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Paracrystallization and Dispersion of Murine Mucosal Mast Cell Granules after *Strongyloides ratti* Infection or IL-3 Administration

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Abstract

Ultrastructural morphological changes of the granules of mucosal mast cells (MMC) in the intestinal mucosa was examined in mice either infected with *Strongyloides ratti* or treated with IL-3. MMC of normal mice had round- or oval-shaped granules. On day 7 post-infection (PI), granules of some, but not all, MMC began to deform into polygonal shape and showed paracrystalline structures. On days 9 to 14 PI, when intestinal worms were expelled from mice, MMC granules frequently fuse each other to form large paracrystalline structures (rhomboid- or rod-shaped), and their matrix portions finally disappeared. Such sequential changes were commonly seen in MMC in the epithelial layer and those in the lamina propria. Some MMC, located nearby the intestinal lumina contained numerous fine paracrystalline structures dispersed in the cytoplasm. MMC induced by IL-3 administration for 7 to 14 days showed granule changes similar to those observed on days 9 and 14 PI. Sequential changes of granule morphology in MMC e.g., paracrystallization and subsequent dispersion, is possibly associated with the mediator release of MMC.

Key words: mucosal mast cells; granules; paracrystallization; *Strongyloides ratti*; IL-3; mouse.

Introduction

Intestinal mastocytosis is one of the features in some parasitic infections in mice and rats. Mucosal mast cells (MMC), which are different from connective tissue type mast cells, have been considered to play a role in the expulsion of *Strongyloides* spp. (Abe and Nawa, 1988; Nawa *et al.*, 1994). IL-3-injected mice showed an increased level of MMC specific proteases in the small intestinal lumen (Abe *et al.*, 1993). MMC are probably involved in protection against *S. ratti* by releasing their mediators without antibody participation. A direct evidence of degranulation of MMC has not been shown yet in *S. ratti*-infection in mice. Therefore, we studied ultrastructural morphological changes of intestinal MMC in mice infected with *S. ratti* or injected with IL-3.

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The results show that granules of intestinal MMC, either induced by *S. ratti*-infection or by IL-3 administration, also exhibit paracrystallization and subsequent dispersion.

Materials and Methods

Animals

Male C57BL/6 mice were raised from the original colony obtained from Japan SLC Co. (Shizuoka, Japan) in the Animal Facilities for Experimental Medicine, Akita University School of Medicine and used at 14–15 weeks of age. Wistar rats were obtained from Japan SLC Co. All animal experiments followed the Guidelines for Animal Experimentation, Akita University School of Medicine.

Parasite

S. ratti was maintained by serial passage in Wistar rats. Infective larvae were obtained by faecal culture on filter papers, washed three times with saline and injected subcutaneously with 0.2 ml of saline into mice.

Preparation of recombinant IL-3

Recombinant IL-3 was prepared from a culture supernatant of myeloma cell line transfected with murine IL-3 cDNA, X63BMG 14-17, as described previously (Abe *et al.*, 1992). Briefly, the myeloma cells were cultured in a serum-free medium containing antibiotics for 2–3 days at 37°C in 5% CO_2. Culture supernatants were pooled, concentrated 10 times by ultrafiltration with Minitan-S (Millipore, Tokyo, Japan) and stored at –20°C until used. One unit of IL-3 was defined as the amount of factor required to support half-maximal [^{3}H] thymidine incorporation by 2×10^4 FDC-P2 cells in 0.2 ml culture.

Treatment of mice

An osmotic mini-pump (Alzet 2001; Alza Corp., Palo Alto, CA, USA) filled with 0.2 ml of rIL-3 (1.7×10^5 U) was implanted in the dorsal subcutaneous tissues of normal mice for 7 days. In order to continue IL-3 injection for additional 7 days, the old mini-pump was replaced with a new one at 7 days after the first implantation in another experiment. When mice were killed, the mini-pump was confirmed to be empty. To examine morphological changes of MMC at an early stage of IL-3 treatment, a group of mice were injected i.p. with 1.1×10^5 U rIL-3 daily for 3 or 5 days and killed. Another group of mice were infected s.c. with 2000 larvae of *S. ratti* and killed on Days 7, 9 or 14 post infection (PI).

