Nippostrongylus brasiliensis: Mast Cell Kinetics at Small Intestinal Sites in Infected Rats

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MacDonald, T. T., Murray, M., and Ferguson, A. 1980. Nippostrongylus brasiliensis: Mast cell kinetics at small intestinal sites in infected rats. Experimental Parasitology 49, 9–14. Mast cell kinetics during infection with the nematode Nippostrongylus brasiliensis were studied at various sites in the small bowel of rats and in heterotopically transplanted isografts of foetal small intestine placed under the kidney capsule. Infection produced an increase in the number of mast cells not only in the proximal jejunum, where most of the worms are located, but also in the distal ileum and in isografts of small intestine. However, globule leucocyte infiltration of the gut epithelium was confined to the proximal small intestine and did not occur in the distal ileum or isografts. These results show that the mast cell increase in the small bowel of N. brasiliensis-infected rats is a property of the whole organ, and is not restricted to sites of worm infection; but that in contrast, globule leucocyte infiltration of the epithelium is dependent upon the presence of worms within the bowel lumen.

Index descriptors: Nippostrongylus brasiliensis; Nematode, parasitic; Rat; Mast Cell; Small intestine.

Introduction

At a site of infection, increase in the number of immunologically competent cells may be induced by division of cells already in the tissues, or by migration of other cells into the site. The types of lymphoid cells which migrate to the gut, and mechanisms involved in their homing patterns, have been the subjects of considerable investigation. In general, principally T and B immunoblasts derived from mesenteric node or thoracic duct tend to be attracted to the lamina propria and the gut epithelium (Gowans and Knight 1964; Guy-Grand et al. 1974; Parrott and Ferguson 1974). However, this attraction is antigen nonspecific, for blasts migrate to antigen-free isografts as well as they do to normally sited gut (Guy-Grand et al. 1974; Parrott and Ferguson 1974). There may be a small component of antigen-related attraction of blasts (Pierce and Gowans 1975). However, if the gut is inflamed, for example by worm infection or a chemical irritant, blasts from peripheral lymph nodes also migrate to the intestine (Rose et al. 1976a).

Factors which attract cell types other than lymphocytes to the gut have not been investigated. This is of special significance in the study of parasite infections, for a variety of cell types including mast cells are important in intestinal parasite immunity. In parasitic worm infections in the rat there is an increase in mast cell numbers in the mucosa at the site of infection (Barth et al. 1966; Murray et al. 1971). We have therefore used this model to study the factors controlling the increase of mast cells in the gut mucosa. In addition, we have also investigated globule leucocyte kinetics within the gut epithelium. These cells also greatly increase in number during worm infection,
and are probably degranulated mast cells which have migrated from the lamina propria into the gut epithelium (Murray et al. 1968). *Nippostrongylus brasiliensis* predominantly localizes in the proximal jejunum, 20 cm from the pylorus (Murray et al. 1971), so we compared the kinetics of mast cells and globule leucocytes at this site with other portions of the small intestine where few or no worms are present (in the ileum), and in heterotopically transplanted isografts of intestine where no antigen is present in the lumen.

**MATERIALS AND METHODS**

**Host animals and parasite infections.** Inbred and outbred female hooded Lister rats, aged 8–10 weeks, were used. Infective *Nippostrongylus brasiliensis* third-stage larvae were prepared as previously described (Jennings et al. 1963). Rats were infected by a single subcutaneous injection of 3000 larvae.

**Implantation of isografts of foetal small intestine.** This was done as previously described (Ferguson and Parrott 1972). 20-day-old foetal rats were dissected from the uterus of an inbred hooded Lister female. The small intestines were removed and placed in cold phosphate buffered saline. Segments of foetal intestine (5–10 mm in length) were then implanted under the kidney capsule of adult syngeneic recipients. The grafts were allowed to grow for 4 weeks before the rats were infected with *N. brasiliensis*.

**Histology.** At various intervals after infection, groups of rats were killed and a segment of intestine, approximately 1 cm long, was taken from the following regions of the small intestine: 10 cm from the pylorus (Site A), 20 cm from the pylorus (Site B), and 20 cm proximal to the caecum (Site C). If grafts were present in the rat these were dissected out along with a piece of underlying kidney. Pieces of small intestine were cut open, laid on dry filter paper, and fixed in Carnoy's fluid. Isografts were placed directly into fixative. Subsequent processing and staining was as previously described (Murray et al. 1971).

