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# Non-specific immunodepression by *Nematospiroides dubius* of concurrent responses to oxazolone and lipopolysaccharide

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**ABSTRACT**

Mice infected with *Nematospiroides dubius* show non-specific immunodepression (NSID) of immune responses to concurrently administered antigens. This project investigated the factors influencing the response of oxazolone (T-dependent antigen) and LPS (T-independent antigen) in infected mice. Depressed responses to both antigens were observed in mice infected with *N. dubius*, although the pattern of immunodepression was influenced by both the level and the relative timing of infection and antigen presentation. Consistent NSID was only achieved when the mice were given the higher levels of infection and there was some evidence that NSID was more severe when infection preceded the administration of antigen by 14 days. In general, although NSID was significant, the degree of immunodepression was not considerable. It was concluded that the underlying mechanism of NSID was likely to be different from that enabling the parasite to survive in the intestine of the chronically infected host, and that altered physiology and host's modulation of the immune responses in heavily infected animals may have given rise to a general weakening of the immune system. It was suggested that future work should investigate NSID and parasite-specific immunodepression locally in the intestine where parasite survival is likely to be determined by events at the mucosal surface.

## INTRODUCTION

There is now substantial evidence in the literature that the murine immune system is altered during infection with the nematode parasite, *Nematospiroides dubius*. However, most of the work to date has concentrated either on concurrent infections with *N. dubius* and other intestinal helminths (BEHNKE *et al.*, 1978; BEHNKE *et al.*, 1984; JENKINS & BEHNKE, 1977; HOPKINS, 1980; COLLWELL & WESCOTT, 1973; JENKINS, 1975; BRUNA & XENIA, 1976) or has studied the effect of *N. dubius* on the response of the mouse to sheep red blood cells (SRBC) (SHIMP *et al.*, 1975; ALI & BEHNKE, 1983, 1984). The immunology of the former is complex but in all cases is believed to be under T cell control (WAKELIN, 1978; MITCHELL, 1982) whereas the immunological processing of SRBC requires the co-operation of T and B lymphocytes and macrophages (ARGYRIS, 1967; GREAVES & MOLLER, 1970; MITCHELL & MILLER, 1968). The results indicate that mice infected with *N. dubius* produce depressed immune responses in all the combinations which have been referred to above, but little is known about the possible mechanisms involved. Recently, PRITCHARD *et al.* (1984) found that *N. dubius*-infected mice had impaired macrophage function and that in consequence/in addition suppressor cells were generated which inhibited the response to SRBC. However, the significance of this finding in respect of parasite survival is uncertain, because even when T suppressor cells were ablated by treatment with 2′ deoxyguanosine, thereby facilitating a normal response to SRBC in infected mice, the animals were still incapable of removing the adult worm population.

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Despite the uncertain significance of non-specific immunodepression (NSID) to the chronicity of infection with *N. dubius*, there is evidence that adult worms survive by interfering with the host's capacity to respond effectively against the homologous infection (CAYZER & DOBSON, 1983; BEHNKE *et al.*, 1983). The possible relationship of parasite-specific and non-specific immunodepression during infection with *N. dubius* is therefore highly pertinent and it was considered of interest to extend our previous work on NSID to antigens which are known to be T-dependent (oxazolone) or T-independent (lipopolysaccharide, LPS). Both of these antigens are widely used in immunology laboratories and are therefore well documented (ASHERSON *et al.*, 1977; POWNALL & KNAPP, 1978; REED *et al.*, 1973; MOLLER, 1965).

## MATERIALS AND METHODS

*Animals*

Random bred CFLP and inbred NIH and $C_{57}$BL/10 mice were used in this work. The animals were bred and maintained under conventional animal house conditions in the Zoology Department's Animal House. All the mice were at least six weeks old when used for experiments.

