PROLIFERATION OF PROTEASE-ENRICHED MAST CELLS IN SARCOPTIC SKIN LESIONS OF RACCOON DOGS

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Introduction and Objective

In Japan, previous study reported that *Sarcoptes scabiei* as the cause of enzootic dermatitis in wild raccoon dogs (*Nyctereutes* pmcyonoides) (Takahashi et al., 2001). These parasites burrow into the skin releasing a massive amount of antigenic material Into the lissue. Recent studies demonstrated the important roles played by tryptase or chymase, or both, secreted from mast cells In the maintenance of tissue integrity and regulation of inflammatory and immune responses in skin (Tomimori et al., 2002). Despite the high prevalence of sarcoptic mange in raccoon dogs, and despite the important role of mast cells in mediating the effects of this dlsease, descriptions of the immunopathogenic importance of mast cell responses are limited. Moreover, protease activity within the mast cells of infected animals has not been evaluated.

Materials and Methods

Five adult male raccoon dogs, with no history or clinical signs of skin disease, were used as a control animals groups. Two adult, hairless, male raccoon dogs that had died were brought to the Veterinary Teaching Hospital, Miyazaki University. The entire skin of both animals was thickened, wrinkled and dry. After macroscopical examination, sarcoptic mites of a number of different developmental stages were found. Specimens from both groups included tongue, lung, liver, jejunum and rectum; and skin of the ventrolateral pinna, dorsal neck, dorsal fore foot, dorsal hind foot, and dorsum. The tissues were separated into two sets, first set specimens fixed in Carnoy's and the second set fixed in formalin. To examine mast cell distribution, sections from each of the specimens fixed in Carnoy's were stained with 0.1% alcian blue and 0.5% safranin O. Sections were stained using naphthol AS-D chloroacetate for chymase identification . Tryptase was demonstrated with Z-Ala-Ala-Lys-4- methoxy-2-naphthylamide as a substrate. Mast cell counts were performed under microscope with a square eyepiece graticule,, covering an area of 0.050625 mm2. Tryptase-positive, alcian blue-positive and chymase-positive mast cells were compared in respect of count and distribution by means of successive staining.

Results and Discussion

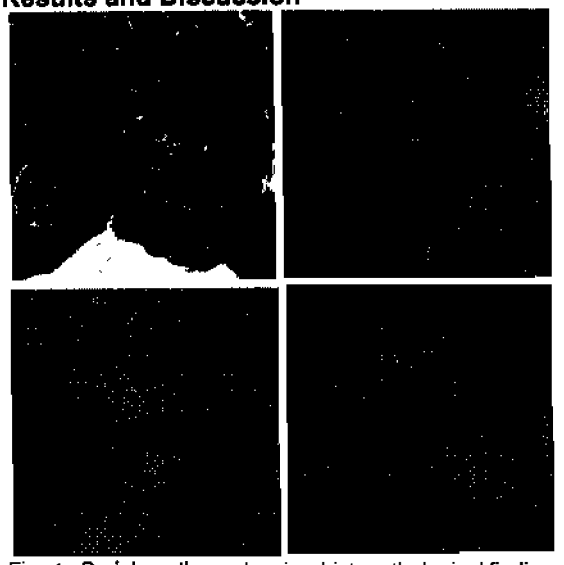

Fig. 1. Serial sections showing histopathological findings within skin lesions from raccoon dogs infested with S. scabiei. (a) HE stain. (b) AB-S O stain. (c) & (d) Enzyme-histochemical stain for chymase and tryptase. Bar, 100 um.

This study clearly showed a cellular infiltrate of mast cells, mononuclear cells and eosinophils in the thickened, hyperplastic, and hyperkeratinized epidermis of *S. scabiei*-infested raccoon dogs. (Fig. Ia). As shown in Fig. Ib, mast cells appeared blue on a pale red background. Increased numbers of mast cells and eosinophils were found around the mites, and mast cells were observed to infiltrate the dermal stratum papillae of the skin. Mast cells and eosinophils were predominated,

suggesting an immediate hypersensitivity reaction. Mast cell numbers within the skin of the dorsum, dorsal neck, dorsal hind foot, and dorsal fore foot of infested raccoon dogs were significantly higher ($P<0.01$) than in controls (Fig. 2). The numbers of mast cells demonstrated in the ventrolateral pinna, liver, jejunal submucosa and rectal mucosa were also greater in mite-infected animals than in controls.

