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# Parameters of intestinal inflammation in mice given graded infections of the nematode *Trichinella spiralis*

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**Abstract**

Four parameters of the intestinal inflammatory response (numbers of mucosal mast cells (MMC) and Paneth cells, villus:crypt ratios and mitotic figures) were measured in mice exposed to varying doses of infective larvae of *Trichinella spiralis.*The aim of the experiments was to determine whether generation of these components of inflammation required a threshold level of infection and whether, once triggered, inflammation became pan-mucosal. Near maximal MMC and Paneth cell responses were elicited even with infections as low as 35 larvae; changes in villus:crypt ratios and in mitotic indices also occurred at this level of infection, but were progressively greater with increasing levels of infection. In all infected mice, including those infected with 35 larvae, MMC and Paneth cell responses extended over most of the small intestine. These data are interpreted as showing: (i) that the intestinal mucosa is highly responsive to *T. spiralis* infection; (ii) that once triggered, components of the inflammatory response are amplified by T cell-dependent mechanisms, becoming pan-mucosal; and (iii) that MMC and Paneth cell responses, which require cell division and differentiation, become maximal at a lower infection threshold than changes in the villus:crypt ratio or in mitotic indices, which directly reflect increased rates of division in crypt cells.

## Introduction

The parasitic nematode *Trichinella spiralis* is known to act as a potent stimulus for intestinal inflammatory responses in infected mice. These responses undoubtedly exert a profound influence on the host–parasite relationship. Their contribution to expulsion of the parasite is disputed (Garside *et al.*, 2000) but they have important pathological consequences for the host by disturbing normal patterns of feeding and nutrition. Although parasite invasion causes direct damage to the epithelial cells in which the worms live, the generation of inflammatory changes is largely dependent upon the activities of T cells of the T helper 2 (Th2) subset, and is mediated through the release of cytokines (Grencis, 1997). The antigens that elicit these changes are known to be released from the stichosome and are also present on the cuticle of the worm (Ortega-Pierres *et al.*, 1996). The antigenic stimulus is therefore confined initially to the immediate vicinity of the worm (Despommier, 1993). It can be assumed that the antigens are then made available to mucosal T cells, as antigen-specific T cells can later be identified both in the lamina propria (Lawrence *et al.*, 1998) and in the draining mesenteric lymph node (Grencis & Wakelin, 1982). Nothing is known of the route by which antigens are presented to T cells or of the spatial relationships of activated T cells and mucosal inflammatory events. An important question is whether inflammation is limited to the sites occupied by the worms or, once elicited, is effectively pan-mucosal, because this implies that there is an effective T cell-mediated amplification step in the host's response. Data relevant to this question can be gained by following the inflammatory process in mice given graded levels of

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infection. In an earlier paper (Dehlawi & Wakelin, 1995) it was shown that the increase in numbers of mucosal mast cells (MMC – a major component of the intestinal response to *T. spiralis*) was similar in mice exposed to infections of 100, 300 and 600 larvae, rising sharply between days 6 and 9 after infection in each case. In mice given only 30 larvae, and in which only a very small number of worms established (< 5 in this experiment) numbers of MMC did increase by day 12, but the increase was significantly smaller than in mice given larger infections.

A fuller picture of the dose-dependency of inflammatory responses to *T. spiralis* can be obtained by monitoring a broader range of inflammatory components. This paper describes experiments in which four parameters of inflammation – numbers of MMC, numbers of Paneth cells, villus:crypt ratios and crypt mitoses – were measured in mice exposed to various infection levels.

## Materials and methods

### *Mice*

Female NIH strain inbred mice were obtained from Harlan-Olac (Bicester, Oxon, UK) and used at approximately six weeks of age. They were maintained under conventional animal house conditions.

### *Parasite*

The London isolate of *T. spiralis* (ISS 25) was used throughout. Techniques for maintenance, infection and recovery were as described previously (Wakelin & Lloyd, 1976).

### *Histology*

Intestinal tissues were fixed in Carnoy's fixative for general histology and MMC counts or in 10% buffered formalin for Paneth cell counts. Blocks were made from 10 cm pieces of small intestine, using the 'Swiss Roll' technique, and sectioned at 5 $\mu$m (or 2 $\mu$m for Paneth cells).