*Nematospiroides dubius*

The origin and maintenance of our strain of *N. dubius* and the methods used for infection and recovery of adult worms have already been described (BEHNKE & WAKELIN, 1977; BEHNKE & PARISH, 1979). Throughout this work cultures of third-stage larvae of *N. dubius* were found to be extremely infective and in some experiments worm recoveries at autopsy were higher than had been intended, indicating that larval inocula had been underestimated. For this reason, mean worm recoveries accompany the results of each experiment and should be referred to, rather than the number of larvae administered.

*Measurement of the response to oxazolone*

Mice were sensitized to oxazolone (2-phenyl-4-ethoxymethylene oxazolone; BDH Ltd) by applying 0·1 ml of a 3% solution of oxazolone in 50:50 vol. acetone/ethanol solution to their shaved abdomen (ASHERSON & PTAK, 1968). Eight days later the mice were challenged on the right ear with 0·025 ml of 1% oxazolone in 80:20 vol. acetone/olive oil, and the increase in ear thickness was determined after a further 24, 48 and 72 hours. Ear thickness was measured using an automatic dial guage (Mitutoyo Mfg. Co. Ltd, Tokyo, Japan) to ensure that the force applied to the ear would be independent of the observer.

*Measurement of the response to LPS*

Mice were injected i.v. with 2·5 $\mu$g of LPS. Antibody titres to LPS were determined by haemagglutination using SRBC coated with LPS (*Escherichia coli* serotype 0·55 : B5, Sigma Chemical Corp.). 1·0 mg of LPS was dissolved in 1 ml of saline by boiling for 1 hr immediately prior to use and 0·2 ml was added to the packed and washed SRBC. The cells were incubated in the presence of LPS for 30 min at 37°C, washed three times in saline and diluted to the required concentration. Serum was serially diluted in microtitre plates and coated SRBC were added. The plates were incubated for two hours at 37°C after which the titres were read. Wells containing immune serum together with uncoated cells and control serum with coated cells were included as additional controls in all experiments.

*Statistical analysis of results*

Unless otherwise stated groups of six mice were used throughout this work and the results are expressed as the group mean ± S.E. Statistical significance was determined by the non-parametric Wilcoxon test (SOKAL & ROHLF, 1969). Detailed analysis of all the results was carried out but significance levels are only given in particular instances. A value of $P < 0.05$ was considered to be significant.

## RESULTS

*The response to oxazolone in mice infected with* N. dubius

Several experiments were carried out to evaluate the effect of *N. dubius* on the immune response to oxazolone. Fig. 1 presents the results of an experiment in which groups of five CFLP mice were infected with 450 larvae of *N. dubius* at various time intervals relative to sensitization. One group was infected two days before sensitization, another on day −7 and the third group on day −14. All the groups together with the control groups were sensitized with oxazolone on day 0 and eight days later they were given the challenge dose of oxazolone. The results show that all the infected, sensitized mice had a significantly depressed response to oxazolone as assessed by the increase in ear thickness 24 hours following challenge.

Two further experiments were carried out, one with CFLP mice and the other using NIH mice. Groups of animals were infected with different numbers of *N. dubius* (50, 250 or 500) at various time intervals relative to sensitization. The experimental details and the results of both experiments are shown in Table I. The mice which were infected 14 days before sensitization responded poorly to oxazolone, even the group given 50 larvae showing a significantly smaller increase in ear thickness. Among the groups given the infection 28 and 42 days before sensitization, only infection levels of 250 and 500 larvae significantly depressed the response to oxazolone.

The mouse strains used in the preceding experiments (CFLP and NIH) rank as moderate and strong responder strains to infection with *N. dubius* (see BEHNKE & WAKELIN, 1977; ROBINSON & BEHNKE, 1983). It was felt that a weak responder strain should also be examined in this system. The result of an experiment in which $C_{57}$BL/10 mice were used are therefore presented in Table II. Thus groups of five mice were infected with 50, 150 or 250 larvae of *N. dubius*, 14 days before sensitization and together with the control groups, all the animals were challenged on day 0. It can be seen from the results that $C_{57}$BL/10 mice did not react to oxazolone as vigorously as did CFLP or NIH mice. Nevertheless a significant reduction in the response was observed in mice infected with 150 and 250 larvae of *N. dubius*.