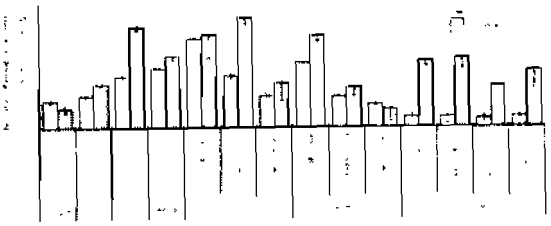

Figure 2. Number of mast cells /mm2 in **various** organs of control **and infested** raccoon **dogs with S. scabiei. Sections were fixed in Camoy's fluid and stained with alcian blue-**safranin O. Vertical bars indicate the **mean ± SD. Musc+Ser: muscularis and serosa.**

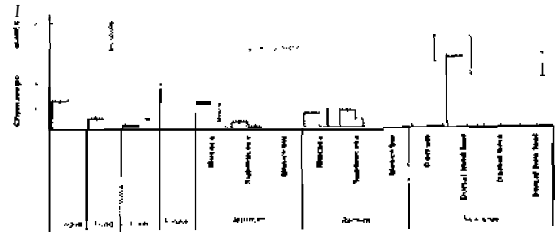

Figure 3. Percentage of chyrnase positive mast cells in various **organs of** control **and** infested raccoon dogs **with** S. scabiei. Vertical bars indicate **the mean ± SD Musc+Ser: muscularis and serosa.**

Figures 3 and 4 show that the observed increase of mast cells in the skin of mite-infected animals was accompanied by an increase in granule protease activity, mast cells showing chyrnase and tryptase activity being significantly more numerous ($P<0.05$) in the skin of infected animals than in controls. Chymase-containing mast cells were apparent within the sections by their pink and purple coloured granules (Fig. 1c). The percentage of mast cells demonstrating chymase activity was 53.0 ± 27.4 % in control animals, and 73.8 ± 19.4 % in mite-infested animals. Tryptase activity was detected in the same tissue regions by the presence of intensely reddish granules (Fig. 1d) The percenlage of mast cells demonstrating tryptase activity was 53.5 ± 25.2 % in control animals, and 89.4 ± 9.8 % in infected animals.

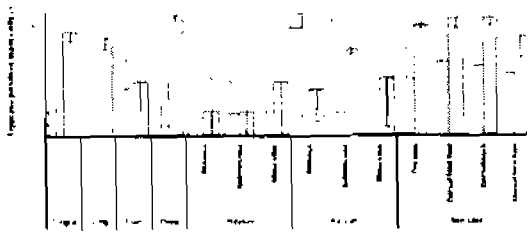

Figure 4. **Percentage of tryptase positive** mast cells in various organs **of control and infested** raccoon **dogs with** S scabiei. Vertical bars indicate the mean ± SD Musc+Ser muscularis and serosa

Mast cells with incresed staining intensity for both chymase and tryptase were also observed in the ventrolateral pinna, liver, lung and tongue specimens of infected animals. Increased numbers of chymase- and tryptase. positive mast cells were detected in tongue and lung specimens from infected animals, even though increased numbers of metachromatic mast cells were not observed in these organs as compared with normal animals. In conclusion, infestation of raccoon dogs with S. scabiei mites results in cutaneous lesions, characterized by mast cell hyperplasia and elevation of mast cell chymase and tryptase activity. Both enzymes have important roles in maintenance of skin integrity and regulation of inflammatory and immune responses, thus, an increase in their activity might explain several of the progressive cutaneous histopathological changes observed in mange infestation.

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