Sections were stained for MMC with a 1:1 mixture of Astra Blue:Alcian Blue followed by counterstaining with Safranin O essentially as described by Dehlawi *et al.* (1987); for villus-crypt measurements and counts of mitoses sections were stained with haematoxylin and eosin using standard methods. To count Paneth cells, formalin-fixed tissues were stained with phloxine-tartrazine (Subbuswamy, 1973). Briefly, sections were hydrated, stained for 5–7 min in Carazzi's haematoxylin (Nustain, Nottingham), differentiated in 1% HCl in 70% ethanol, blued in tap water and then stained in 0.5% phloxine solution (Nustain) for 10–12 min, washed and then differentiated for 5 min in a saturated solution of tartrazine (Nustain) in 2-ethoxyethanol. After staining, the sections were washed in 95% alcohol, dehydrated, cleared and mounted.

Histological data are based on counts from three (MMC, Paneth cells) or four (villus:crypt ratio and mitotic index) mice per group. Mucosal mast cells were counted in a total of 20 villus-crypt units (vcu) per mouse. Villus:crypt ratios were calculated from 10 measurements of villus height and crypt depth per mouse, using a calibrated micrometer eyepiece. Mitotic figures in 10 crypts per mouse were counted to give a mitotic index. Paneth cells were counted in 20 crypts per mouse. In uninfected mice the granules of Paneth cells are predominantly yellow, indicating immature cells, whereas in infected mice mature granules stain red. The counts made included both immature and mature cells.

Data were analysed using the non-parametric Mann-Whitney test. A value of $P < 0.05$ was considered statistically significant.

## Results

Data from two experiments are described. In the first, mice infected with 35, 70, 140 or 280 *T. spiralis* larvae were used to determine the dose-dependency of the components of the inflammatory response; uninfected mice were used to provide control base-line data. Worm establishment was measured on day 6, the remaining mice were killed (three per group) on day 10 for histological examination, using tissue taken from the first 10 cm of the small intestine. The data from this experiment showed little difference in cellular aspects of inflammation between the highest and lowest infection dose. The second experiment was therefore designed to follow both the time course and the distribution of MMC and Paneth cell changes in mice given low (35 larvae) infections and killed on days 4, 8 and 12. Lengths of intestine approximately 10 cm long were taken from each of the duodenum, jejunum and ileum and processed for histology. Changes in mice infected with 35 larvae were compared to those seen at day 8 in mice given 350 larvae, which acted as a positive control. Worm counts were again measured on day 6.

### *Experiment 1*

At day 6 after infection adult worm recoveries from the different infection levels were 48% (280 larvae), 59% (140 larvae), 79% (70 larvae) and 71% (35 larvae). Values for numbers of MMC, Paneth cells, mitotic indices and villus:crypt ratios are given in table 1. Control mice had few or no MMC present (< 5 MMC per 20 vcu), but all infected mice had large numbers present on day 10. Whereas numbers of MMC showed no significant differences between groups infected with the different dose levels, Paneth cell numbers, although significantly elevated compared with controls (mean 28 per 20 crypts), were lowest in mice infected with 35 larvae and greatest in mice infected with 280 larvae. Mice infected with 70 or 140 larvae had intermediate numbers of cells. In infected mice the granules of the Paneth cells stained red, and cells were present not only in the bases of the crypts but also higher up on the crypt walls. Numbers of mitotic figures were increased in all infected mice, the groups infected with 35 and 70 larvae having approximately twice as many mitoses per crypt as controls, and those infected with 140 or 280 larvae having approximately three times as many. The villus:crypt ratio was decreased in all

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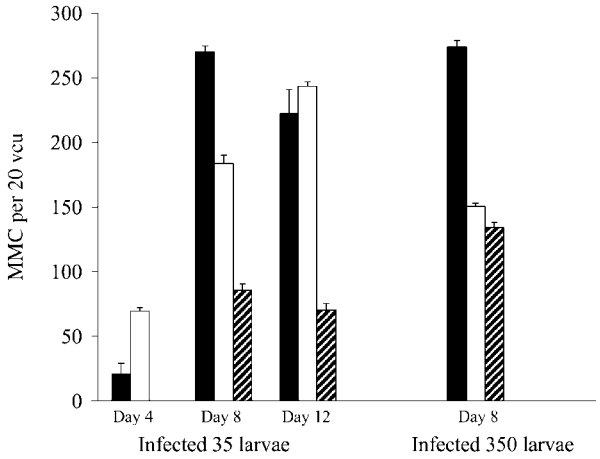