A final experiment was carried out to investigate the possibility that the serum of mice with a primary infection of *N. dubius* contains non-specific blocking factors which interfere with the response to concurrently administered oxazolone. Serum was obtained from CFLP mice which had undergone a four-week infection with 250 *N. dubius*. Recipient mice were treated (i.p. injection) with primary infection serum (PrS) at different times in relation to sensitization with oxazolone as shown in Table III. All the mice, together with appropriate control groups, were challenged on day 8 and ear thickness was determined 24 hours later. The results show that PrS had no depressive activity in this system.

*The response to LPS in mice infected with* N. dubius

An experiment was carried out in which two groups of six CFLP mice were given 3·5 μg of LPS i.v. One of these groups had been infected with 500 *N. dubius* two weeks previously. The animals were bled at four-day intervals for 20 days and the antibody

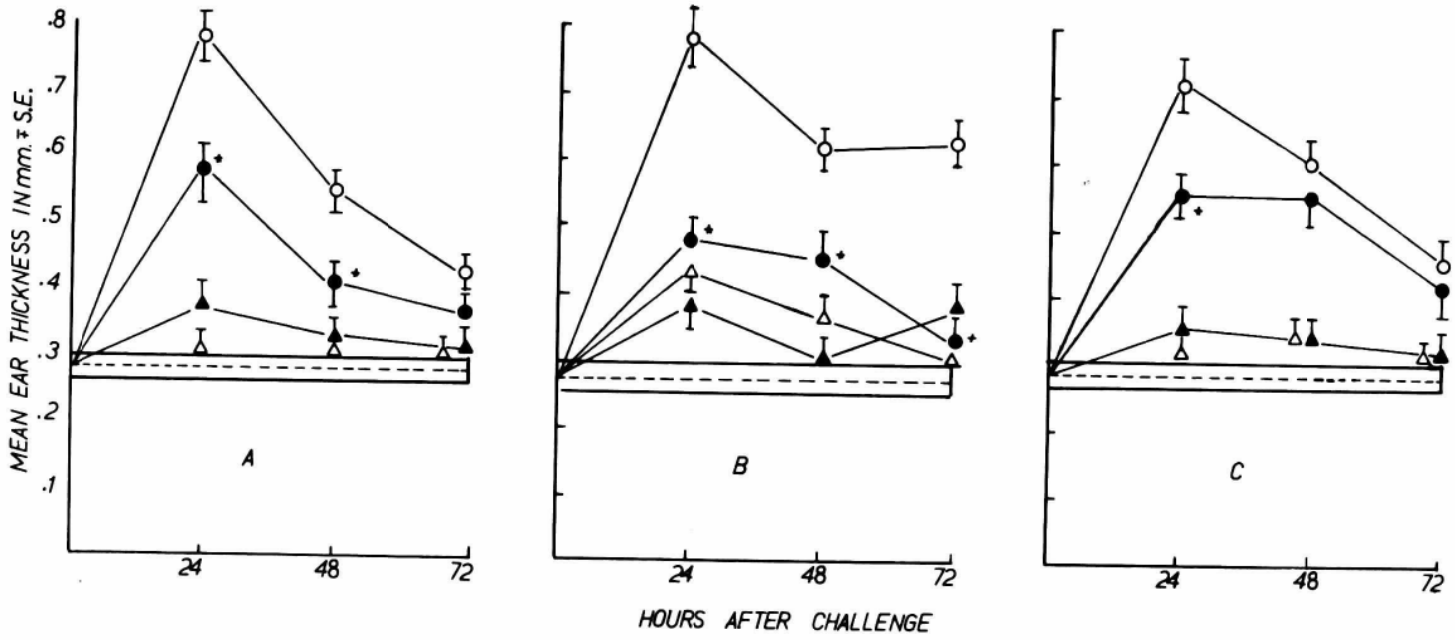


FIG. 1. The response to oxazolone in mice infected with *N. dubuis* at various time intervals relative to sensitization. In Fig. 1a the infection was given on day 2; Fig. 1b on day −7 and Fig. 1c on day −14. The key to the symbols used is as follows:

| Symbol | Infection | Sensitization | Challenge |
|---|---|---|---|
| ● | + | + | + |
| ○ | − | + | + |
| ▲ | + | − | + |
| △ | − | − | + |