**Counts of mast cell and globule leucocyte numbers.** The number of mast cells in the lamina propria of 20 villus: crypt (V:C) units of each piece of tissue was counted as before (Miller and Jarrett 1971). In addition, the number of globule leucocytes within the epithelium of 20 V:C units was also counted. Mast cells and globule leucocytes were easily recognizable in histological sections because of their blue-staining cytoplasm.

**RESULTS**

Small intestinal infection with *Nippostrongylus brasiliensis* followed the usual time course (Ogilvie and Love 1974). Worms were first present in the gut at 2 days, where they remained until Day 12. There was then a rapid period of worm elimination so that by Day 20 few remained in the gut. Grafts of small intestine took and grew, and were easily recognizable as white swellings under the kidney capsule. Histological examination of the grafted intestine revealed a reduction in villus height, and fewer lymphoid cells in the lamina propria of the grafts, when compared to normal gut, but in other respects the tissues were essentially normal.

**Mast Cell Numbers in Normal and Grafted Intestine**

The influence of worm infection on the mast cell kinetics in normally sited intestine is shown in Fig. 1. Mast cells were present at all three sites in the small intestine at Day 0, the highest number being 10 cm distal to the pylorus (Site A). This is probably a reflection of the lamina propria volume at these sites, villus height being much greater in the duodenum than in the ileum (Altmann and Enesco 1967). At Sites A and B there was a drop in mast cell numbers on Days 4–10, but thereafter their numbers increased dramatically, maximal at Days 16–20, and remaining high even until Day 40. There was no reduction in mast cell
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numbers during the first 10 days at Site C, but again, starting on Day 12, there was an increase in the numbers of mast cells in the lamina propria.

The effect of worm infection on mast cell numbers in isografts of small intestine is shown in Table I. Mast cells were present in all of the grafts at Day 0. Seven days after infection there was no change in their numbers, but by 19 days postinfection there was a twofold increase in mast cells in the lamina propria of the grafts. Normally sited intestine (proximal jejunum) taken from these same rats showed the same pattern of mast cell kinetics as Site B in Fig. 1.

Globule Leucocyte Numbers in Normally Sited and Grafted Intestine

There were no globule leucocytes in the epithelium at any of the sites in normally sited intestine until 10 days after the start of the worm infection. At Sites A and B, their numbers increased dramatically in the next few days peaking at Day 16, and falling back to very low levels by Day 40. At Site C there was a small increase in the number of globule leucocytes in the epithelium at Days 16 and 20, but the levels never reached that observed in the proximal small intestine. These results are illustrated in Fig. 2.

Table II shows the results of globule leucocyte counts in isografts of small intestine at various times after worm infection. There was no change in their numbers over the time course of the experiment. In normally sited small intestine (proximal jejunum) taken from these animals there

| TABLE I |
| Mast Cell Numbers per V:C Unit in Isografts and Normally Sited Intestine at Various Times after Infection with Nippostrongylus brasiliensis |

<table>
<thead>
<tr>
<th>Mast cells per V:C unit (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Isografts*</td>
</tr>
<tr>
<td>Normally sited small intestine (proximal jejunum)*</td>
</tr>
</tbody>
</table>

* At least 6 grafts or 5 rats per group.

* P < 0.001 when compared to the mast cell numbers in grafts at Day 0 and Day 7 (Student’s t test).

* P < 0.001 when compared to the mast cell numbers in normal small intestine at Day 0 and Day 7 (Student’s t test).
were virtually no globule leucocytes present at Days 0 and 7, but their numbers increased by Day 19.

**Discussion**

It is well established that there is an increase in mast cell numbers at the site of infection during the expulsion phase of *Nippostrongylus brasiliensis* from the small intestine of rats. In addition, there is also an increase in mast cell numbers at other sites in the body e.g., the mesenteric lymph node (Keller et al. 1974), lung (Wells, 1977), and transverse colon (Mayrhofer et al. and Gowans, 1976). We have now demonstrated that the increase in mast cells in the small bowel is not restricted to the site of infection, but occurs at other sites in the gut where there is no worm burden. In addition, there is an increase in mast cells in sterile antigen-free grafts of small intestine heterotopically transplanted under the kidney capsule. These data then suggest that the mast cell increase at the site of worm infection is not antigen specific, but may represent an increased homing of mast cell precursors to intestinal tissue, regardless of the presence of worm antigen. Alternatively, the presence of the worms or the immune response against them could by some unknown mechanism cause systemic mast cell multiplication, for during worm infection there is an increase in mast cell numbers at other sites in the body (Mayrhofer et al. 1976; Wells 1977).