Ultrastructural examination

Two mice were euthanized by an overdose of ether on the designated days. A part of small intestine at about 10 cm distal to the pylorus was removed immediately and cut into several pieces of 1 mm in width. The pieces were fixed in cold 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 for 3 hr, followed by washing twice with the sodium cacodylate buffer. And then, they were post-fixed in 1% osmium tetraoxide in 0.1 M sodium cacodylate buffer for 1.5 hr at 4°C, dehydrated with ethanol and embedded in the epoxy resin, TAAB812. Ultrathin sections were prepared by an ultramicrotome (LKB, Sweden) with a diamond knife. Those sections were stained with uranyl acetate and lead citrate, and then examined with an electron microscope, Akashi LEM 2000 (Japan). In order to identify intestinal MMC on a particular ultrathin section, an adjacent section (1 μm) was prepared for light microscopy and stained with both alkaline-Giemsa diluted 1:10 in borate buffer (pH 7.25) by the method of Dvorak *et al.* (1970) and toluidin blue-O. The cytoplasmic granules of MMC, which were stained metachromatically with Giemsa at alkaline pH, were stained with toluidine blue as well.

Results

MMC of mice infected with S. ratti

Only few MMC were seen in the epithelial layer of normal mice. They consistently contained few round granules (Fig. 1a). Although most of granules of MMC on Day 7 PI were generally round- or oval-shaped, granules of some MMC turned to polygonal shape and seemed to fuse each other (Fig. 1b). During Days 9 and 14 PI MMC granules showed drastic changes. Extensive fusion of granules (Fig. 1c) and paracrystallization were characteristic changes observed during this period (Fig. 1d). Such sequential changes were commonly seen in MMC in the epithelial layer and those in the lamina propria.

Throughout the course examined, no obvious signs of degranulation were seen in MMC.

MMC of mice treated with IL-3

Continuous administration of IL-3 by an osmotic mini-pump induced a characteristic changes of MMC granules, similar to those observed in MMC after *S. ratti*-infection. The cells containing these altered granules were MMC, because these granules showed metachromasia by alkaline-Giemsa and toluidine blue staining (Fig. 2a). No basic difference was observed in overview of granule changes between mice treated with IL-3 for 7 days and those treated for 14 days. Typical changes in those groups were formation of a large mass by granule fusion (Fig. 2a), followed by paracrystallization (Fig. 2b) and subsequent dispersion of paracrystalline structures into the cytoplasm (Figs. 2c, d and 3a). Long-rhomboid shaped or rod shaped paracrystalline structures were observed in some MMC granules, in which matrix components disappeared (Fig. 2d). Some MMC showed a drastic morphological change having paracrystalline structures broken up into small pieces and scatteredly dispersed within the