The MWR ± S.E. from the infected mice in Fig. 1a, 1b and 1c was 422·2 ± 11·4, 438·7 ± 7·5, 435·5 ± 7·7 respectively. *Denotes a significant depressed response.

**TABLE I.** The response of mice infected with different numbers of *N. dubius* to challenge with oxazolone. (This Table shows the results from 2 separate experiments, one in CFLP mice and the other in NIH mice. A representative number of mice was killed from each infected group for worm counts. The mean worm recovery in mice given 500 *N. dubius* ranged from 447 ± 8·2 to 529 ± 2·8; in mice given 250 *N. dubius* from 202 ± 8·3 to 298 ± 17·6; in mice given 50 *N. dubius* from 40·0 ± 12·1 to 68·0 ± 1·4.)

| Day of infection with *N. dubius* | Strain of mice | Mean ear thickness in mm ± S.E.† (% increase relative to naive control group)* | | | |
|---|---|---|---|---|---|
| | | 500 *N. dubius* | 250 *N. dubius* | 50 *N. dubius* | No Infection |
| −14 | CFLP | 0·64 ± 0·02 (93·9%) | 0·77 ± 0·03 (133·3%) | 0·68 ± 0·03 (106·1%) | 0·86 ± 0·01 (160·6%) |
| | NIH | 0·46 ± 0·09 (48·4%) | 0·48 ± 0·01 (54·8%) | 0·59 ± 0·02 (90·3%) | 0·66 ± 0·01 (112·9%) |
| −28 | CFLP | 0·63 ± 0·03 (90·9%) | 0·61 ± 0·02 (84·8%) | 0·72 ± 0·02 (118·2%) | 0·78 ± 0·05 (136·4%) |
| | NIH | 0·42 ± 0·02 (35·5%) | 0·48 ± 0·02 (54·8%) | 0·57 ± 0·04 (83·9%) | 0·59 ± 0·02 (90·3%) |
| −42 | CFLP | 0·55 ± 0·02 (66·7%) | 0·63 ± 0·04 (90·9%) | 0·66 ± 0·02 (100%) | 0·67 ± 0·02 (103·0%) |
| | NIH | 0·50 ± 0·01 (61·3%) | 0·51 ± 0·01 (64·5%) | 0·58 ± 0·02 (87·1%) | 0·59 ± 0·01 (90·3%) |

† The mean ear thickness of four control CFLP mice was 0·33 ± 0·05 and for four NIH mice the figure was 0·31 ± 0·03.

* % increase in ear thickness was calculated as $\frac{\text{Experimental} - \text{naive control}}{\text{naive control}} \times 100$.

**TABLE II.** The response to ozaxolone in C57 BL/10 mice infected with different numbers of *N. dubius* 14 days before sensitization

| Group | No. of *N. dubius* given | No. of mice | Sensitized | Challenged | Mean ear thickness* in mm ∓ S.E. | MWR ∓ S.E. |
|---|---|---|---|---|---|---|
| A | 250 | 5 | + | + | 0·38 ∓ 0·09 (1) | 269 ∓ 4·2 |
| B | 150 | 5 | + | + | 0·42 ∓ 0·09 (2) | 149·5 ∓ 23·3 |
| C | 50 | 5 | + | + | 0·46 ∓ 0·06 (3) | 49·5 ∓ 10·6 |
| D | — | 5 | + | + | 0·48 ∓ 0·09 | — |
| E | — | 5 | — | + | 0·27 ∓ 0·06 | — |

* Ear thickness measured 24 hours after challenge.
(1) P = 0·004. (2) P = 0·004. (3) P = 0·210.