Fig. 1. Time course of mucosal mast cell (MMC) response in NIH mice infected with 35 or 350 *Trichinella spiralis* larvae on day 0. Tissues were taken from the duodenum (■), jejunum (□) and ileum (▨) MMC counts are expressed as mean numbers (±S.E.) per 20 villus-crypt units (vcu) taken from three mice per group.

infected mice, showing a negative correlation with the number of larvae given.

*Experiment 2*

Mean worm recoveries on day 6 were 20.8 (59%) in mice given 35 larvae and 168.3 (48%) in mice infected with 350 larvae. The time course of the MMC and Paneth cell responses are shown in figs 1 and 2.

In mice given 35 larvae MMC numbers had increased in the duodenum and jejunum on day 4, but none were recorded in the ileum (fig. 1). A major increase occurred by day 8 in all regions and this was maintained on day 12.

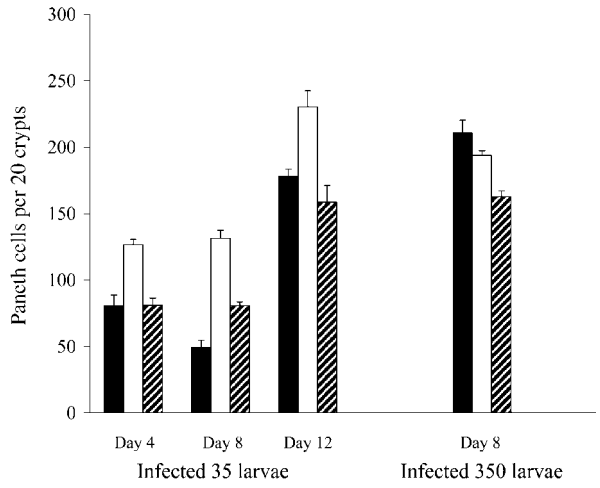


Fig. 2. Time course of Paneth cell response in NIH mice infected with 35 or 350 *Trichinella spiralis* larvae on day 0. Tissues were taken from the duodenum (■), jejunum (□) and ileum (▨). Paneth cell counts are expressed as numbers (±S.E.) per 20 crypts taken from three mice per group.

Mucosal mast cell numbers in the ileum were significantly lower than in the other two regions of the intestine. Overall MMC counts were similar at day 8 in mice given 35 or 350 larvae, although the latter had significantly more MMC in both the jejunum and ileum.

In control mice mean Paneth cell numbers were: duodenum 16 per 20 crypts, jejunum 64 per 20 crypts and ileum 42 per 20 crypts. In mice given 35 larvae there were significant increases on days 4 and 8, with the largest numbers seen on day 12 (fig. 2). Mice given 350 larvae had significantly greater numbers of Paneth cells on day 8 in all regions of the intestine than those given the smaller infection.

## Discussion

The intestinal inflammatory response induced in mice by infection with *T. spiralis* is complex and has profound effects on the structure and function of the mucosa. The degree of the response reflects genetically-determined characteristics of the host, NIH mice being high responders and developing pronounced inflammatory changes rapidly after infection (Alizadeh & Wakelin, 1982). Three of the four components of the inflammatory response studied here can all be related to changes in the kinetics and differentiation of epithelial stem cells. Increased cell division in the crypts, measured here in terms of mitotic indices, leads to a more rapid transit of epithelial cells along the surface of the villi and a higher rate of cell loss at the villal tips. In consequence villi become shorter and crypts longer, resulting in a decreased villus:crypt ratio. A direct consequence of altered stem cell behaviour is an increase in the number, distribution and maturation of Paneth cells, which has only recently been identified as a consequence of *T. spiralis* infection (Kamal *et al.*, 2001a). These changes are accompanied by a marked infiltration of the mucosa by MMC, cells that develop from bone marrow-derived precursors.