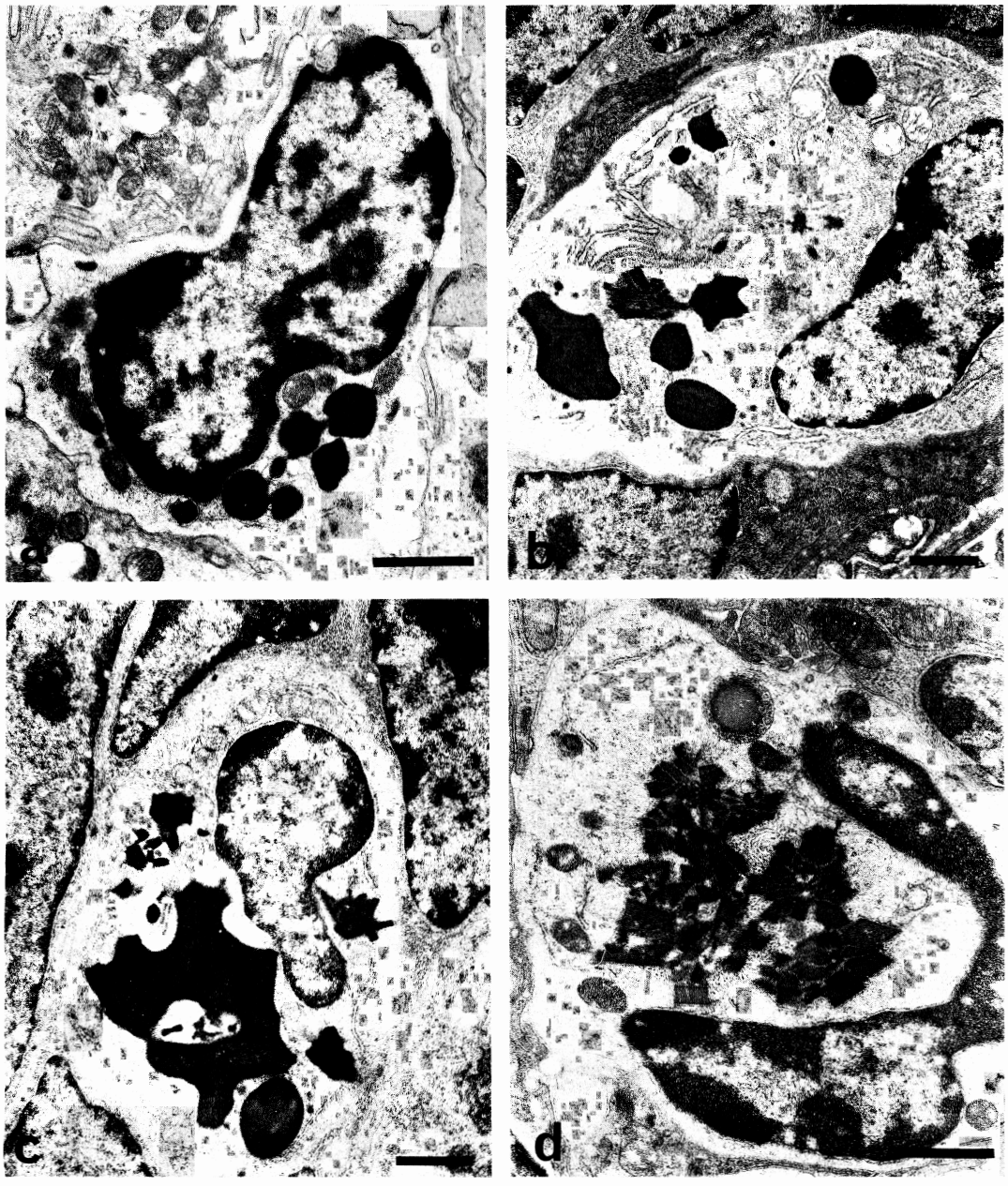

Fig. 1 (a) A mucosal mast cell (MMC) observed in the intestine of a normal mouse containing some small and round granules. ×17,600. *Bar*: 1 μm. (b) A MMC observed in the intestine of a mouse infected with *S. ratti* for 7 days. Granule number in this MMC is few and some granules are polygonal shape. Some granules are fused. ×11,800. *Bar*: 1 μm. (c) A MMC found in the intestine of a mouse infected with *S. ratti* for 14 days. Note progressive granule fusion. ×13,700. *Bar*: 1 μm. (d) A MMC found in the intestine of a mouse infected with *S. ratti* for 14 days. Note appearance of paracrystalline materials due to the disintegration of matrix components. ×17,600. *Bar*: 1 μm.