**TABLE III.** The response to oxazolone in mice treated with primary infection serum (PrS)

| Group* | Day on which mice were sensitized | Day on which mice were given PrS | Mean ear thickness ∓ S.E. |
|---|---|---|---|
| A | day 0 | — | 55·6 ∓ 2·6 |
| B | day 0 | −1, 0, +1, +2, +3+ | 55·6 ∓ 3·43 |
| C | day 0 | 6, 7, 8, 9+ | 54·9 ∓ 1·74 |
| D | — | – | 38·0 ∓ 1·93 |

* Groups of six CFLP mice.
+ 0·5 ml of PrS was injected on each occasion.

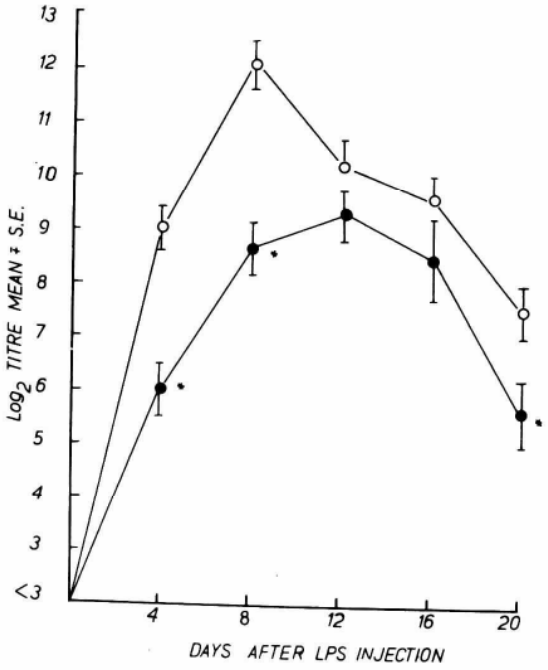


FIG. 2. The response to LPS in mice infected with *N. dubius*. The key to the symbols used is as follows: ○—control group; ●—mice given *N. dubius* on d−14. The MWR ± S.E. in this group was 462·2 ± 2·8. *Denotes a significantly depressed response.

titres to LPS were measured using LPS-coated SRBC. The results of this experiment are presented in Fig. 2. Mice harbouring the infection showed a significantly reduced response on days 4 and 8. By days 12 and 16 the two groups had comparable anti-LPS titres, although on day 20 the infected group was significantly lower.

A second experiment was carried out in which groups of five CFLP mice were infected with *N. dubius* at various time intervals relative to immunization with LPS. Details of the experimental design together with the results are presented in Table IV. Four days following the administration of LPS all the infected groups showed a reduced response to LPS. On day 8 the most severely depressed group was the one which had been infected on day −14. However, most of the infected animals behaved similarly irrespective of the time of infection and showed depressed responses throughout the course of the experiment.

In a final experiment the response to LPS was investigated in groups of mice infected with 75, 200 or 500 *N. dubius* respectively. The results in Table V establish that there was a significantly reduced antibody response to LPS only in the more heavily infected animals. The group which had been given 75 larvae responded normally to LPS.

## DISCUSSION

An understanding of the mechanisms used by parasitic nematodes to evade host defence mechanisms is likely to prove useful in the development of immunoprophylaxis against the species which affect man. One possible mechanism which has been a particular focus of attention in recent years is parasite-induced immunodepression. There is evidence in both filarial (LAMMIE & KATZ, 1983; OTTESEN *et al.*, 1977; PORTARO *et al.*, 1976) and gastro-intestinal nematodes (ALI & BEHNKE, 1983; HAIG *et al.*, 1980)

