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Suppression of muscosal mastocytosis by infection with the intestinal nematode *Nematospiroides dubius*

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Summary Mice exposed to primary infections with the parasitic intestinal nematode *Nematospiroides dubius* failed to show the mucosal mast cell (MMC) response which is characteristic of infections with other species of intestinal nematode and which was readily induced in these mice by infections with *Nippostrongylus brasiliensis* or *Trichinella spiralis*. The failure to generate a mucosal mastocytosis was independent of host strain or sex. When infections with *N. dubius* were established before, or concurrently with, *T. spiralis* or *N. brasiliensis*, the MMC response elicited by these species was delayed and/or depressed as was expulsion of the worms themselves. Infection with *N. dubius* given when a MMC response was already established, by exposure to *T. spiralis*, had no effect on MMC numbers. The possibility that the effects of *N. dubius* upon MMC responses reflect a lack of mastocytopoietic potential, rather than an active interference, was excluded by showing that SJL mice, which expel primary infections with *N. dubius* and express strong immunity to reinfection, developed marked mastocytosis during secondary infections. The depression of MMC responses by *N. dubius* is discussed in relation to the known immunosuppressive properties of this parasite and in relation to the T cell mediated control of MMC development.

Keywords: nematodes, mouse, *Nematospiroides dubius, Trichinella spiralis, Nippostrongylus brasiliensis,* mucosal mast cells, mastocytosis, immunosuppression, concurrent infection, intestinal inflammation

Introduction

The accumulation of mucosal mast cells (MMC) is a characteristic and well-defined response to infection with intestinal nematodes (Miller 1984). The response is mediated by a population of T-lymphocytes, which stimulate the proliferation and differentiation of bone marrow-derived precursor cells present in the intestinal mucosa (Guy-Grand *et al.* 1984). Normal mice have very few MMC; after infection, for example with the nematode *Trichinella spiralis*, there is a very rapid appearance of MMC, within 4 to 6 days, and by day 8 there may be as many as 50 cells per villus-crypt unit (Alizadeh & Wakelin 1982). A causal association between the appearance of MMC and the expulsion (spontaneous cure) of intestinal nematodes has long been suspected, and this association has been

strengthened by the demonstration that MMC are functionally active during the expulsion of both *Nippostrongylus brasiliensis* and *Trichinella spiralis* from infected rats (Woodbury *et al.* 1984). The basis of this association remains obscure at present, and it is clear that MMC accumulation by itself is neither sufficient nor necessary for worm expulsion to take place.

The development of mucosal mastocytosis has been investigated most thoroughly in infections involving worms which generate strong spontaneous cure responses. Little is known of the MMC response in nematodes such as *Nematospiroides dubius* which give rise to long-term, chronic infections. *N. dubius* is known to exert immunosuppressive effects upon the host (Behnke, Hannah & Pritchard 1983) and is capable of delaying the expulsion of other nematodes in concurrent infections (Jenkins 1975, Behnke, Wakelin & Wilson 1978). The present paper is concerned with a study of the MMC response to primary and challenge infections with *N. dubius* in mice. It also investigates in concurrent infections the effects of *N. dubius* upon the MMC responses generated by *N. brasiliensis* and *T. spiralis*.

Materials and methods

MICE

Three inbred strains of mice were used, NIH and B10.G, which are respectively high and low responders to *T. spiralis*, and SJL, which are high-intermediate responders. All the mice were bred in the Department of Zoology at Nottingham. Male mice were used, unless stated otherwise, and were 6- to 8-weeks old at the beginning of each experiment.

PARASITES

The techniques for maintenance, infection with, and recovery of *Nematospiroides dubius*, *Nippostrongylus brasiliensis* and *T. spiralis* were essentially as described elsewhere (Jenkins & Behnke 1977, Jennings, Mulligan & Urquhart 1963, Wakelin & Wilson 1977).

HISTOLOGY

Blocks were made of 5–10 cm pieces of the anterior small intestine, using the 'swiss roll' technique. Immediately after the mouse has been killed the length of intestine was removed, flushed through with Carnoy's fixative, slit longitudinally and rolled onto a piece of syringe plunger. After fixation for 6–12 h, the intestine was gently removed from the plunger and transferred to 70% ethanol. Standard techniques were used to dehydrate, embed and prepare 5μm sections of the tissue. Sections were stained with Alcian Blue and counterstained with Safranin O essentially as described by Alizadeh and Wakelin (1982) with the following modifications. Sections were stained for 1 h in a 50 : 50 mixture of 1% Alcian Blue and 1% Astra Blue in 0·7 M HCl (pH = 0·3) which had been pre-warmed to 60°C. They were then counterstained in prewarmed 0·5% Safranin in 0·125 M HCl (pH = 1) for 30–45 min, before processing and mounting in DPX.