There are few detectable mast cells present at the site of infection until Day 10, however, at this time there appears in the intestinal mucosa a population of blast cells which undergoes rapid division and differentiates into mast cells (Miller 1971; Miller and Jarrett 1971). These cells could have

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**Table II**

Globule Leucocyte Numbers per V:C Unit in Isografts and Normally Sited Intestine at Various Times after Infection with *Nippostrongylus brasiliensis*

<table>
<thead>
<tr>
<th></th>
<th>Isografts(^a)</th>
<th>Normally sited small intestine (proximal jejunum)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Globule leucocytes per V:C unit (mean ± SE)</td>
<td>0.15 ± 0.12</td>
<td>0.16 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.05</td>
<td>0.06 ± 0.04</td>
</tr>
</tbody>
</table>

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\(^a\) At least six grafts or 5 rats per group (mean ± 1 SE).

\(^b\) *P* < 0.001 when compared to the Day 7 levels in normal small intestine (Student's *t* test).
differentiated from precursor cells, not recognizable as mast cells, but already present in the lamina propria. Alternatively, they might represent an influx of newly formed cells, perhaps derived from cells of the lymphoid series, into the gut mucosa (Miller 1971). If the mast cell increase in the gut does reflect increased homing of precursor cells, then the mast cell expansion seen in the gut grafts shows that proliferation and differentiation of the mast cell in the lamina propria are not dependent upon the presence of worm antigen from the lumen.

It is relevant to compare our results on mast cell kinetics in the bowel during worm infection with the observations made by other workers on the homing of lymphoblasts to worm-infected gut. The salient point of recent papers by Rose and her coworkers (1976a, b) on lymphocyte migration to Trichinella spiralis-infected gut is that at Day 4 postinfection there is increased lodging of lymphoblasts in the gut mucosa. This response is not antigen specific for lymphoblasts from normal mice also home in increased numbers to infected gut, as do blasts from oxazolone-stimulated peripheral lymph nodes (Rose et al. 1976a, b). In addition, it has been known for several years that lymphoblasts home to antigen-free grafts of small intestine placed under the kidney capsule as readily as they do to normal gut (Guy-Grand et al. 1974; Parrott and Ferguson 1974). Our present data on mast cells therefore indicate that there are similarities between the homing of lymphoblasts and mast cells: for increases in mast cell numbers are also antigen nonspecific, and they are also found in antigen-free grafts of small intestine.

During parasitic worm infection, as well as an increase in mucosal subepithelial mast cells, there is also an increase in the numbers of a morphologically related cell, the globule leucocyte, within the gut epithelium (Murray et al. 1968). We therefore also investigated globule leucocyte kinetics in the various tissues during worm infection. There were virtually no globule leucocytes in the epithelium of normal intestine, at any site, or in parasitized gut until 12 days postinfection. Starting at Day 12, however, there was a rapid increase in their number in the proximal small intestine, which was greatest at Day 16 and then rapidly declined. There was only a slight increase in globule leucocyte numbers in the distal ileum at this time, and no increase in their numbers in isografts. The globule leucocyte is thought to be derived from the lamina propria mast cell, and increases greatly in numbers during worm expulsion, at the time when there is extensive mast cell degranulation (Murray et al. 1968). There was no obvious mast cell degranulation in isografts of small intestine during worm expulsion, and very little in the terminal ileum (MacDonald, personal observation). Therefore, while mast cell expansion in the gut seems not to be directly related to the presence of worms in the gut lumen, local factors related to the worms, or the immune response against them, seem necessary before there is a globule leucocyte increase within the epithelium. In support of this, we observed a slight increase in globule leucocyte levels in the ileum at Days 16 and 20 after infection, the time when the worms are passing down the intestine after being expelled from the proximal bowel.

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References


