 1965). It is pertinent that initially the spleens in NIH mice increased rapidly in size (Fig. 3) but once the MLN achieved maximum size, the spleens began to regress indicating that there was subsequently less stimulation of the spleen, and suggesting that the MLN were coping adequately with the parasite antigens. In $C_{57}BL/10$ mice the slow response of the MLN necessitated the continuous involvement of the spleens which therefore remained enlarged throughout the six-week long experiment. CFLP mice, which are regarded as a moderate/strong responder strain behaved almost like NIH mice, although the response of their secondary
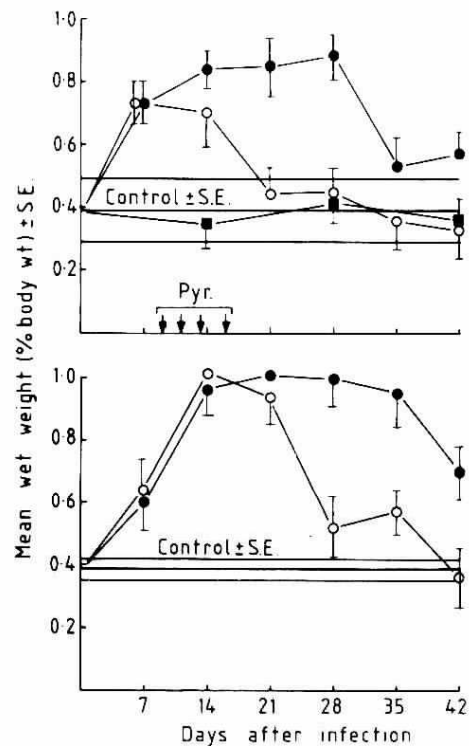
FIG. 6. (a, top) Increase in the wet weight of the spleen in CFLP mice given a single infection with 500 larvae of *N. dubius* abbreviated by treatment with anthelmintic or, (b, bottom) infected with irradiated larvae. All symbols as in Fig. 5.

lymphoid organs was not quite so vigorous. Nevertheless, mice of this strain were readily available for the project and therefore the remaining experiments were carried out entirely in CFLP mice.

The dose response experiment (Fig. 4) provided further evidence that the spleen's involvement was secondary to the capacity of the MLN to handle the products of infection. Thus in mice given 65 larvae, there was a small increase in the size of the MLN but no appreciable change in the size of the spleen. The MLN of CFLP mice could cope with this infection level and the systemic circulation was hardly affected.

When adult worms were removed by treatment with an anthelmintic, both the MLN and the spleen returned rapidly to normal size. It was therefore, the presence of the adult worms within the intestinal lumen and presumably the continuing release of toxins and/or antigens which necessitated the maintenance of enlarged MLN throughout infection. However, when mice were given irradiated larvae, the MLN remained enlarged whilst the spleens returned to normal size by day 28. The destiny of irradiated larvae is not known but mice infected with larvae exposed to 25 krad of irradiation acquire solid immunity to challenge infection (HAGAN *et al.*, 1981), despite the fact that almost no worms develop to the adult stage (BEHNKE *et al.*, 1980). Therefore, some irradiated larvae must establish in the host's intestinal walls, and the present finding that the MLN remain enlarged for up to six weeks following infection, provides strong evidence that the irradiated worms survive, perhaps as arrested larvae in the intestinal tissues (BEHNKE & PARISH, 1979). It is difficult to explain otherwise the continuing stimulus for the enlarged MLN. In contrast the spleens of mice given irradiated larvae returned to normal quite rapidly. Presumably the antigens

released by irradiated larvae were processed in and restricted to the intestine and the MLN and hence there was no requirement for the continuing involvement of the spleen.

In conclusion our experiments suggest that the involvement of the spleen during infection with *N. dubius* is secondary to the role of the mesenteric lymph nodes. The spleens enlarge only when the mesenteric lymph nodes are incapable of processing all the toxic/antigenic products of infection emanating from the intestinal tract.

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