COUNTING OF MMC

Blue-staining granulated cells were deemed to be MMC whether located in the epithelium or lamina propria. The numbers of cells present were counted in villus-crypt units (v.c.u.) and a total of 20 v.c.u. was counted for each mouse. In preliminary experiments counts were made of five mice per group. The variance recorded at the peak of mastocytosis in *T. spiralis* infected mice was of the order of 18% of the mean. Because of the labour involved in making sequential counts on different groups of mice, in subsequent experiments MMC counts were usually based on the mean of three animals per time point.

Results

MMC RESPONSE DURING A PRIMARY INFECTION WITH *N. DUBIUS*

An initial experiment was carried out in which NIH and B10.G mice were infected with 300 *N. dubius* larvae and killed on days 8, 14 and 22 to follow the development of the mast cell response. Adult worms were present in number on days 14 and 22. No MMC were detected in either control or infected B10.G mice and very few (maximum of 25 cells/20 v.c.u. on day 8) in infected NIH mice. The experiment was then repeated with the addition of groups of NIH and B10.G mice infected with 300 larval *T. spiralis* to act as positive controls for the development of intestinal mastocytosis. Mice were killed on days 3, 6, 9, 12 or 15 after infection and the presence of adult *N. dubius* or *T. spiralis* confirmed on days 12 and 15 or day 6 respectively. The numbers of MMC present after infection are shown in Table 1. Again *N. dubius* elicited no response whereas, as expected, NIH, and to a lesser extent B10.G, responded strongly to *T. spiralis*. The failure of *N. dubius* to stimulate intestinal mastocytosis was confirmed in a number of other experiments with NIH mice, some of which followed the infection for a total of 60 days.

Table 1. MMC response of NIH and B10.G mice to infection with 300 *N. dubius* or 300 *T. spiralis* larvae.

		Mean no. of MMC/20 v.c.u. in three mice				
		Days after infection				
Infection	Strain	3	6	9	12	15
N. dubius	NIH	0	0	<5	<5	10
	B10G	0	0	0	<5	<5
T. spiralis	NIH	0	25	395	474	217
	B10G	0	11	36	183	nd

nd Not done.

Table 2. Effect of concurrent infection with 300 *N. dubius* upon the MMC response and expulsion of worms in NIH mice infected with 300 *T. spiralis*

Group of mice			Mean no. of MMC/20 v.c.u. in three mice				
	T. spiralis infection given day	*N. dubius* infection given day	Days after *T. spiralis* infection 6	9	12	15	18
1	0	−8	32	273	330	406	162
2	0	0	0	110	120	132	298
3	0	+8	nd	nd	566	344	202
4	0	none	84	404	567	438	130
5	none	none	<5	nd	nd	<5	nd
			Mean no. (±s.d.) *T. spiralis* in five mice				
1	0	−8	211 (14)	188 (18)	173 (27)	108 (22)	57 (8)
2	0	0	158 (17)	165 (10)	158 (16)	114 (8)	83 (18)
3	0	+8	nd	nd	16* (6)	2* (2)	1* (2)
4	0	none	206 (27)	94* (19)	7* (9)	0·4 (1)	1* (2)

nd Not done.

* Significantly different from groups 1 and 2.

EFFECT OF *N. DUBIUS* INFECTION UPON THE EXPULSION OF *T. SPIRALIS* AND THE MMC RESPONSE IN CONCURRENTLY INFECTED MICE

N. dubius is known to depress the spontaneous cure of *T. spiralis*, and in concurrent infections expulsion of *T. spiralis* is dealyed by several days (Behnke *et al.* 1978). The effect of *N. dubius* upon the MMC response elicited by *T. spiralis* was studied in five groups of mice, four of which were infected with 300 *T. spiralis* on day 0. Three of the infected groups were additionally infected with 300 *N. dubius* either 8 days before, on the same day as, or 8 days after *T. spiralis*. Mice were killed on days 6, 9, 12, 15 and 18 after *T. spiralis*, three mice per group being used for histology and five mice per group for worm recoveries. The results are shown in Table 2.