**TABLE IV.** Antibody response to LPS on different days in CFLP mice infected with *N. dubius* at different time intervals relative to immunization with LPS

| Group | Day on which *N. dubius* given | Log 2 Titre, Mean ∓ S.E. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 4 | Day 8 | Day 12 | Day 16 | Day 20 | MWR ∓ S.E. |
| A | −28 | <3 | 5·8 ∓ 0·2 (S) | 7·8 ∓ 0·2 (S) | 9·8 ∓ 0·4 (S) | 8·0 ∓ 0 (S) | 7·5 ∓ 0·5 (S) | 451 ∓ 5·01 |
| B | −21 | <3 | 6·0 ∓ 0·3 (S) | 9·2 ∓ 0·3 (S) | 10·4 ∓ 0·2 (S) | 8·3 ∓ 0·3 (S) | 6·6 ∓ 0·3 (S) | 434 ∓ 14·4 |
| C | −14 | <3 | 6·0 ∓ 0·3 (S) | 6·8 ∓ 0·3 (S) | 9·8 ∓ 0·4 (S) | 8·8 ∓ 0·3 (S) | 6·0 ∓ 0·4 (S) | 425·5 ∓ 10·4 |
| D | −7 | <3 | 6·0 ∓ 0·3 (S) | 9·6 ∓ 0·5 (S) | 9·8 ∓ 0·3 (S) | 9·0 ∓ 0·4 | 7·8 ∓ 0·3 | 452 ∓ 6·1 |
| E | 0 | <3 | 5·8 ∓ 0·4 (S) | 9·0 ∓ 0·3 (S) | 10·6 ∓ 0·4 (S) | 9·2 ∓ 0·3 | 6·6 ∓ 0·4 | 464 ∓ 13·9 |
| F | No *N. dubius* | <3 | 8·4 ∓ 0·2 | 11·0 ∓ 0·4 | 12·2 ∓ 0·2 | 10·2 ∓ 0·3 | 8·2 ∓ 0·3 | — |

5 mice per group were infected on the given days with 450 larvae of *N. dubius*.
*Titre:* Uncoated cells + immune serum, 3; Coated cells + control serum, 3. LPS was given intravenously on Day 0.
(S) Statistically significant difference relative to control group.

**TABLE V.** Antibody response to LPS on different days in CFLP mice infected with different numbers of *N. dubius* two weeks before immunization with LPS

| Group | No. of *N. dubius* given | Log 2 Titre, Mean + S.E. | | | | | | MWR ∓ S.E. |
|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 4 | Day 8 | Day 12 | Day 16 | Day 20 | |
| A | 500 | <3 | 6∓0·25 (S) | 8·1∓0·30 (S) | 10·3∓0·31 | 8·5∓0·4 | 7·1∓0·30 | 441∓7·2 |
| B | 200 | <3 | 6·6∓0·42 (S) | 8·5∓0·40 (S) | 10·5∓0·42 | 8·6∓0·5 | 7·0∓0·36 | 202∓7·7 |
| C | 75 | <3 | 7·6∓0·30 | 9·3∓0·42 | 11·3∓0·21 | 9·5∓0·3 | 7·8∓0·30 | 67·3∓3·4 |
| D | No *N. dubius* | <3 | 7·8∓0·31 | 9·8∓0·30 | 11·3∓0·3 | 9·6∓0·33 | 8·1∓0·40 | — |
| Immune serum + uncoated cells | | <3 | <3 | <3 | <3 | <3 | <3 | |
| control serum + coated cells | | <3 | <3 | <3 | <3 | <3 | <3 | |

Groups of 6 CFLP mice were used in this experiment.
* Groups were infected with *N. dubius* 14 days before immunization with LPS which was given i.v. on day 0.
(S) Statistically significant difference relative to control group.

that immunodepression of the host may contribute to parasite survival. However, the role of non-specific immunodepression (NSID) in promoting survival is controversial because NSID can also be readily demonstrated in species which are effectively controlled by the host, e.g., *Nippostrongylus brasiliensis* (see HAIG *et al.*, 1980; MCELROY *et al.*, 1983; JENKINS, 1975; BRUNA & XENIA, 1976). Nevertheless in the case of *N. dubius* the observation that adult parasites depress the expression of homologous immunity prompted us to extend our previous work on NSID of the response to SRBC in infected mice in order to learn more about the relationship between these two phenomena.