Previous workers have shown that alterations in the villus:crypt ratio and infiltration of MMC are T cell-dependent (Garside *et al.*, 1992), being regulated through the activity of a number of cytokines, including IL-4 and TNF-$\alpha$ (Lawrence *et al.*, 1998). The increase in Paneth cells is also T cell-dependent, but appears to require a rather different population of mucosal T cells (Kamal *et al.*, 2001b). Although not demonstrated specifically, it can be assumed that the increased rate of mitosis in crypt cells is also T cell-influenced.

The MMC data described here confirm a previous study (Dehlawi & Wakelin, 1995) which showed that only a low threshold level of infection (considered to be about 20 worms) was necessary to induce the maximum MMC response, infection levels above this threshold having little effect on the level of response obtained. It was concluded from this that there must be a very effective amplification step in induction of the MMC response, initial release of antigen leading to a rapid T cell response with cytokine release, MMC degranulation then releasing cytokines to boost the response further. Figure 1 implies that these amplification steps, once initiated, must operate over a considerable length of the small intestine

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Table 1. Inflammatory changes at day 10 in the intestines of mice infected with different numbers of *Trichinella spiralis* larvae.

| | MMC per 20 vcu | | Paneth cells per 20 crypts | | Villus:crypt ratio | Mitotic index | |
|---|---|---|---|---|---|---|---|
| Group | Mean | S.E. | Mean | S.E. | Mean | Mean | S.E |
| Control | 0.0 | 0.0 | 28.0 | 1.2 | 5.73 | 3.56 | 0.57 |
| 35 larvae | 439.0* | 5.6 | 145.3** | 6.4 | 2.30** | 6.19** | 0.33 |
| 70 larvae | 473.7* | 22.0 | 203.3* | 11.7 | 1.40* | 6.43** | 0.25 |
| 140 larvae | 427.7* | 16.0 | 195.7* | 6.3 | 0.63* | 9.19* | 0.57 |
| 280 larvae | 445.3* | 116.3 | 245.0* | 9.5 | 0.31* | 11.00* | 0.91 |

*Group mean significantly different from control ($P < 0.05$).
**Group mean significantly different from both control and mice infected with 280 larvae ($P < 0.05$).
MMC, mucosal mast cells; vcu, villus-crypt units.
MMC and Paneth cell counts based on data from three mice per group. Villus:crypt ratio and mitotic index based on counts from four mice per group.

to produce the mastocytosis seen in the duodenum and jejunum, given that the small number of worms present (each only a few mm in length) would occupy a very small part of the mucosal surface.

It is clear from table 1 and fig. 2 that the Paneth cell response is also triggered at a low threshold level of infection, and once triggered occurs in all regions of the intestine. This again implies the existence of a powerful amplification step, in this case affecting the patterns of division and differentiation of crypt stem cells.

In contrast to both the MMC and Paneth cell responses, changes in the villus:crypt ratio and in mitotic indices showed a clear dose dependency. All infection levels, including the lowest at 35 larvae, induced significant changes in these parameters compared with uninfected controls, but the magnitude of the changes was directly related to the number of worms established. Mucosal mast cell responses are not directly related to changes in stem cell activity, but Paneth cell numbers are. Altered rates of division and changed patterns of stem cell differentiation result in the appearance of more Paneth cells, extension of cells onto the walls of the crypts and maturation of the cytoplasmic granules. However, the fact that there is an infection dose-related threshold in Paneth cell changes implies that there is a maximum capacity for change and that continuing increases in cell division in the crypt, while affecting villus and crypt lengths, has no further affect on Paneth cell numbers.

The data presented here extend our knowledge of the influence of *T. spiralis* on the host's intestine, confirming the view firstly that only small amounts of parasite antigen are necessary to elicit specific changes in T cell activity and, secondly, that powerful amplification steps rapidly induce maximal inflammatory changes even when only small numbers of worms are present. Although the contribution of these changes to the survival of worms in the intestine is still debated, our results suggest strongly that hosts have highly sensitive mechanisms for detecting intestinal infection, and that, although these are antigen-specific initially, they rapidly and effectively trigger a wide range of non-specific inflammatory events that can only make the intestine a less favourable environment for worm survival.

## Acknowledgements

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