Previous or simultaneous infection of mice with *N. dubius* (groups 1 and 2) delayed the expulsion of *T. spiralis*, which followed an expected pattern in the controls (group 4). Infection with *N. dubius* given 8 days after *T. spiralis* (group 3) did not affect expulsion of that worm, neither did it influence the MMC response. In contrast, the MMC response was either delayed in onset or both delayed and depressed in groups 1 and 2 respectively. These results were confirmed in two further experiments.

EFFECT OF *N. DUBIUS* INFECTION UPON EXPULSION OF *NIPPOSTRONGYLUS BRASILIENSIS* AND THE MMC RESPONSE IN CONCURRENTLY INFECTED MICE

Two groups of NIH mice were used, one (Group A) infected with *N. brasiliensis* alone, and one (group B) with both *N. dubius* and *N. brasiliensis*. Infections were given on the

Table 3. Effect of concurrent infection with 300 *N. dubius* upon the MMC responses of NIH mice infected with 600 *N. brasiliensis*

	Mean no. of MMC/20 v.c.u. in three mice			
	Days after infection			
Group	7/9	12	16	22
N. brasiliensis only	85·0	488·0	200·0	76·0
N. brasiliensis + *N. dubius*	20·0*	92·6	126·3	27·3

* Killed day 9.

same day, using 300 *N. dubius* and 600 *N. brasiliensis* larvae. Faecal egg counts were carried out over a period of 22 days to monitor the course of the infections in each group. The numbers of *N. brasiliensis* present were determined in small groups of three mice killed on days 7 and 14 (group A) and 9 and 14 (group B). The mean values obtained for worm recoveries were 125·0 and 0·0. (group A) and 110·1 and 65·0 (group B). Small numbers of *N. brasiliensis* ($\simeq 20$) were still present in group B mice killed on day 22, and the persistence of this infection was confirmed from egg count data. Groups of three mice were killed at intervals after infection for histology and the results are shown in Table 3. *N. brasiliensis* alone (group A) stimulated a strong MMC response, which was maximal on day 12. In concurrently infected mice (group B) this response was considerably depressed.

MMC RESPONSE DURING PRIMARY AND CHALLENGE INFECTIONS IN SJL MICE

The results of the experiments described above show that infections with *N. dubius* depress MMC responses in mice concurrently infected with *T. spiralis* and *N. brasiliensis*. They suggest, also, that the absence of MMC responses in mice harbouring primary infections of *N. dubius* alone may similarly reflect an active interference by the worm with the inflammatory response. However, it is difficult to test this hypothesis in strains of mice such as NIH and B10.G, which maintain long-term primary infections and develop only limited resistance to reinfection (Behnke & Robinson 1985). SJL mice respond strongly to primary infections, expelling adult worms within 10 weeks of infection and develop high levels of resistance to reinfection (Prowse *et al.* 1979; Behnke & Robinson 1985). They therefore constitute a better strain in which to examine the question of MMC suppression in *N. dubius*-only infections.

Four experiments were carried out in male or female mice given primary infections of 200–300 larval *N. dubius* and one experiment in male mice given 300 larval *T. spiralis* as a positive control. The progress of the infections was followed by worm counts and egg counts (where appropriate) and the MMC response determined in groups of three to five mice killed at intervals after infection. The results of these experiments can be summarized as follows:

1. Uninfected SJL mice had no or very few MMC present.

Table 4. MMC response of SJL mice to primary and challenge infections with 200 and 300 larvae of *N. dubius*

Day after challenge	Mean no. of MMC/ 20 v.c.u. in three mice	Mean no. (±s.d.) of worms in five mice
0(=day 65 of 1°)	68·5	0 —
10	34·0	77±18
15	116·3	17±2
20	456·7	6±2
25	446·0	1±1
30	283·7	0 —
Uninfected controls	0·0	0 —

Mean no. of *N. dubius* day 14 after 1° infection = 140·6.

2. Female SJL expelled *N. dubius* within approximately 40 days of infection. MMC numbers remained low (maximum 30 cells per 20 v.c.u.) or undetectable throughout.
3. Male SJL expelled *N. dubius* within 60 to 65 days of infection. MMC numbers remained low (maximum 68·5 cells per 20 v.c.u.) throughout.
4. SJL mice responded strongly to *T. spiralis*, worm loss beginning after day 9, and produced a moderate MMC response, reaching a maximum of 290 cells per 20 v.c.u. on day 12.