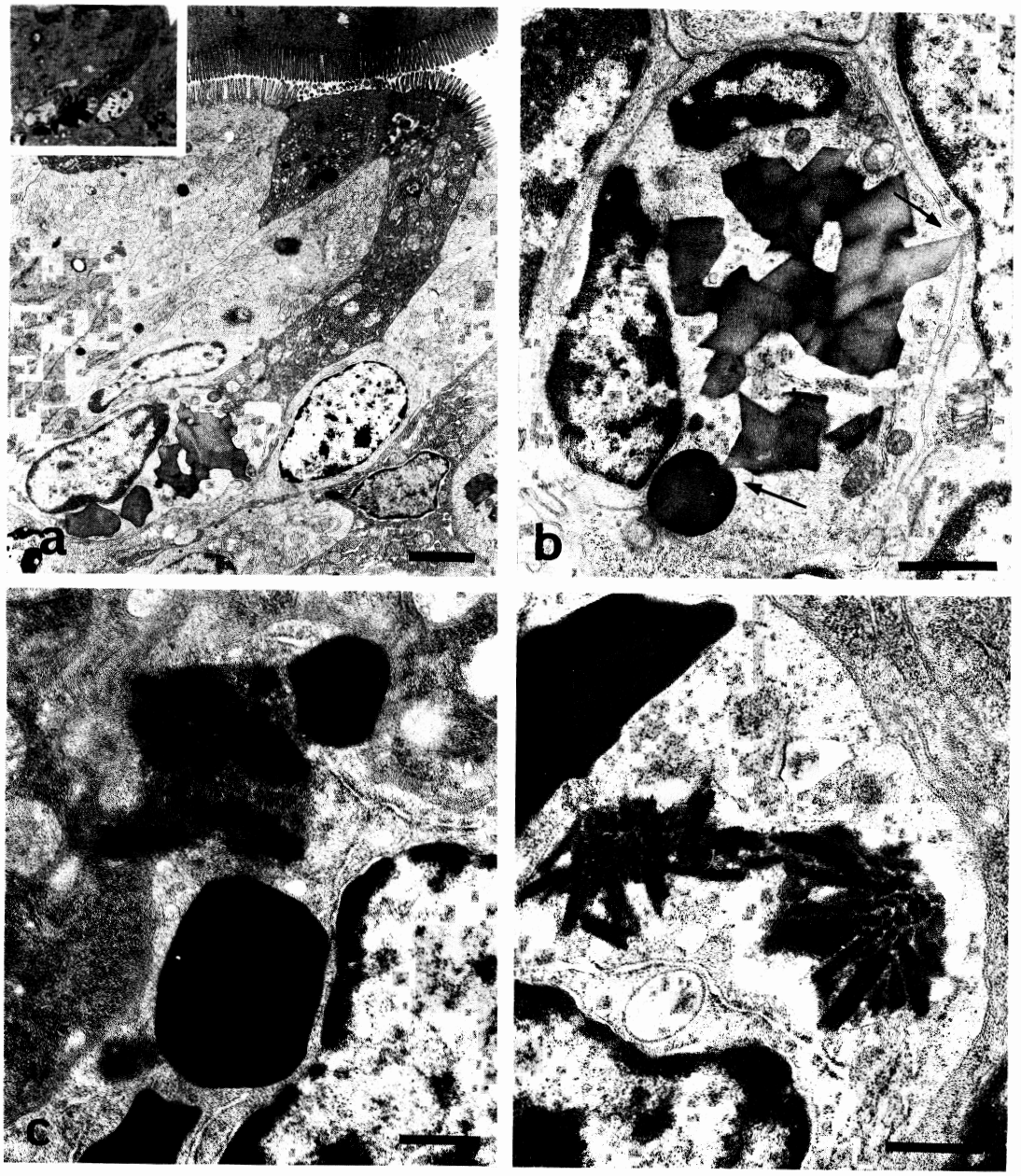

Fig. 2 Mucosal mast cells (MMC) in the intestine of mice transplanted with an osmotic mini-pump containing IL-3 for 7–14 days. (a) Fusion of granules. ×5,500. Inset: toluidine blue staining of the identical MMC. Metachromatic reaction is noted in the granules. ×700. *Bar*: 2 μm. (b) Appearance of paracrystalline structure. Dents (*arrows*) of cytoplasmic membrane and granule wall due to the attachment of paracrystalline structures are visible. ×17,600. *Bar*: 1 μm. (c) Granular matrix components are solubilized. ×28,400. *Bar*: 0.5 μm. (d) Rhomboid- or rod-shaped crystalline materials are visible. ×35,300. *Bar*: 0.5 μm.

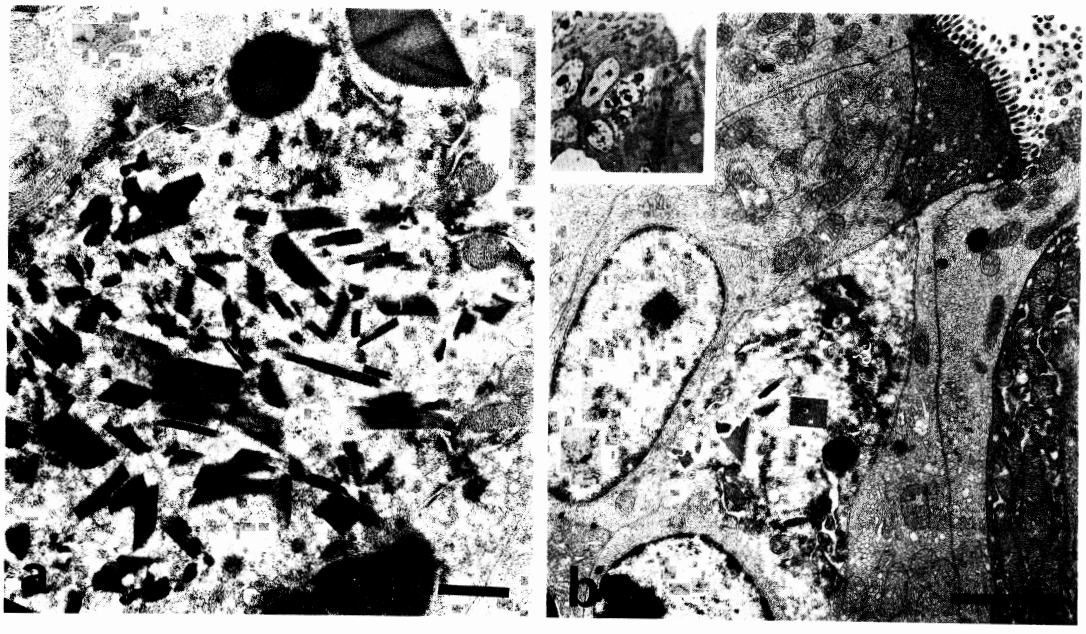

Fig. 3 Mucosal mast cells (MMC) in the intestine of mice transplanted with a mini-pump containing IL-3 for 14 days. (a) A MMC has numerous paracrystalline structures scatteredly dispersed within the cytoplasm. ×23,200. *Bar*: 0.5 μm. (b) A necrobiotic MMC containing some crystalline materials is noted nearby intestinal lumen. ×8,670. *Bar*: 2 μm. Inset: a toluidine blue stained specimen of the identical MMC. ×700.