The results reported here demonstrated that concurrent infection with *N. dubius* had a significant effect on the immune response of the host to both T-dependent and T-independent antigens. However, in both cases significant depression was achieved consistently only when the mice were infected at the higher levels of infection. 75 *N. dubius* had no detrimental effect on the response to LPS. 50 worms did not depress the response to oxazolone in $C_{57}$BL/10 mice although a significant depression was observed at this level of infection in CFLP and NIH mice infected 14 days before sensitization. Thus both the level of infection and the relative timing of infection and sensitization were important (although the latter was less evident in the case of LPS).

It is likely that immunodepression of responses to LPS and oxazolone was brought about by interference with a component central to both, and one possibility is that sensitized lymphocytes were diverted away from the sites where they would have exerted their normal role during the response, i.e., oxazolone-sensitive cells may have been prevented from localizing normally in the ear. There is some support for this hypothesis in the work of HAGAN & WAKELIN (1982) who found that the homing of lymphocytes to the intestine was impaired in mice concurrently infected with *N. dubius* and *Trichinella spiralis*. The increased proportion of cells homing to the intestines of mice infected with *T. spiralis* alone was absent in concurrently infected animals and the authors concluded that *N. dubius* interfered with the inflammatory components of the host's response to *T. spiralis* which influence cell traffic to the intestine.

A further possibility is suggested by the work of PRITCHARD *et al.* (1984) who demonstrated that macrophages exposed to SRBC in the peritoneal cavity of infected animals were inefficient, compared to controls, in adoptively transferring the SRBC response to naive recipient mice. Such an impairment of macrophage function may have contributed to the host's modulation of responses to both oxazolone and LPS.

Whilst the present work has demonstrated that the ability of mice to respond to both T-dependent and T-independent antigens is affected by infection with *N. dubius*, the degree of depression in these experiments was not very pronounced and only consistently detectable in heavily infected animals. This suggests that the underlying mechanism of NSID may be different from that enabling *N. dubius* to survive in the host, since infections of fewer than 50 larvae are chronic in mice despite the apparent absence of NSID. If survival of *N. dubius* is dependent on immunomodulatory factors (IMF) secreted by the parasite in the intestinal lumen, as has been suggested (BEHNKE *et al.*, 1983) the primary target of such factors is likely to be local expression of immunity by the host, in the environment of the parasite. Furthermore infection with *N. dubius* is chronic lasting for some eight to ten months and presumably therefore either the parasite maintains the secretion of IMF throughout its existence or long-lasting changes in immune responsiveness are brought about in the early stages of infection. It is conceivable that the significantly enhanced NSID in mice infected 14 days before challenge with antigen (this paper and ALI & BEHNKE, 1983) is related to this early phase of the existence of adult worms in the intestinal lumen. However, the intestine undergoes severe damage in the preceding week when worms emerge from

their sites of development in the muscularis externa. This damage to the structure of the intestinal walls may allow IMF access to the circulation and may be responsible for the enhancement of NSID at this time. Trauma and physiological stress which the animals undergo at this time may also exacerbate NSID of immune responses to non-parasite antigens.

Our results in this and our previous work (ALI & BEHNKE, 1983, 1984; PRITCHARD *et al.*, 1984) have focused attention on NSID during infection with *N. dubius*, but they also suggest that the continuation of this line of investigation is unlikely to clarify parasite survival. We conclude that a more fruitful approach may be to investigate NSID and the modulation of immune responses locally in the intestine where parasite survival is far more likely to be determined by events at the mucosal surface (MCELROY *et al.*, 1983; BEFUS & BIENENSTOCK, 1982).

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