Male SJL mice that had rejected a primary infection of 200 *N. dubius* (mean recovery day 14 = 140·6 worms) were challenged on day 65 with a further 300 larvae. Mice were killed 10, 15, 20, 25 and 30 days later, the numbers of worms determined in groups of five and the MMC response in groups of three. The results are shown in Table 4. Recovery of worms was low on day 10 and the majority had been lost by day 20 after challenge. In contrast to the primary infection, the challenge stimulated a very strong MMC response which was maximum on day 20 (mean value of 456·7 cells per 20 v.c.u.).

Discussion

Mucosal mast cells are now recognized as a distinct subset of mast cells, characterized by differences in development, morphology, ultrastructure, mediator composition and response to activation (Lee *et al.* 1985). In contrast to connective tissue mast cells, which are present in considerable numbers in normal tissues, MMC are absent or relatively infrequent in the normal mouse intestine. Their appearance is characteristic of immunologically-mediated responses to infection with parasitic organisms. Digeneans, cestodes and protozoa such as coccidia, all stimulate a mucosal mastocytosis, but the most dramatic responses occur following infections with nematodes (Miller 1984). Two species in particular, *Nippostrongylus brasiliensis* and *Trichinella spiralis*, have been used extensively to study the parasitological correlates of mastocytosis and to produce material for a variety of other studies concerned with MMC structure, function and development (Lee, Swieter & Befus 1986). From such studies have come data supporting and amplifying the T-cell-dependent nature of the response and the requirement for bone marrow-derived, lamina propria-located precursor cells in generating the explosive

appearance of cells after infection. In the context of the potent mastocytopoietic effects which most nematodes exert, it is surprising to record the virtual absence of a MMC response in mice infected with *Nematospiroidies dubius*. However, this nematode differs markedly from the majority of intestinal nematodes used experimentally in the mouse host in that, in most strains, it establishes as a chronic infection, capable of persisting for many months (Behnke & Robinson 1985). Even in the relatively few strains which do eliminate a primary infection, the time course of worm expulsion is much slower than that seen in infections with *T. spiralis* or *N. brasiliensis*.

The absence of MMC in *N. dubius* infections could be interpreted as showing that this worm, unusually, has little MMC-stimulating activity. However, the volume of evidence which shows not only that *N. dubius* depresses the immune responses of the host, but by doing so enhances its own survival (Behnke *et al.* 1983, Behnke 1987) suggests an alternative explanation, namely that *N. dubius* actively depresses the potential for expression of a MMC response. Three of the experiments described above show clearly that this indeed is the case. In concurrent infections with *T. spiralis* or *N. brasiliensis, N. dubius* markedly depressed and/or delayed the MMC response that these species elicit in NIH mice (Tables 2 & 3). This effect was seen only when infection with *N. dubius* was given before or simultaneously with the other parasite, infection with *N. dubius* given when a MMC response had been elicited (Table 2, group 3) had no effect, showing that the presence of the parasite did not cause the disappearance of mature cells.

Primary infections with *N. dubius* were studied in three inbred strains (NIH, B10.G and SJL) all of which produce a mastocytosis on infection with *T. spiralis*. The failure to detect a significant MMC response with *N. dubius* in these strains shows that the effect is parasite-determined and independent of host genotype. Host genotype was, however, an important factor in the choice of SJL for the experiment using a challenge infection, as this strain can expel primary infections and displays strong immunity to challenge (Prowse *et al*, 1979, Behnke & Robinson 1985). Expulsion of a primary infection from SJL mice was not accompanied by mastocytosis, showing that, despite the activation of a host-protective response, the parasite was still capable of inhibiting some components of the mucosal reaction. It also follows from this observation that a MMC response *per se* is not necessary for expulsion of adult *N. dubius*. SJL mice clearly have the capacity to overcome the immunodepressive effect of *N. dubius* and, under these conditions, if the parasite is capable of inducing mastocytosis, a marked response should be evident on reinfection. As shown in Table 4 this did occur. Other experiments (paper in preparation) have shown that good MMC responses can be elicited in susceptible NIH mice, if the immune status is manipulated appropriately by exposure to drug-abbreviated or irradiation-attenuated primary infection. The data in Table 4 imply that challenge infections are largely inhibited at the mucosal stage in SJL mice, and MMC may contribute to the destruction of larvae in the granulomata which form around them. Certainly mast cells are numerous within these granulomata (Dehlawi, unpublished).