cytoplasm (Fig. 3a). Moreover, some necrotic or necrobiotic MMC containing paracrystalline structures were sometimes observed in the epithelia close to the intestinal lumen (Fig. 3b). Meanwhile, MMC observed after daily administration of IL-3 for 3 or 5 days possessed round- or oval-shaped granules with mild granule fusion, but did not show any other noticeable changes.

Discussion

After *S. ratti*-infection or IL-3 administration, murine intestinal MMC showed an unique sequential changes in granule morphology, which were markedly different from those seen in degranulation of connective tissue mast cell (Dvorak *et al.*, 1983; Dvorak *et al.*, 1992; Dvorak, 1994). Regardless of either infection with *S. ratti* or IL-3 administration, the early change was fusion of granules in some MMC. In addition, some intact MMC granules started paracrystallizing individually while some other granules fused with each other and then start paracrystallizing. Granule matrices surrounding these paracrystalline structures then disappear gradually and paracrystalline structures themselves are broken up into small pieces, finally scattering in the cytoplasm. The morphological changes of granules, therefore, may imply a functionally activated state of MMC. Similar to our observations, presence of rhomboid-shaped structures of granules, have been reported in murine MMC (Silva, 1967; Crowle and Phillips, 1983), in MMC of *N. brasiliensis*-infected rats (Miller, 1971) and *Trichostrongylus colubriformis*-infected guinea pigs (Handlinger and Rothwell, 1984). However, drastic granule fusions and subsequent dispersion of granule materials in the cytoplasm have been found in only murine MMC at the later stage of infection with *N. brasiliensis*. (Crowle and Phillips, 1983). The granule fusion and the cytoplasmic dispersion of crystalline structures therefore may be the most severe granule change in MMC and be characteristic to mice.

In the present study, neither granule nor

paracrystalline structures were observed outside MMC, although granule-to-granule fusions (Figs. 1c and 2b) and attachment of paracrystalline structures to the plasma membrane (Fig. 2b) were notable. As far as our study is concerned, therefore, MMC did reveal neither anaphylactic degranulation showing granule-to-plasma membrane fusion, extrusion of membrane-free granule contents and formation of cytoplasmic canalicular structures (Dvorak *et al.*, 1983; Dvorak *et al.*, 1992; Dvorak, 1994) nor piecemeal degranulation showing progressive losses of granule contents in the absence of granule-to-granule or granule-to-plasma membrane fusions with the retention of empty granule containers (Dvorak *et al.*, 1992; Dvorak, 1994), which were all characteristically seen in connective tissue mast cell. Hence, MMC themselves may disintegrate and then release granule materials into the extracellular spaces (Crowle and Phillips, 1983). Meanwhile, we noticed the disappearance of granule matrix portions following paracrystallization (Fig. 2d). This finding impels the possible liberation of some active materials in the matrix portions into the outside of the cell. MMC differentiated and proliferated from the progenitor cells in the presence of IL-3 *in vivo* (Abe *et al.*, 1988) or *in vitro* (Gurish *et al.*, 1992). However, granules of bone marrow-derived cultured mast cells generated by IL-3 showed no paracrystalline structures (Combs, 1971; Pitton *et al.*, 1988). We also could not detect significant granule changes in cultured mast cells (unpublished observation). Several distinct proteases, mouse mast cell protease-1 (MMCP-1) to MMCP-6, are present in different subtypes of murine mast cells (Reynolds *et al.*, 1990) and are expressed differently in intestinal MMC and bone marrow-derived cultured mast cells (Gurish *et al.*, 1992; Ghildyal *et al.*, 1992). Difference in protease expression between intestinal MMC and cultured mast cells may thus be associated with the above described diversity in their granule morphology. Alternatively, some unknown stimuli may be responsible for inducing granule morphological changes only *in vivo*. Although the mechanism capable of inducing granule changes are still unclear, IL-3 itself and some other cytokines, but not parasitic antigen, may be involved. Biochemical identification of paracrystalline structures, disappearing granule matrices, and of factors triggering granule crystallization, and possible significance of granule changes all remain to be determined.

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