The observation that *N. dubius* exerts such a powerful influence upon MMC responses has a number of important implications for analysis of the control and regulation of this cell population. Nematode infections remain the most effective and reliable means of stimulating MMC responses, yet it is also clear that certain species have evolved ways of interfering with the development of mastocytosis. The net result of this interference may be enhancement of parasite survival, but it is unlikely that the suppressive effect is directed specifically at the MMC response, especially in view of the fact that SJL mice can expel worms in the absence of a mucosal mastocytosis. It is more probable that the effect is

exerted via the T-cell control of mastocytosis and reflects a more general inhibition by *N. dubius* upon immunological interactions, acting both at the mucosal level and systemically, through which the worms prolong their survival (Hagan & Wakelin 1982, Behnke 1987). The stages of *N. dubius* responsible for the effects upon MMC and an analysis of the mechanisms through which the effect is expressed will form the subject of a subsequent paper.

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References

ALIZADEH H. & WAKELIN D. (1982) Comparison of rapid expulsion of *Trichinella spiralis* in mice and rats. *International Journal for Parasitology* **12,** 65.

BEHNKE J.M. (1987) Evasion of immunity by nematode parasites causing chronic infections. *Advances in Parasitology* in press.

BEHNKE J.M. & ROBINSON M. (1985) Genetic control of immunity to *Nematospiroides dubius:* a 9-day anthelmintic abbreviated immunizing regime which separates weak and strong responder strains of mice. *Parasite Immunology* **7,** 235.

BEHNKE J.M., HANNAH J. & PRITCHARD D.I. (1983) *Nematospiroides dubius* in the mouse: evidence that adult worms depress the expression of homologous immunity. *Parasite Immunology* **5,** 397.

BEHNKE J.M., WAKELIN D. & WILSON M.M. (1978) *Trichinella spiralis:* delayed rejection in mice concurrently infected with *Nematospiroides dubius. Experimental Parasitology* **46,** 121.

GUY-GRAND D., DY M., LUFFAU G. & VASSALLI P. (1984) Gut mucosal mast cells. Origin, traffic and differentiation. *Journal of Experimental Medicine* **160,** 12.

HAGAN P. & WAKELIN D. (1982) *Nematospiroides dubius:* effect of infection on lymphocyte responses to *Trichinella spiralis* in mice. *Experimental Parasitology* **54,** 157.

JENKINS D.C. (1975) The influence of *Nematospiroides dubius* on subsequent *Nippostrongylus brasiliensis* infection in mice. *Parasitology* **71,** 349.

JENKINS S.N. & BEHNKE J.M. (1977) Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius. Parasitology* **75,** 71.

JENNINGS F.W., MULLIGAN W. & URQUHART G.M. (1963) Variables in X-ray inactivation of *Nippostrongylus brasiliensis* larvae. *Experimental Parasitology* **13,** 367.

LEE T.D.G., SWIETER M. & BEFUS A.D. (1986) Mast cell responses to helminth infection. *Parasitology Today* **2,** 186.

LEE T.D.G., SWIETER M., BIENENSTOCK J. & BEFUS A.D. (1985) Heterogeneity in mast cell populations. *Clinical Reviews in Immunology* **4,** 143.

MILLER H.R.P. (1984) The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. *Veterinary Immunology and Immunopathology* **6,** 167.

PROWSE S.J., MITCHELL G.F., EY P.L. & JENKIN C.R. (1979) The development of resistance in different inbred strains of mice to infection with *Nematospiroides dubius. Parasite Immunology* **1,** 277.

WAKELIN D. & WILSON M.M. (1977) Transfer of immunity to *Trichinella spiralis* in the mouse with mesenteric lymph node cells: time of appearance of cells in donors and expression of immunity in recipients. *Parasitology* **75,** 215.

WOODBURY R.G., MILLER H.R.P., HUNTLEY J.F., NEWLANDS G.F.J., PALLISER A.C. & WAKELIN D. (1984) Mucosal mast cells are functionally active during the spontaneous expulsion of primary intestinal nematode infections in the rat. *Nature (Lond.)* **312,** 450.